TABLE I
Kidney and Liver Lipid Values

Lipid value	Kidney			Liver		
	March	April	May	March	April	May
Total Lipids [a]	17.0 ± 0.7 [b]	17.0 ± 0.9	17.3 ± 0.8	20.1 ± 0.9	20.2 ± 0.7	20.0 ± 0.4
Phospholipids [a]	10.3 ± 0.4	10.1 ± 0.2	10.3 ± 0.3	11.9 ± 0.3	12.0 ± 0.2	12.1 ± 0.1
Total cholesterol [a]	1.52 ± 0.03	1.53 ± 0.02	1.55 ± 0.03	1.02 ± 0.02	1.05 ± 0.02	1.05 ± 0.03
Iodine value of total lipids	71.5 ± 1.8	72.7 ± 2.0	72.9 ± 1.7	69.5 ± 0.8	70.1 ± 2.8	70.0 ± 1.5

[a] Expressed as percentage of dry tissue.
[b] Standard deviations.

species (7). The percentage of phospholipids in the kidney of antelope is very similar to that of beef, but this value for antelope liver is lower than that of beef (6).

The iodine values listed in Table I can only be used to indicate that there was no major change in the unsaturation of kidney and liver lipids during the latter part of the gestation period. Listing average iodine values for the lipids in tissues that are directly affected by dietary lipids is not possible.

Summary

The percentages of total lipids, phospholipids, and cholesterol in the kidney and liver of pregnant antelope are reported. The iodine values of the total lipids in these two organs are also listed. By the use of three collections of the antelope made about one month apart during the latter part of the gestation period, it was shown that there were no significant changes in the above lipid values during this period.

The lipid values were very similar to those values obtained for beef with the exception that the antelope livers contained less phospholipids. Iodine values were not compared since they are so easily influenced by dietary lipids.

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Studies in the Development of Antibacterial Surfactants. I. Institutional Use of Antibacterial Fabric Softeners

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OVER the past decade or two, institutions and hospitals in particular have been burdened with an ever-increasing problem of controlling bacterial cross-contamination. The development of powerful antibiotics has been countered in nature with the emergence of an increasing number of antibiotic-resistant strains, especially of *Staphylococcus aureus*. Carl W. Walter and co-workers at the Peter Bent Brigham Hospital in Boston (1, 2) have postulated a plausible mechanism for cross-contamination. The increasing morbidity and mortality rates attributable to staphylococcal infections have been well documented in the medical literature (3).

The present series of studies was undertaken in an effort to combat bacterial cross-contamination in hospitals by chemical means. It was recognized that a three-pronged method of attack was required, namely, treatment of hospital linens to render them bacteriostatic, treatment of the patients' skin, and, lastly, treatment of all hard surfaces, such as walls, floors, etc.

The present study deals with the first phase of this program, the treatment of linens. A rinse treatment of institutional linens appeared to be a good approach and, in view of previously reported studies (4), cationic fabric softeners were selected as the vehicle for the incorporation of antibacterial agents. As a rule, the cationic softener is the last step in the wet processing of linens. This practice minimizes the neutralization of the cationic agent by anionic (detergent) substances used in washing. Furthermore a cationic softener which is exhausted onto cellulosic fibers is a better carrier for an antibacterial agent than a carrier which is not preferentially adsorbed. Lastly the softening of the linens adds to the comfort of hospital patients and at the same time aids in the processing of the linens in the hospital laundry.

It was realized that antibacterial activity should extend over a broad spectrum of organisms. An organo-mercurial, phenylmercuric propionate was selected because of its effectiveness against gram-positive organisms, gram-negative organisms, and fungi. Various resistant strains of *S. aureus*, as well as the standard F.D.A. strain, were used as the test organisms representative of the gram-positive class of micro-organisms.

In order to combat the threat of staphylococcal infections in hospitals, the treatment of the linens must inhibit effectively the growth of this class of organisms. Among the gram-negative organisms the proteus types are perhaps the most troublesome, particularly in nurseries. The proteus organisms will hydrolyze urea to produce ammonia, which is irritating to the skin of infants and otherwise offensive because of its odor. An effective treatment of linens must inhibit this class of micro-organisms. Lastly inhibition of mildew is required in a good antibacterial treatment since soiled institutional linens are frequently stored in warm, damp places for periods of several days. If the temperature and humidity of the atmospheric air are high enough, extensive damage by mildew can be expected. In many southern hospitals this poses a serious problem.

The first phase of this study was one of range-finding in order to establish an application level where all three types of micro-organisms would be effectively inhibited. The second phase was a toxicity study, required to make certain that the level of effectiveness established in the first phase would be harmless to humans. The last, and perhaps most important, phase was a study of the effectiveness of the treatment under actual-use conditions in hospitals.

Experimental

Method of Fabric Treatment. A suitable softener concentrate was prepared by blending a dilute aqueous solution of the active cationic softener with an aqueous alcohol solution of phenylmercuric propionate. The actual formulation of such a concentrate is unimportant since the determining factor in this entire study is the amount of total cationic softener and the amount of mercurial available to fabric.

In such a formulation the active softener was chosen from among the dialkyldimethylammonium chlorides where the alkyl chains are derived from hydrogenated tallow or from the class of the fatty acid amide of 1-aminoethyl-3-alkylimidazolinium methosulfates, in which again both the alkyl chain and fatty acid group were derived from hydrogenated tallow. The two types of softeners showed no difference in performance in this study. The mercurial content of the formulation was varied in accordance with the desired level of mercurial on the fabric, as will be explained.

The bulk of the treatments were carried out in laundry machines of various designs, some with completely automatic feed and others with manual feed. In each case the formulated softener was applied at a rate of 1 g. of active softener per kilogram of soiled linens, which corresponds to a level of 0.1% softener on weight of fabric. It was pointed out in a previous publication (4) that complete exhaustion of softener upon fabric cannot be attained within a reasonable period of time; however, for the sake of uniformity of the experiments, all tests were run at the 0.1% available softener level on weight of fabric. To obtain numerical values for the mercurial level it was assumed that complete exhaustion upon the fabric occurred, and accordingly the levels of available mercurial are expressed in parts per million on weight of fabric. Preliminary tracer studies with tagged phenylmercuric propionate however have indicated that the exhaustion of the mercurial upon fabric is not quantitative for the period of time allotted to the rinse treatment in a laundry.

In all cases the formulated antibacterial fabric softener was applied in the final rinse of the laundering process at a temperature of about 70°F., and the treatment time was usually 5 min. The test pieces for bacteriological evaluation consisted either of Indian Head swatches or of diapers. In the early work test pieces were withdrawn wet from various locations in the washwheel and were dried in the laboratory. In the later stages of the study the test pieces passed through the regular laundry drying and ironing process.

Bacteriological Evaluation Methods. The effectiveness against growth of various strains of *Staph. aureus* was tested by the standard zone of inhibition technique (5). Both the standard F.D.A. strain No. 209 and three antibiotic-resistant hospital strains were used as test organisms. *Proteus mirabilis* was chosen as a typical representative of the gram-negative class of micro-organisms. Since proteus organisms hydrolyze urea to produce ammonia, inhibition could be determined very readily by the method of Latlief, Goldsmith, Friedl, and Stuart (6). Lastly the inhibition of mildew was determined by using *Chaetomium globosum* as the test organism (7). For this purpose 20 ml. of standard mineral salts agar were poured into culture flasks, steam-sterilized, and allowed to harden. One-by-four-inch swatches were placed on the agar surface, and 1 ml. of an aqueous suspension of *Chaetomium globosum* was seeded uniformly over the fabric surface. The flasks then were incubated at 30°C. for 12 days. Inhibition of growth was evidenced by the absence of black sporulent areas on the fabric surface.

Range Finding Tests. In the initial phase of this study it was discovered that the cationic softener exerts some potentiating effect to the activity of the mercurial; in addition, it appears to aid somewhat in obtaining a more uniform distribution of the mercurial. Table I illustrates this effect. Tests 1 and 2 were run by adding the mercurial to the final rinse in the absence of cationic softener. All other tests were run with a formulated product containing both mercurial and softener. The relative unevenness of application in Tests 1 and 2 is indicated by the variation of zones of inhibition on test pieces withdrawn from different areas of the washwheel. If the size of the zone of inhibition is to be used as a criterion of effectiveness, it would appear that the mercurial formulated in a cationic softener is at least as effective at 25 p.p.m. on weight of fabric as is the mercurial alone at 50 p.p.m.

The mercurial formulated in the cationic medium then was applied at levels varying from 30 to 63 p.p.m. in several hospital laundries. The results ob-

TABLE I
Potentiating Effect of Cationic Softener on Antibacterial Activity of Phenylmercuric Propionate

Test No.	Available mercurial, p.p.m.	Cationic present	Zones of inhibition for *S. aureus* F.D.A. No. 209 on test piece from various areas of the washwheel in mm. (5)				
			Outside left	Inside left	Outside right	Inside right	Average
1	50	No	5	4	1	3	3.3
2	50	No	5	4	1	5	3.8
19	20	Yes	4	4	4	2	3.5
20	20	Yes	4	4	1	2	2.8
13	25	Yes	5	6	3	4	4.5
14	25	Yes	5	9	3	3	5.0
7	38	Yes	5	5	6	6	5.5
8	38	Yes	4	6	6	7	5.7
4	50	Yes	5	7	7	6	6.2

tained at four different hospitals are summarized in Table II. The zones of inhibition are given for the F.D.A. No. 209 strain of *Staph. aureus* as well as for three resistant strains. The results obtained with *Proteus mirabilis* are indicated by a plus sign, which designates ammonia evolution and hence no inhibition, or a minus sign, designating absence of ammonia and hence complete inhibition. Mildew inhibition is recorded as 0 for no growth, 1 for slight growth, 2 for moderate growth, and 3 for heavy growth.

The data given in Table II indicate first of all that the so-called resistant organisms, MS-10, MS-62, and MS-14, are somewhat more resistant to the mercurial. Secondly there is considerable variation between various hospitals, which is partly attributable to differences in equipment as well as to variations in operating procedures at these hospital laundries. On the basis of these range-finding tests it was concluded that an effective cationic softener application at an available mercurial level of 63 p.p.m. on the weight of fabric would give adequate antibacterial protection against a variety of organisms. Hence all subsequent test runs were conducted at that level.

TABLE II
Variation of Antibacterial Activity with Level of Mercurial

Mercurial available to fabric, p.p.m.	Hospital code	Zones of Inhibition for *S. aureus* in mm.				Ammonia inhibition [a]	Mildew inhibition [b]
		FDA 209	MS-10	MS-62	MS-14		
0	CT	0	0	0	0	+	3
30	CT	5	1	1	1	—	3
36	CT	8	1	1	1	—	1
42	CT	9	8	7	7	—	0–1
0	HS	0	0	0	0	+	3
42	HS	5	1	2	2	+	3
52	HS	5	1	1	2	—	
63	HS	10	2	2	2	—	0
0	SF	0	0	0	0	+	
42	SF	2	1	0	1	—	
52	SF	5	2	1	2	—	
63	SF	10	2	2	2	—	0
0	MM	0	0	0	0	+	
42	MM	5	2	2	1	—	
52	MM	5	2	1	2	—	
63	MM	9	2	2	2	—	0

[a] *Proteus mirabilis.* [b] *Chaetomium globosum.*

Toxicology. Although details of a toxicological study on a germicidal fabric softener are beyond the scope of this publication, it is felt that a summary of the data is important since in all application research, particularly in the field of antibacterial agents, safety to humans is a governing factor, and the present study would be meaningless without such information.

Toxicity Data (70% Cationic Softener, 0.85% mercurial)

Acute oral LD_{50} (albino rats)	2.86 g./kg.
Acute oral $LD_{0.01}$	0.258 g./kg.
Acute oral $LD_{99.9}$	31.2 g./kg.

Skin irritation and sensitization: Four of 200 subjects exhibited mild irritation when patch-tested with the concentrate; no evidence of sensitization was obtained.

Skin irritation and sensitization in hospital-use test: No evidence of skin irritation or sensitization during a total of 40,000 patient-days for a period of six months involving 1,500 patients.

Long-Term Use Studies. The last phase of this study is concerned with the phenomena observed upon repeated application of the antibacterial softener treatment. In the first series of tests all linens in a specific hospital laundry received the antibacterial softener rinse treatment for a period of more than six months, during which some of the observations aforementioned on toxicology were made. The data are summarized in Table III. During the washing cycle samples of water were withdrawn during the first suds step and during the first, third, and final (antibacterial treatment) rinse steps. An aliquot portion of the water sample was placed in a culture medium, and a total bacterial count was made after a 24-hr. incubation period. It is noted that at the onset of the test when the antibacterial softener treatment was used for the first time the counts remained high throughout the washing cycle until the final rinse step, where a drop in count is to be expected from the addition of the antibacterial softener. After two weeks there were still some untreated linens; and in the first suds, which in essence contained the entire bacterial load of the soiled linens, the count still was elevated although much lower than the initial test. Initially counts were made weekly, then semi-monthly, and finally monthly. The greatly abbreviated record of these counts (Table III) indicates that, once all of the linens have received the softener treatments, the total bacterial count remains very low.

TABLE III
Use Test of Germicidal Softener

Time interval	Total counts, organisms per ml.			
	First suds	First rinse	Third rinse	Final rinse
Initial	830	425	450	22
After 2 weeks	24	6	2	0
After 3 weeks	4	6	17	2
After 4 weeks	3	1	3	1
After 5 weeks	0	0	4	3
After 2 months	5	0	5	1
After 3 months	1	0	6	4

In repeated applications of the antibacterial softener it was of importance to determine whether or not a build-up of the material would occur on the fabric. Since standard wet methods of mercury analysis are not sensitive enough at the low levels used in these tests, build-up and removal was followed by bacteriological means. The test pieces received five consecutive launderings with the antibacterial softener treatment in the last rinse. Thereupon the test pieces were laundered three more times without any rinse treatment. Small swatches of fabric were cut from the test pieces after each complete laundering, and the zone of inhibition was established by the U.S.D.A. procedure (5), using the F.D.A. No. 209 *Staph. aureus* as the test organism.

It can be concluded from the data that a very slight build-up of the mercurial occurs upon repeated application. On the other hand, after one laundering the mercurial level is substantially reduced, and after two or three consecutive launderings only a trace of

TABLE IV
Build-up and Removal Tests

Number of consecutive treatments	Average zone of inhibition with *S. aureus* F.D.A. No. 209
0	5
1	6.0
2	5.0
3	5.5
4	6.5
5	8.0
Number of washes without treatment	
1	3
2	1.5
3	1.0

the antibacterial agent remains on the cloth. The results are given in Table IV.

Summary

It has been determined that the incorporation of a highly active antibacterial agent, phenylmercuric propionate, into an aqueous solution of a fabric softener of the quaternary ammonium type results in a most effective antibacterial laundry rinse additive. The presence of the quaternary ammonium compound appears to enhance the antibacterial activity of the mercurial. When applied at a level of 63 parts of available mercurial to one million parts of fabric, the treated cloth is rendered bacteriostatic to several strains of *S. aureus* and to ammonia-producing organisms of the *Proteus* group, also mildewstatic to *Chaetomium globosum*. The formulated antibacterial softener as well as the treated linens appear to be entirely safe to humans.

In longer-term application tests in a hospital laundry the total bacterial load carried by the soiled treated linens is practically zero. There is no excessive build-up of antibacterial agent on fabric upon repeated applications, and it is readily washed out of the fabric by conventional laundering.

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Studies in the Development of Antibacterial Surfactants. II. Performance of Germicidal and Deodorant Soaps

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Although the so-called deodorant toilet soaps have been a popular consumer item for more than a decade, little technical information has been published on the antibacterial or deodorant properties of such soaps. It was recognized that these soaps owed their deodorant effectiveness to the retention of the antibacterial additive on the skin at a sufficiently high concentration to inhibit the growth of various odor-producing micro-organisms. The best known of these additives is probably hexachlorophene, and Blank and co-workers (1, 2, 3) published a series of articles in which the effectiveness of hexachlorophene-containing soap was discussed.

Recently it has been discovered by Casely and Noel (4) that certain pairs of chemically-unlike antibacterial agents will exhibit a marked degree of synergisms either *per se* or when incorporated into soap or other surface-active agents. The synergism is particularly striking between certain bisphenols and various halogenated anilides and carbanilides, and a detailed bacteriological study in this area will be published in the near future (5).

The present study covers primarily soaps containing synergistic mixtures of 3,4,4′-trichlorocarbanilide (abbreviated T.C.C.) and hexachlorophene, or mixtures of trichlorocarbanilide and 2,2′-thiobis-4,6-dichlorophenol (known as bithionol). In order to develop an effective antibacterial soap based upon such synergistic mixtures it was necessary to establish the optimum ratio of the two components in each system. Secondly it was of importance to study the effect of the surface-active substrate upon the effectiveness of the antibacterial agents. Among various types of surface-active agents only the nonionic detergents exhibit a peculiar potentiating effect upon certain germicidal agents, which is in conflict with an earlier publication on hexachlorophene (6) but confirms the findings of Gregg and Zopf (7) also on hexachlorophene.

The study of the synergistic effect was broadened to include various types of micro-organisms, among which various resistant strains of *S. aureus* were of special interest in view of the increasing spread of *Staphylococcus* infections in numerous United States and European hospitals.

The scope of the investigation, which up to this point was purely on an *in vitro* basis, was extended to two types of studies. The first was a degerming study by a prescribed handwashing technique, and the other a purely subjective evaluation of deodorant activity. Although beyond the scope of this publication, it should be mentioned that toxicological data were obviously needed and a summary of such data follows.

Experimental

Variation of Synergistic Effect with Changes in the Ratio of Components. All experiments were carried out in a toilet soap medium. For this purpose the individual germicidal compounds or mixtures of compounds were incorporated at a total germicidal level