INHIBITION BY PHOSPHOLIPIDS OF THE ACTION OF SYNTHETIC DETERGENTS ON BACTERIA

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Certain of the synthetic detergents exert a marked inhibitory effect on the metabolism (1) and viability (2) of bacteria. The germicidal action of these compounds and other biological effects have been studied by numerous investigators.[1]

Various explanations have been proposed for the action of these compounds on bacteria, but none of these has been proved experimentally. Kuhn and Bielig (3) have suggested recently that germicidal concentrations of some detergents correspond rather closely with the concentrations necessary to effect denaturation of proteins. However, the relative activity of the detergents in protein denaturation, as reported by Anson (4), does not correspond with the effects which we have observed on bacterial metabolism and viability. Furthermore, anionic detergents which denature proteins readily are highly selective in their action on bacteria. These compounds inhibit only Gram-positive microorganisms.[1] Obviously, one or more factors besides denaturation of proteins must influence the activity of the anionic detergents. Possibly, in the organized cell, there is the additional factor of interaction between the detergents and the lipoid constituents of the cellular membrane.

The rôle of lipoids in the membrane of cells has received considerable attention. It has been suggested that the protoplasmic membrane consists of a continuous lipoid structure (5), a lipoprotein mosaic (6) or a layer of lipoid molecules between adsorbed protein layers (7–9). The exact nature of the lipoid constituent is not known. However, Bungenberg de Jong and Bonner (10) have suggested as a working hypothesis that "the special properties of the protoplasmic membrane depend upon one or more layers of oriented phosphatide ions."

A number of studies on the action of phospholipids on cells have been made. These compounds have been reported to weaken the antiseptic action of mercuric chloride, phenol, and salvarsan on anthrax bacilli (11) and to inhibit the action of staphylococcus bacteriophage (12, 13) and of various bacterial lysins and toxins (14–18). A

[1] See reference 1 for bibliography.

number of workers (19–22) believe that cytolysis and hemolysis by saponins are reduced by phosphatides.

Since the detergents are known to be highly surface-active, and their initial effect, in all probability, is to disorganize the cell membrane, it was of interest to determine the influence of added phospholipids on the inhibitory and germicidal action of detergents. As shown later, it appears that certain surface-active compounds such as the phospholipids can modify the activity of detergents very markedly.

Methods

Bacterial Metabolism.—All experiments were conducted in Warburg manometers as previously described (1). The bacterial suspensions were prepared from bacteria grown on veal infusion agar. Lactobacillus was grown in 1 per cent glucose-meat infusion broth. Respiration and glycolysis experiments were conducted in phosphate and bicarbonate buffers, respectively, containing glucose. The phospholipids were added directly to the bacterial suspension in the vessel; the detergents were pipetted into the side bulb and added at the start of the experiment unless otherwise specified.

Bactericidal Action.—The bactericidal potency of the detergents was determined under precisely the same experimental conditions as were employed in the studies on bacterial metabolism. This makes possible direct comparison between these two phenomena. The bacterial suspension was diluted so as to contain 10 billion cells per cc.; 1.0 cc. of this suspension was added to 1.8 cc. of phosphate buffer, (pH 7.0), and 0.1 cc. of phospholipid solution. Controls were made up in the same manner without phospholipid. The tubes were placed in a water bath at 38°; 0.2 cc. of detergent solution, sufficient to provide the desired final concentration, was then added. After 1 hour, 0.1 cc. of this mixture was pipetted into 5 cc. of veal infusion broth (containing 0.2 per cent glucose for lactobacillus experiments). A second transfer of 0.1 cc. was made from the first tube of veal infusion broth to eliminate the possibility of bacteriostatic action. The tubes were examined for growth after 48 or 72 hours. Only data on the second subculture tubes are recorded.

Detergents.—The detergents used have been described in previous publications (1, 2) and are referred to here as *cationic* or *anionic*, depending on whether the long-chain, hydrophobic group is in the cation or anion. A few experiments were performed with an unionized detergent. The compounds were dissolved in water and neutralized to pH 7.0.

Phospholipids.—Phospholipids from various sources were used for these experiments. We wish to express our indebtedness to the following individuals and companies for supplying purified preparations of these compounds: soy-bean lecithin, (Emulsol Corporation); soy-bean lecithin and cephalin, the latter purified through the cadmium salt (Dr. Percy Julian, Glidden Company); beef heart lecithin and egg lecithin from Dr. Mary C. Pangborn, as prepared by her improved technique (23); brain cephalin and sphingomyelin from Dr. H. N. Christiansen, described in (24); brain cephalin (phosphatidyl serine) from Dr. Jordi Folch (25). Aqueous suspensions of the phospholipids were prepared and diluted as desired.

RESULTS ON BACTERIAL METABOLISM

Effect of Soy-Bean Lecithin

Most of our first experiments were performed with a purified preparation of soy-bean lecithin (obtained from Emulsol Corporation) which also contained some cephalin. Upon addition of this lecithin to a suspension of bacteria it was found that the usual inhibition of bacterial metabolism by detergents did not occur. To produce inhibition in the presence of lecithin considerably higher concentrations of detergents were required. These results were obtained with both Gram-positive and Gram-negative microorganisms, and with cationic and anionic detergents (Tables I and II). Thus, in Experiment 1 of Table I on *aerobic acid production* by lactobacillus, 0.1 mg. Zephiran inhibited 100 per cent; on the addition of 3.0 mg. lecithin, no inhibition was observed. Similarly, 0.1 mg. Emulsol-660 B and 0.1 mg. Emulsol-609 inhibited 43 and 91 per cent, respectively; in the presence of lecithin, there was no inhibition. As shown also in Table I, the same type of results was obtained with *Micrococcus tetragenus* (Experiment 2). The inhibitory effect of Zephiran on the *anaerobic acid production* of *Staphylococcus aureus* was also prevented by lecithin (Experiment 3).

Experiments 4 and 5 demonstrate that lecithin prevents the inhibitory action of cationic detergents on the *respiration* of the Gram-positive organisms, *Staphylococcus aureus* and *Sarcina lutea*. Experiments 6 and 7 demonstrate a similar action on the respiration of the Gram-negative microorganisms, *Proteus vulgaris* and *Escherichia coli*.

The three cationic detergents were chosen to provide a wide variation in chemical structure: Zephiran (alkyl dimethyl benzyl ammonium chloride) represents the *acyclic* quaternary nitrogen type; Emulsol-660 B (lauryl pyridinium iodide), the *cyclic* quaternary nitrogen type; and Emulsol-609 (lauryl ester of alpha-amino isobutyric acid hydrochloride), the non-quaternary nitrogen type of cationic detergent. All of these detergents were influenced to the same degree by lecithin.

The experiments in Table II show that lecithin can also prevent the inhibitory action of the anionic detergents. Only the Gram-positive microorganisms, *Staphylococcus aureus* and lactobacillus, are included in this table since previous studies demonstrated that the anionic detergents inhibit the metabolism of Gram-positive organisms only. It can be seen from Table II that the inhibitory action of the anionic detergents, Tergitol-7, cetyl sulfate, Duponol LS, and Triton W-30, is largely prevented by 3.0 mg. of lecithin. When the concentration of Tergitol-7 was increased to 0.5 mg., this concentration of lecithin was sufficient to protect *Staphylococcus aureus*, but not lactobacillus.

It can be seen from Tables I and II (and subsequent tables) that in a number

of instances, *stimulation* of acid production or respiration occurred in control experiments with lecithin. In general, this is a genuine stimulation. For example, in the experiments on acid production by lactobacillus, analysis

TABLE I

Effect of Soy-Bean Lecithin on Inhibition of Bacterial Metabolism by Cationic Detergents

Experiment	Bacteria	Detergent	Concentration of detergent	Concentration of lecithin	Inhibition*
			mg./3 cc.	*mg./3 cc.*	*per cent*
1	Lactobacillus‡	Zephiran	0	3.0	+9
			0.1	0	−100
			0.1	3.0	0
		Emulsol-660 B	0	3.0	+5
			0.1	0	−43
			0.1	3.0	+5
		Emulsol-609	0	3.0	+5
			0.1	0	−91
			0.1	3.0	0
2	*Micrococcus tetragenus*‡	Zephiran	0	3.0	+3
			0.1	0	−89
			0.1	3.0	+5
		Emulsol-660 B	0	3.0	+3
			0.1	0	−83
			0.1	3.0	−2
3	*Staphylococcus aureus*§	Zephiran	0	3.0	+36
			0.1	0	−85
			0.1	3.0	+8
4	*Staphylococcus aureus*‖	Zephiran	0	3.0	+45
			0.05	0	−81
			0.05	3.0	+40
			0.05	0.3	+32
			0.05	0.1	−30
			0.05	0.05	−48
		Emulsol-660 B	0	3.0	+48
			0.1	0	−86
			0.1	3.0	+46
		Emulsol-609	0	3.0	+48
			0.2	0	−85
			0.2	3.0	+41

TABLE I—*Concluded*

Experiment	Bacteria	Detergent	Concentration of detergent	Concentration of lecithin	Inhibition*
			mg./3 cc.	*mg./3* cc.	*per cent*
5	*Sarcina lutea*‖	Zephiran	0	3.0	+65
			0.1	0	−85
			0.1	3.0	+112
		Emulsol-660 B	0	3.0	+65
			0.1	0	−71
			0.1	3.0	+114
		Emulsol-609	0	3.0	+65
			0.2	0	−85
			0.2	3.0	+119
6	*Proteus vulgaris*‖	Zephiran	0	0.3	+14
			0.05	0	−41
			0.05	0.3	+18
7	*Escherichia coli*‖	Zephiran	0	3.0	+13
			0.05	0	−93
			0.05	3.0	+13
		Emulsol-660 B	0	3.0	+13
			0.1	0	−88
			0.1	3.0	−6
		Emulsol-609	0	3.0	+13
			0.2	0	−84
			0.2	3.0	−2

* Minus signs represent inhibition; plus signs represent stimulation.
‡ Aerobic glycolysis.
§ Anaerobic glycolysis.
‖ Respiration.

showed that the increase in acid, indicated by manometric readings, was accounted for by increased glucose utilization and increased lactic acid production, (the latter estimated by the method of Miller and Muntz (26)); no acid was produced from lecithin by bacterial action. Although this effect has been observed with highly purified preparations, it is impossible at present to say with certainty whether the stimulation is caused by the phospholipid or traces of catalytic impurity. In a few cases in which glucose estimations were made in respiration experiments, however, a "sparing" action was observed in the presence of lecithin, *i.e.*, less glucose was used than in the control vessel. Presumably oxidation of lecithin accounted for the extra oxygen uptake.

Other Phospholipids

Essentially the same results as those presented in Tables I and II were obtained with other phospholipids studied. The most active compounds were

TABLE II

Effect of Soy-Bean Lecithin on Inhibition of Bacterial Metabolism by Anionic Detergents

Bacteria	Detergent	Concentration of detergent	Concentration of lecithin	Inhibition*
		mg./3 cc.	*mg./3 cc.*	*per cent*
Staphylococcus aureus‡	Tergitol-7	0	3.0	+26
		0.3	0	−90
		0.3	3.0	−5
		0.5	0	−88
		0.5	3.0	+25
Lactobacillus§	Tergitol-7	0	3.0	+79
		0.2	0	−94
		0.3	0	−91
		0.5	0	−96
		0.2	3.0	+63
		0.3	3.0	+27
		0.5	3.0	−81
	Cetyl sulfate	0	3.0	+60
		0.1	0	−94
		0.2	0	−94
		0.1	3.0	+42
		0.2	3.0	+48
	Duponol LS	0	3.0	+60
		0.1	0	−36
		0.2	0	−79
		0.1	3.0	+52
		0.2	3.0	+46
	Triton W-30	0	3.0	+60
		0.2	0	−84
		0.2	3.0	+38

* Minus signs represent inhibition; plus signs represent stimulation.

‡ Respiration.

§ Anaerobic glycolysis.

cephalins and soy-bean lecithin. Some typical results with Zephiran are reproduced in Table III. Similar results have been obtained with other detergents. The data in this table indicate that the phospholipids are effective at very low concentrations. Thus, in several instances, 0.15 mg. of cephalin or lecithin (approximately M/20,000) was sufficient to reduce considerably the

inhibitory action of 0.05 mg. of Zephiran. In some cases as little as 0.05 mg. phospholipid reduced the inhibition.

TABLE III

Effect of Various Phospholipids on Inhibition by Zephiran

Bacteria	Phospholipid	Inhibition by 0.05 mg. Zephiran* / Concentration of phospholipid, *mg./3 cc.*				
		0.0	0.05	0.15	0.3	0.5
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Staphylococcus aureus‡	Beef lecithin	−87	−54	−19	−12	+8
	Egg lecithin	−87	−77	−79	−71	−54
	Soy lecithin	−87	−55	−26	−12	−2
	Soy cephalin	−87	−75	−48	−15	−5
	Sphingomyelin	−87	−75	−70	−42	−36
	Brain cephalin§	−78	−45	−21	+17	+29
Escherichia coli‡	Beef lecithin	−81	−72	−57	−50	−23
	Egg lecithin	−81	−76	−69	−58	−33
	Soy lecithin	−81	−71	−41	−8	+3
	Soy cephalin	−81	−70	−29	−6	−1
	Sphingomyelin	−81	−73	−47	−26	−5
	Brain cephalin§	−89	−86	−36	−4	−6
Lactobacillus‖	Beef lecithin	−85	−61	−45	−41	−31
	Egg lecithin	−85	−63	−47	−37	−35
	Soy lecithin	−85	−50	−30	−3	−2
	Soy cephalin	−85	−58	−41	−21	−1
	Sphingomyelin	−85	−48	−44	−33	−32
	Brain cephalin**	−79	−48	−45	−19	−11
	Brain cephalin§	−89	−38	−26	−10	+1

* Minus signs represent inhibition; plus signs represent stimulation.

‡ Respiration.

§ Phosphatidyl serine.

‖ Aerobic glycolysis.

** Cephalin, prepared by Dr. H. N. Christiansen.

Time Relationship and Period of Exposure

Previous experiments have shown that the detergents act very rapidly and that their inhibitory effect on bacterial metabolism is completed usually within 5 minutes. We have found in the present study that if bacteria are exposed to the detergent *first*, subsequent addition of phospholipid cannot prevent the inhibitory action. The phospholipid must be added *before or simultaneously with* the detergent. Thus, in an experiment with *Staphylococcus aureus*, 0.05 mg. of Zephiran produced an inhibition of 81 per cent; 3.0 mg. of soy-bean lecithin, added 15 minutes later, had no effect on the inhibition. On the other

hand, when either 0.1 mg. or 0.3 mg. of lecithin was added *before* the detergent, the phospholipid prevented the inhibition by Zephiran.

If bacteria are exposed to a solution containing lecithin for several minutes, and then *removed* from the lecithin solution and washed, they continue to be resistant to concentrations of detergents which are normally inhibitory. Protection induced in this manner is dependent on the concentration of both the phospholipid and detergent. A typical experiment was carried out as follows: a suspension of *E. coli* (1.0 cc.) was treated with 3 mg. of soy-bean lecithin; after 5 minutes the cells were centrifuged, washed, recentrifuged, and suspended in their original volume. A similar suspension, treated in the same manner but without lecithin, served as a control. Subsequent experiments on respiration in the presence of glucose plus 0.05 mg. Zephiran gave the following results: control, 88 per cent inhibition; lecithin-treated, 2 per cent inhibition. Similar results were obtained with *Staphylococcus aureus*, *Proteus vulgaris*, and lactobacillus.

Addition of Lecithin to Culture Medium

Several organisms were grown in the presence of soy-bean lecithin (1 mg./cc.) to determine if this would affect subsequent sensitivity to the detergents. The results obtained with *Staphylococcus aureus*, *E. coli*, and lactobacillus are indicated in Table IV. It is apparent that there was no change in the sensitivity of *Staphylococcus aureus* and *E. coli*. The inhibitions with Zephiran at various concentrations were almost precisely the same as those of the controls (grown normally, in the absence of lecithin). On the other hand, two experiments with lactobacillus indicated that considerably higher concentrations of Zephiran or Tergitol-7 were necessary to inhibit cells grown in the presence of lecithin. Possibly, further experiments with *Staphylococcus aureus* and *E. coli* at different concentrations of phospholipid might lead to the same result obtained with lactobacillus.

Effect of Phospholipids on the Bactericidal Action of Detergents

A few experiments were performed to determine whether the phospholipids could prevent the *bactericidal* action of the detergents. The results obtained are presented in Table V. Bactericidal action (absence of growth) is indicated by a minus sign. The values recorded were obtained by examination of the second subculture tubes after 48 or 72 hours incubation. It is apparent that a considerably higher concentration of detergent is required to kill the microorganisms in the presence of phospholipid than in its absence, and that the phospholipid is effective in a relatively low concentration.

With *Staphylococcus aureus* and *E. coli*, the bactericidal action of 0.3 and 0.5 mg. of Zephiran and Phemerol was prevented by 3.0 mg. of lecithin. With lactobacillus the following results were obtained: 3 mg. of lecithin prevented

the bactericidal action of 0.1 mg. of Zephiran or Phemerol (1/30,000). Moreover, 1.0 mg., but not 0.5 mg., of lecithin protected against 0.1 mg. of Zephiran. 3.0 mg. of lecithin was not sufficient to protect against 0.5 mg. of Zephiran, but was effective against 0.3 and 0.5 mg. of Tergitol-7 (but not against 1.0 mg. of

TABLE IV

Effect of Inclusion of Lecithin in Culture Medium

Bacteria	Culture medium	Concentration of detergent		Inhibition of metabolism by detergents	
		Zephiran	Tergitol-7	Cultured in absence of lecithin	Cultured in presence of lecithin*
		mg./3 cc.	*mg./3 cc.*	*per cent*	*per cent*
Staphylococcus aureus‡	Broth	0.05		70	67
		0.10		84	85
		0.20		86	87
		0.30		87	82
E. coli‡	Agar	0.05		80	80
		0.10		89	91
		0.20		92	91
Lactobacillus§	Broth	0.10		82	8
		0.15		91	15
		0.20		92	13
		0.30		92	42
Lactobacillus§	Broth	0.05		79	9
		0.10		92	11
		0.20		91	32
		0.30		94	83
			0.10	90	9
			0.20	96	17
			0.30	95	96

* 1.0 mg./cc.

‡ Respiration.

§ Aerobic glycolysis.

Tergitol-7). It is apparent from these results that, just as in the experiments on bacterial *metabolism*, there is a close relationship between the concentration of bactericidal agent and the effective concentration of phospholipid.

Effect of Phospholipids on Inhibition by Other Germicidal Compounds

The effect of lecithin on the inhibition of metabolism by mercuric chloride and by two organic mercury compounds, Merthiolate and Metaphen, has been studied. The results are summarized in Table VI. In no instance did 3 mg.

of lecithin adequately protect against 0.05 or 0.1 mg. of these compounds (in 3 cc. volume). However, when the concentration of mercuric chloride was very low (*viz.* 0.01 mg.) 3.0 mg. of lecithin prevented the inhibition (Experiment 5). Even at the very lowest concentrations of Metaphen and Merthiolate, however, lecithin did not protect.

TABLE V

*Effect of Phospholipids on Bactericidal Action of Detergents**

Bacteria	Detergent	Concentration of detergent	Concentration of lecithin			
			0	0.5	1.0	3.0
		mg./3 cc.	*mg./3 cc.*	*mg./3 cc.*	*mg./3 cc.*	*mg./3 cc.*
Staphylococcus aureus	Zephiran	0.5	−			+
		0.3	−			+
		0.1	−			+
	Phemerol	0.5	−			+
		0.3	−			+
E. coli	Zephiran	0.5	−			+
		0.3	−			+
	Phemerol	0.5	−			+
		0.3	−			+
Lactobacillus	Zephiran	0.1	−			+
	Phemerol	0.1	−			+
Lactobacillus	Zephiran	0.1	−	−	+	+
		0.5	−			−
		1.0	−			−
		3.0	−			−
	Tergitol-7	0.3	−			+
		0.5	−			+
		1.0	−			−

* A minus sign in the table indicates bactericidal action (no growth in subculture tube).

Some experiments have been performed with the selective bactericidal agents isolated by Dubos (27) and by Hoogerheide (28).[2] Both preparations were dissolved in a small volume of alcohol and diluted with water to give a

[2] Gramicidin was purified from a preparation kindly supplied by Eli Lilly and Company. This probably contained several of the bactericidal substances described by Dubos and coworkers (29–31). We wish to thank Dr. J. C. Hoogerheide for a sample of his material.

TABLE VI

Effect of Soy-Bean Lecithin on Inhibition of Bacterial Metabolism by Mercury Compounds

Experiment	Bacteria	Concentration of mercury compound			Inhibition by mercury compound alone	Inhibition by mercury compound + 3.0 mg. lecithin
		$HgCl_2$	Metaphen	Merthiolate		
		mg./3 cc.	*mg./3 cc.*	*mg./3 cc.*	*per cent*	*per cent*
1	*Staphylococcus aureus**	0.05			89	88
			0.05		84	86
				0.05	76	74
2	*Sarcina lutea**	0.10			92	60‡
			0.10		91	58‡
3	Lactobacillus§	0.05			89	90
		0.10			91	98
				0.10	91	98
4	*Micrococcus tetragenus*§	0.10			90	91
5	*Escherichia coli**	0.05			91	85
		0.025			91	77
		0.010			90	11
		0.005			81	1
		0.001			6	5
			0.05		93	96
			0.025		93	94
			0.010		90	90
			0.005		78	61
			0.001		16	11
				0.05	82	83
				0.025	75	72
				0.010	63	70
				0.005	50	55
				0.001	13	8

* Respiration.

‡ 6.0 mg. lecithin.

§ Aerobic glycolysis.

stable, milky emulsion. Control experiments showed that the low concentration of alcohol present did not influence the results. It was found that lecithin could effectively prevent the inhibition of bacterial metabolism caused by these compounds (Table VII). Other phospholipids were also active. All the experiments reported in Table VII were performed with a phospholipid

concentration of 1 mg. per cc. No effort was made to determine the minimum effective phospholipid concentration.

TABLE VII

Effect of Phospholipids on Inhibition by Gramicidin and Hoogerheide's Compound

Bacteria	Phospholipid	Concentration of inhibitor		Inhibition* by inhibitor alone	Inhibition* by inhibitor plus 3 mg. phospholipid
		Gramicidin	Hoogerheide		
		mg./3 cc.	*mg./3 cc.*	*per cent*	*per cent*
Staphylococcus aureus‡	Lecithin (beef)	0.2	—	−68	+4
	Lecithin (soy)	0.1	—	−45	+18
		0.2	—	−81	+53
		—	0.1	−51	+37
		—	0.2	−69	+29
		—	0.3	−73	+42
Sarcina lutea‡	Lecithin (soy)	0.1	—	−83	+92
		—	0.2	−77	+40
Lactobacillus§	Lecithin (soy)	0.1	—	−99	−2
		—	0.1	−93	−9
	Lecithin (beef)	0.1	—	−91	−28
		—	0.1	−47	−17
	Cephalin (soy)	0.1	—	−93	−7
		—	0.1	−70	−9
		—	0.2	−97	+11
	Sphingomyelin	0.1	—	−90	−12

* Minus signs represent inhibition; plus signs represent stimulation.

‡ Respiration.

§ Aerobic glycolysis.

Protective Action by Surface-Active Compounds Other than Phospholipids

An unionized detergent, Demal,[3] was found to be very effective in preventing the inhibitory action of both cationic and anionic detergents. This detergent is described (32, 33) as a mixture of polyglycerol esters, with the following type formula: R—COO—CH_2—CHOH—CH_2—O—CH_2—CHOH—CH_2OH, in which R represents a long-chain alkyl radical. This compound possesses the typical polar-nonpolar structure of surface-active compounds. On the other hand, it differs markedly from the cationic and anionic detergents be-

[3] A sample of this compound was kindly supplied by the Emulsol Company.

cause it does not ionize. Furthermore, it appears to have no effect on bacterial metabolism.

TABLE VIII

Effect of Demal on Inhibition of Bacterial Metabolism by Detergents

Bacteria	Detergent	Concentration of detergent	Concentration of Demal	Inhibition*
		mg./3 cc.	*mg./3 cc.*	*per cent*
Staphylococcus aureus‡	Zephiran	0	3.0	−4
		0.05	0	−85
		0.05	3.0	0
		0.05	1.0	+12
		0.05	0.5	+11
		0.05	0.3	+3
	Tergitol-7	0.3	0	−85
		0.3	3.0	−14
Escherichia coli‡	Zephiran	0	1.0	0
		0.05	0	−82
		0.05	1.0	−15
		0.05	0.5	−42
		0.05	0.3	−57
		0.05	0.1	−72
Lactobacillus§	Zephiran	0	1.0	0
		0.05	0	−77
		0.05	1.0	−9
		0.05	0.5	−11
		0.05	0.3	−32
		0.05	0.1	−83
	Tergitol-7	0	1.0	0
		0.05	0	−92
		0.05	1.0	−4
		0.05	0.5	−2
		0.05	0.3	−20
		0.05	0.1	−80

* Minus signs represent inhibition; plus signs represent stimulation.

‡ Respiration.

§ Aerobic glycolysis.

The results obtained with this compound are illustrated in Table VIII. It can be seen that relatively low concentrations of Demal prevent inhibition of bacterial metabolism by both Zephiran (cationic) and Tergitol-7 (anionic).

It is of interest to note that other experiments (not included in Table VIII) demonstrate that the unionized detergent, Demal, also prevents the inhibition caused by gramicidin. Thus, in an experiment with lactobacillus, 0.1 mg. of

gramicidin inhibited aerobic acid production 99 per cent; in the presence of 3 mg. of Demal, the inhibition was only 19 per cent.

Some experiments were performed with cholesterol to determine whether it protected against the detergents in a manner similar to lecithin. Due to its insolubility in water, it was necessary to use alcoholic solutions. A concentration of 1.2 mg. was unable to prevent the inhibitory action of 0.05 mg. of Zephiran on *Staphylococcus aureus* or *E. coli*.

It is known that detergents of opposite ionic charge precipitate each other, and therefore the inhibition of bacterial metabolism by a cationic detergent can be prevented by the simultaneous addition of an anionic detergent, and *vice versa*. Thus, the action of a cationic detergent such as Zephiran can be prevented by the simultaneous addition of an equivalent quantity of an anionic detergent, decyl sulfate. On the other hand, a combination of anionic detergents, one of which is inhibitory and the other which is not, (such as Tergitol-7 and decyl sulfate), still inhibits bacterial metabolism. Similarly, the surface-active compound, sodium taurocholate, prevents the action of Zephiran but not of Tergitol-7; it behaves like a typical anionic synthetic detergent. The neutralization of oppositely charged detergents appears to be a different phenomenon from the protective action of the phospholipids and Demal.

Effect of Compounds Which Are Not Surface-Active

It was thought of interest to determine if compounds which are not surface-active but are known to be involved in growth or metabolism of bacteria or in some manner related to the phospholipids, could act like phospholipids in protecting bacteria against the action of detergents. In experiments on *Staphylococcus aureus* and lactobacillus (respiration and acid production studies, respectively) it was found that nicotinic acid and nicotinamide, thiamine, riboflavin, diphosphopyridine nucleotide,[4] and yeast extract were unable to prevent the inhibition by Zephiran. Negative results were also obtained with the following compounds: methylene blue, choline, glycerol, and the ethanolamines (mono-, di-, and tri-).

DISCUSSION

It appears to us that the most reasonable working hypothesis to explain the rapid action of synthetic detergents on bacterial metabolism and viability would be one based on a twofold action: first, a disorganization of the cell membrane by virtue of the great surface activity of these compounds, and second, a denaturation of certain proteins essential to metabolism and growth. The effects of detergent-like compounds on lysis and agglutination of red cells have been investigated by Schulman (34). He concluded from studies on model systems that compounds which penetrate lipoprotein monolayers

[4] We are indebted to Dr. A. Altschul for a sample of purified material.

increase surface pressure markedly and cause lysis, whereas compounds which do not penetrate but are adsorbed cause agglutination. It is conceivable that similar disorientations and alterations in surface forces may occur in bacterial cells. Denaturation of proteins and inactivation of viruses have been reported by a number of investigators (3, 4, 35–40). Very low concentrations of detergents have been shown to denature proteins.

If such an explanation were correct, then it is reasonable to expect that compounds which could significantly alter the affinity of detergents for bacteria would influence their effect on the cell membrane and their tendency to denature cell proteins. Phospholipids possess a characteristic polar-nonpolar structure and, presumably, have an affinity for bacterial cells similar to that of the detergents. They have been shown to produce a marked lowering of surface tension at very low concentrations (41). Since they do not inhibit bacterial metabolism even at quite high concentrations, the phospholipids could protect the bacterial cell, perhaps by altering the structure of the membrane in such a manner as to prevent penetration by the detergents. As some evidence for this, it should be noted that our experiments demonstrate that the phospholipids are ineffective unless they are added before or at the same time as the detergent. It will be of interest to establish whether or not the phospholipids can prevent denaturation of proteins by detergents.

We have found that an unionized detergent, Demal, functions very similarly to the phospholipids, protecting bacteria against both cationic and anionic detergents. On the other hand, the action of taurocholate is confined to cationic detergents, and cholesterol is relatively inactive.

We have not had the opportunity to study phospholipids derived from bacterial cells. Such an investigation would be of value in elucidating a possible relationship between cellular phospholipids and the resistance or susceptibility of various cells and bacterial species to the detergents.

There is a striking contrast between the action of phospholipids against the synthetic detergents and the bactericidal compounds of Dubos and Hoogerheide, as compared with the very low activity of phospholipids against mercuric salts and derivatives. Fildes (42) has demonstrated that mercuric ions act on bacterial cells by combining specifically with sulfhydryl groups. The inhibitory action can be reversed even after long periods of time by the addition of sulfhydryl compounds which form soluble compounds with mercury, such as glutathione, cysteine, and thiolacetate. As shown above, the action of the detergents on bacteria appears to be of a different type, and does not seem to involve combination with a specific group.

SUMMARY

1. Lecithin, cephalin, and sphingomyelin prevent the *inhibition of bacterial metabolism* which is caused by synthetic anionic and cationic detergents. The phospholipids must be added either before or simultaneously with the deter-

gent. Addition after the detergent is without effect. Bacteria still exhibit this phenomenon after they have been exposed to the phospholipid and thoroughly washed.

2. A similar action of the phospholipids has been demonstrated towards the bactericidal compounds isolated by Dubos and Hoogerheide from soil bacteria. There is very little effect with bactericidal mercury compounds.

3. The effect of lecithin against the *bactericidal action* of synthetic detergents was also determined. It was found that germicidal quantities of the detergents were not effective in the presence of the phospholipids.

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