Influence of Various Surfactants on the Antimicrobial Activity of Bromsalans and Other Ring-Halogenated Substances[1]

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Abstract

Test methods used in the assessment of activity are discussed, utilizing the gradient plate dilution technique of Curry (1) for screening work. The various classes of surfactants studied included anionics, nonionics, amphoterics, and cationics.

A study was made in vitro of the influence of these surface-active agents on five antimicrobial agents. Based upon these tests it appears that, in general, Temasepts I, II, and IV show greater activity and a broader spectrum than G-11 and TCC. This is particularly evident in the presence of conventional soap and the newer non-soap bars.

An interesting pattern of results was observed when nonionic BC720 was used in the system. All of the antimicrobial agents were equal in activity against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans,* but Temasept I showed the highest activity against *Pityrosporum ovale* and *Aspergillus niger,* with TCC occupying an intermediate position.

The cationic vehicles alone, in addition to any possible enhancement of the ring-halogenated aromatics, contribute significantly in the system to inhibit all of the test strains.

With the amphoteric vehicles, the antimicrobial agents for the most part were equally effective, with G-11 being slightly less effective. In striking contrast, the three Temasepts showed far greater activity against *P. ovale, C. albicans,* and *A. niger,* especially in Miranol CS.

The data presented may be utilized for the formulation of fabric softeners, and the tests employed aid in the selection of the proper surfactant for textile and skin applications. The work demonstrates that in any technical appraisal of the antimicrobial potency of a compound, the influence of the surfactant vehicle is of paramount importance.

[1] Presented at the AOCS Meeting, Chicago, October 1967.

Introduction

THE INFLUENCE of surfactants on the antimicrobial activity of various ring-halogenated antimicrobial agents has been a subject of considerable interest. Workers in Switzerland, Germany, the United Kingdom, Japan, Hungary, and the United States have contributed to the field (2–11). The work on which I am reporting, done in our laboratories, concerns a systematic study of currently used antimicrobial agents of ring-halogenated character, i.e., the brominated salicylanilides, G-11 and TCC. The surfactants (Table I) used in the study are commercially available and belong to the class of anionics (including a commercial soap bar and a neutral toilet bar containing as principal component acyl isethionate

TABLE I

Surfactants Tested

Trade name	Chemical nomenclature	Activity
		%
Anionics		
Standapol SHC101	Anionic condensate of di-basic half-ester	30
Stepan DS-60	Dodecylbenzene sodium sulfonate	50
Ivory soap	Sodium oleostearate	90
Neutral toilet bar	Principally acyl isethionate and stearic acid	
Amphoterics		
Monateric LA	2-C_{11}-1-(ethyl-beta-oxipropanoic acid) imidazoline	100
Miranol CS	Sulfonated imidazolinium compound (coconut acid derivative)	40
Miranol HS	Sulfonated imidazolinium compound (lauric acid derivative)	40
Nonionic with some cationic characteristics		
Monazoline C	1-Hydroxyethyl, 2 C_{9-17} imidazoline	100
Cationics		
Bromat	Cetyl trimethyl ammonium bromide	100
Mytab	Myristyl trimethyl ammonium bromide	100
Nonionic		
Emulphogene BC720	Tridecyl-oxypoly(ethyleneoxy) ethanol	100

TABLE II

Activity of Surfactants Alone in ppm

	S. aureus	*E. coli*	*Ps. aeruginosa*	*S. typhosa*	*P. ovale*	*C. albicans*	*A. niger*
Anionics							
Standapol SHC 101	1,467	>8,000	>8,000	>8,000	>8,000	>8,000	4,000
Stepan DS-60	111	>8,000	>8,000	>8,000	2,445	1,200	400
Ivory soap	1,511	>8,000	>8,000	5,689	1,867	4,000	1,867
Neutral toilet bar	2,000	>8,000	>8,000	>8,000	800	1,867	800
Amphoterics							
Monateric LA	453	1,511	>8,000	1,022	236	>8,000	285
Miranol CS	>8,000	>8,000	>8,000	>8,000	>8,000	>8,000	>8,000
Miranol HS	>8,000	>8,000	>8,000	>8,000	1,333	>8,000	1,911
Nonionic with some cationic characteristics							
Monazoline C	80	187	>8,000	107	13	164	20
True cationics							
Bromat	<5	667	1,778	516	<5	22	18
Mytab	<5	178	1,378	156	<5	26	13
Nonionic							
Emulphogene BC720	253	>8,000	>8,000	>8,000	>8,000	>8,000	>8,000

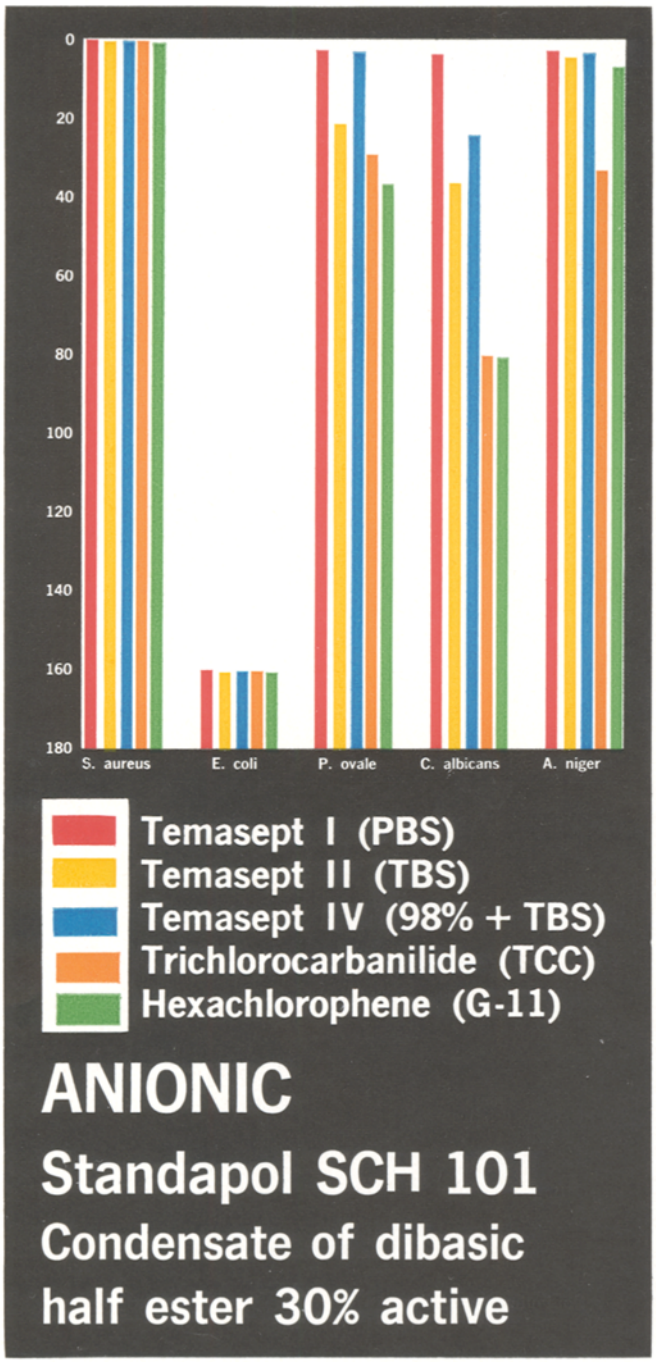

FIG. 1.

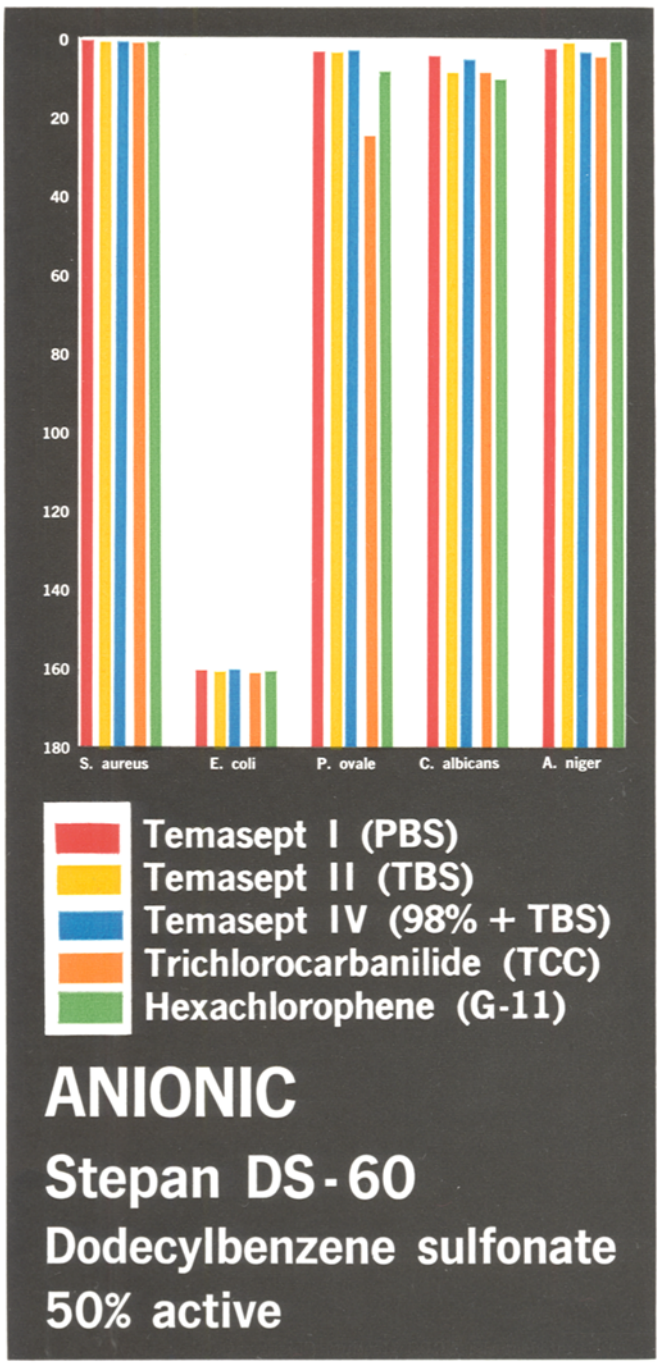

FIG. 2.

and stearic acid), amphoteries, nonionic, nonionics with some cationic characteristics and cationics.

I call to your attention the fact that I use the term antimicrobial agents to encompass not only bacteria but yeast-like organisms, such as *Pityrosporum ovale* and *Candida albicans*, and fungi, such as *Aspergillus niger*. Usually the term bacteriostat has been applied in the literature, particularly in relation to soap, but I believe that antimicrobial agents has the proper broad, descriptive connotation.

The antimicrobial products tested were:

Temasept I (Fine Organics, Inc., Lodi, N.J.), an approximately 50:50 mixture of Tribromsalan (3,4′,5-tribromosalicylanilide) and Dibromsalan (4′,5-dibromosalicylanilide)

Temasept II (Fine Organics, Inc., Lodi, N.J.), 90% minimum Tribromsalan, the balance being 3,5-dibromosalicylanilide (also known as Metabromsalan), with less than 0.1% 4′5-dibromosalicylanilide (Dibromsalan)

Temasept IV (Fine Organics, Inc. and Dow Chemical Co., Midland, Mich. under the name Tuasal 100), a Tribromsalan containing a minimum 98% Tribromsalan and less than 0.1% 4′,5-dibromosalicylanilide (Dibromsalan), other isomers representing the balance

Hexachlorophene (Givaudan Corporation, New York, N.Y.), which is *bis*(2-hydroxyl-3,5,6-trichlorophenyl)methane, also called G-11

Trichlorocarbanilide (Monsanto Co., St. Louis Mo.), 3,4,4′-trichlorocarbanilide, also called TCC

Experimental Procedures

The test method of Curry of Lever Brothers Research Center allows fast screening on a number of organisms (1). This procedure, a gradient plate

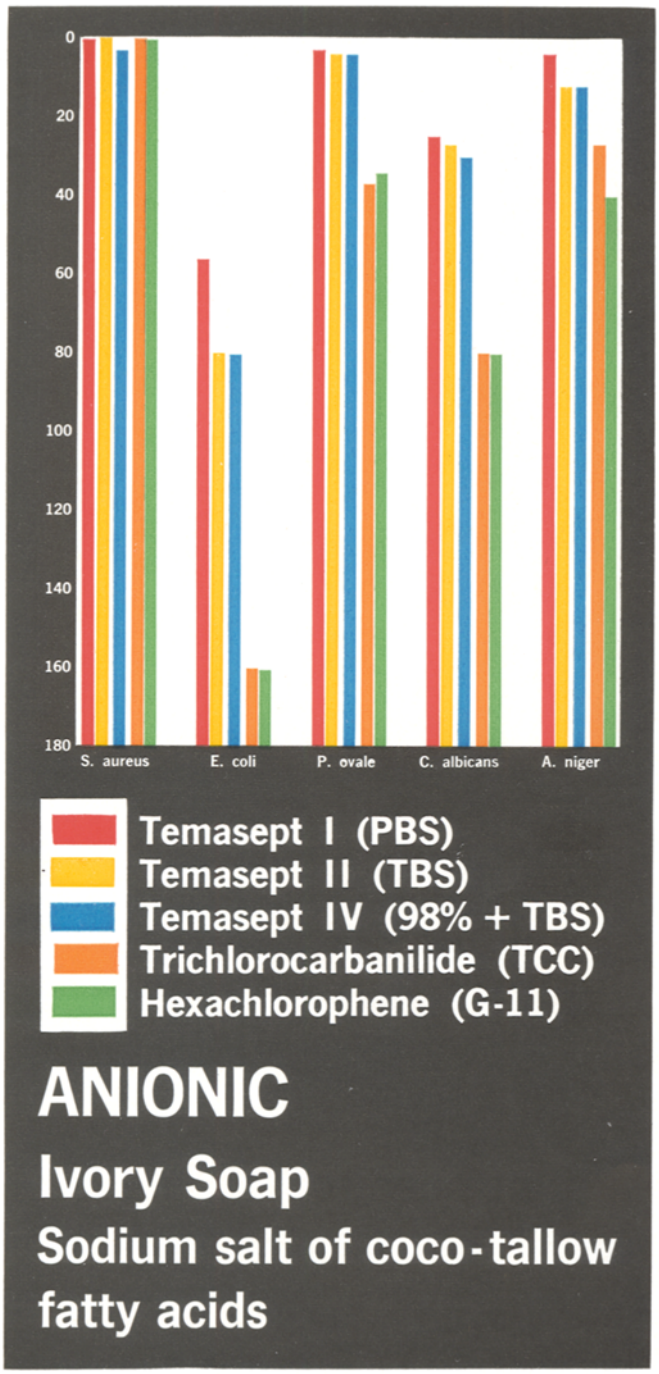

FIG. 3.

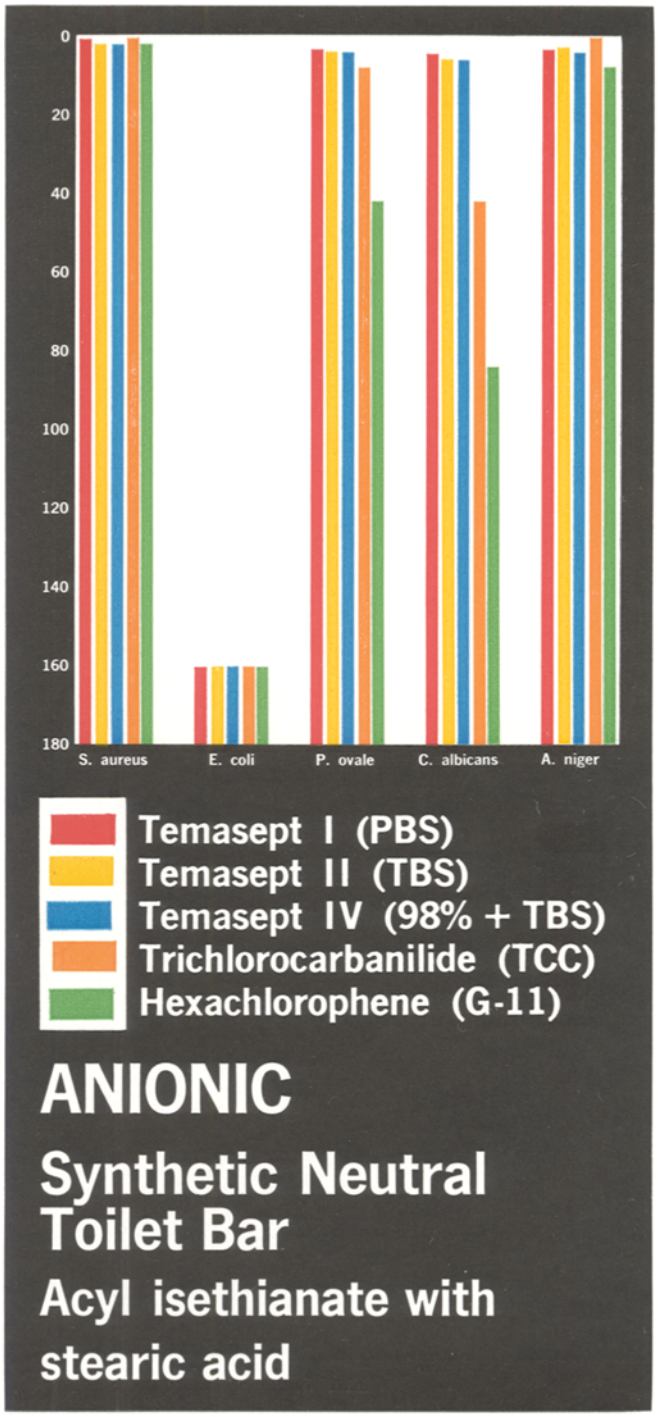

FIG. 4.

technique, is predicated upon pouring agar as a base layer into a square Petri dish at an incline of 1:16 and allowing it to set. Then with the dish on a horizontal surface, a second layer of agar is poured. This layer, however, contains a definite concentration level of the antimicrobial agent. This is allowed to set. Because the thickness of the agar containing the antimicrobial agent from the bottom of the plate to the top of the surface of the agar varies with the degree of incline, each plate allows reading at various dilution levels. After the plate has set, a streak inoculation of the surface is made by brushing on the various organisms in a parallel line to each other. The length of the area showing heavy growth is measured. Assuming linearity, the Minimum Effective Concentration (MEC) values are calculated by direct proportion according to a formulation:

$$\frac{\text{GP Concentration (ppm)}}{\text{Width of plate (90 mm)}} = \frac{\text{MEC (ppm)}}{\text{Growth front (mm)}}$$

The test organisms used in this work were *Staphylococcus aureus* ATCC (American Type Culture Collection) 6538, *Escherichia coli* ATCC 11229, *Salmonella typhosa* ATCC 6539, *Pseudomonas aeruginosa* ATCC 14502, *Pityrosporum ovale* ATCC 14521, *Candida albicans* ATCC 2091, and *Aspergillus niger* ATCC 10535. The results against *typhosa* and *coli* were found rather similar, so for illustration of Gram-negative organisms, only *E. coli* was included in the bar graphs. The results on *Ps. aeruginosa* also are not tabulated on the bar graphs but are given in the tables. The two were omitted to achieve

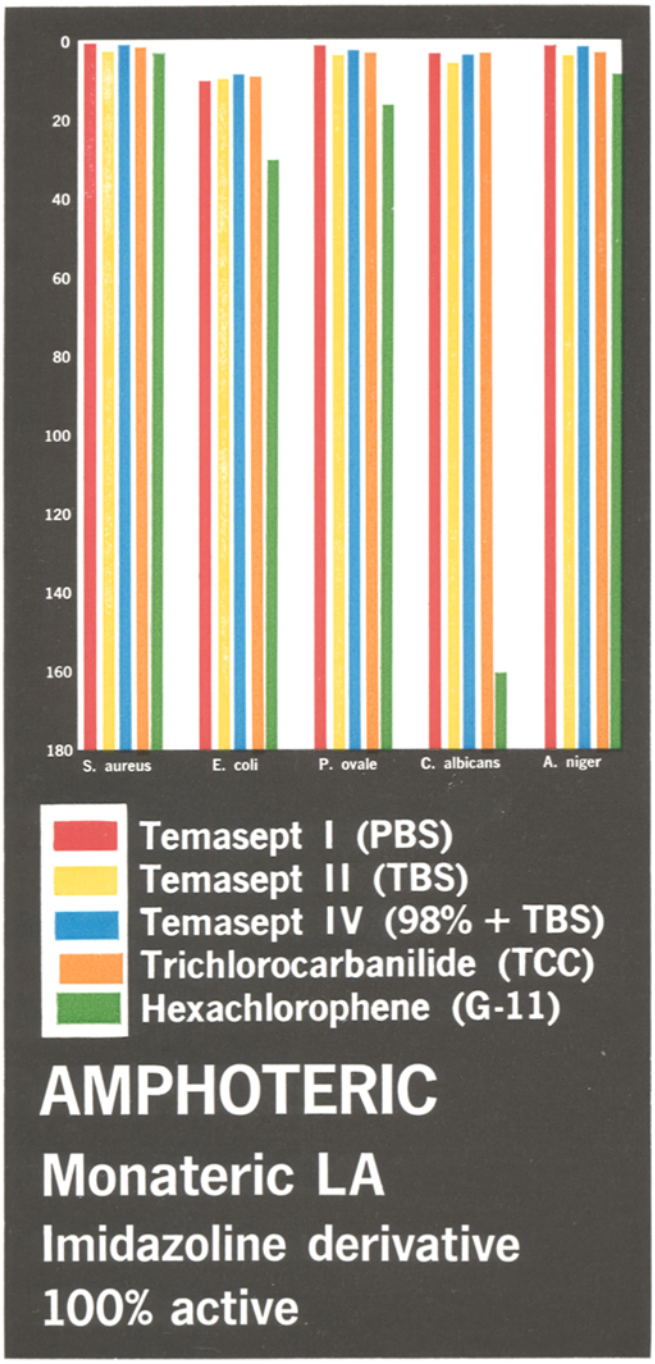

FIG. 5.

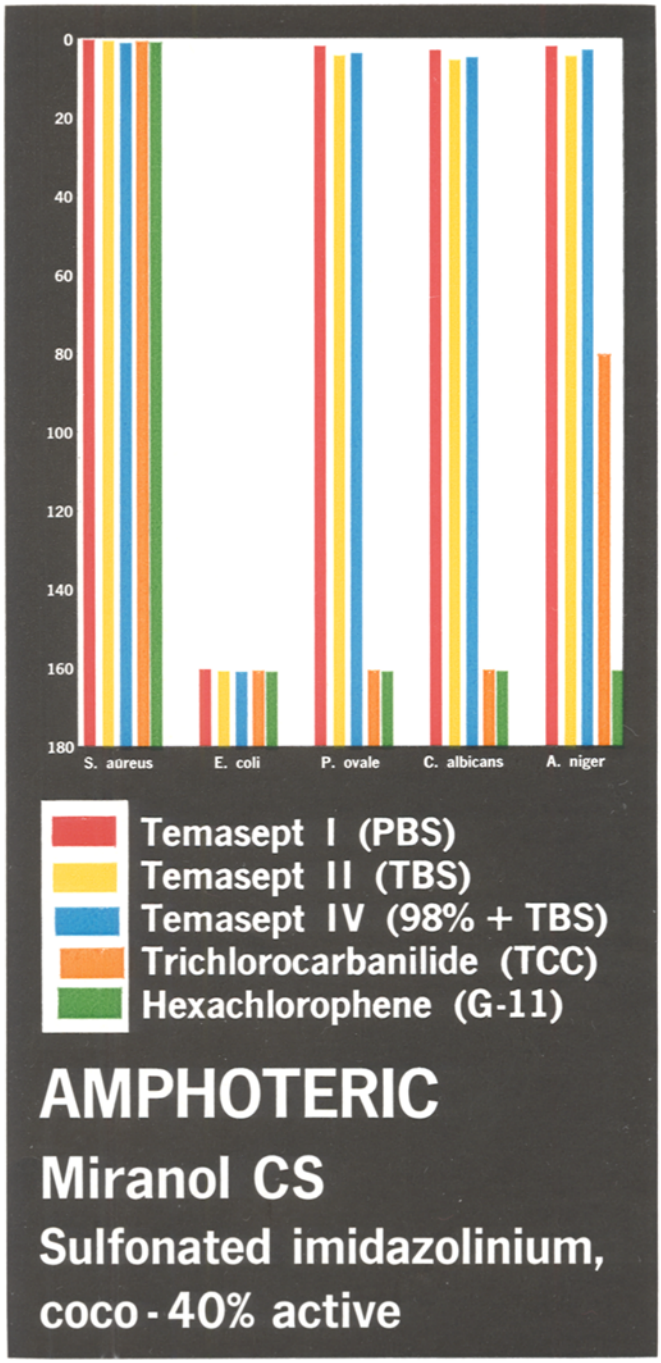

FIG. 6.

good visual comparison on the bar graphs.

An 8% stock solution was prepared of each of the surface-active agents. The 8% solution was based upon the commercially available material. To 100 g of each of these stock solutions, 160 mg of the antimicrobial agent was added in a concentrated solution of acetone. Thus the antimicrobial agent content was 2% on the surfactant basis as commercially sold.

The stock solution was then further diluted in the agar to obtain the various levels of activity. Hence concentration levels in actual reading range from less than 1 to 160 ppm, based upon the absolute concentration of the antimicrobial agent in the agar. Where an MEC of 160 ppm is reported, that may be 160 ppm or higher.

The plates were incubated for 48 hr at 37C. The end point was taken where growth resulting from streaking stopped on the plate.

The surfactants were tested in the same manner as described above without the addition of any antimicrobial agent (Table II). As the highest concentration of the surfactant (as supplied) in the test was 8,000 ppm, the results indicate 8,000 ppm or less MEC.

Results

In the bar graphs showing the results, the ordinates range from less than 1 to 180, representing MEC's from less than 1 to 160 ppm. *The bars are drawn in such a manner that the lower the MEC, the higher the bar.* Hence 1 ppm is represented by the highest bar and 160 ppm (the greatest dilution tested) is represented by the shortest. The shorter the bar

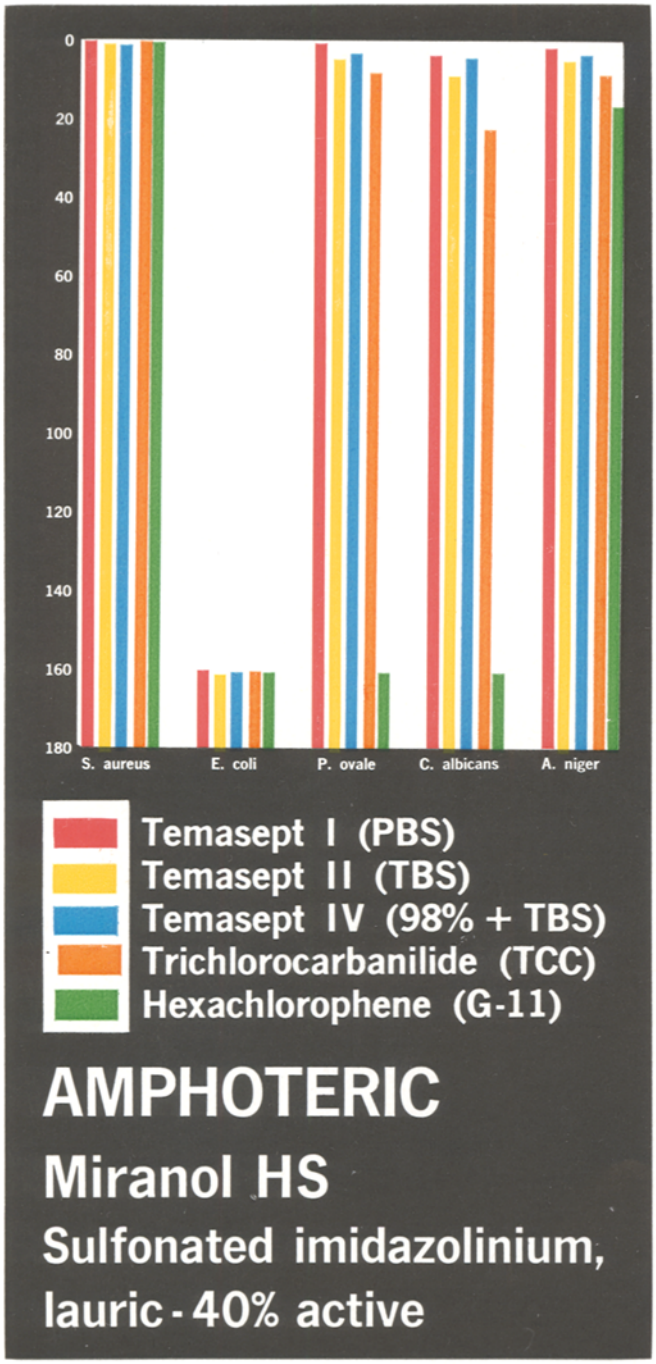

FIG. 7.

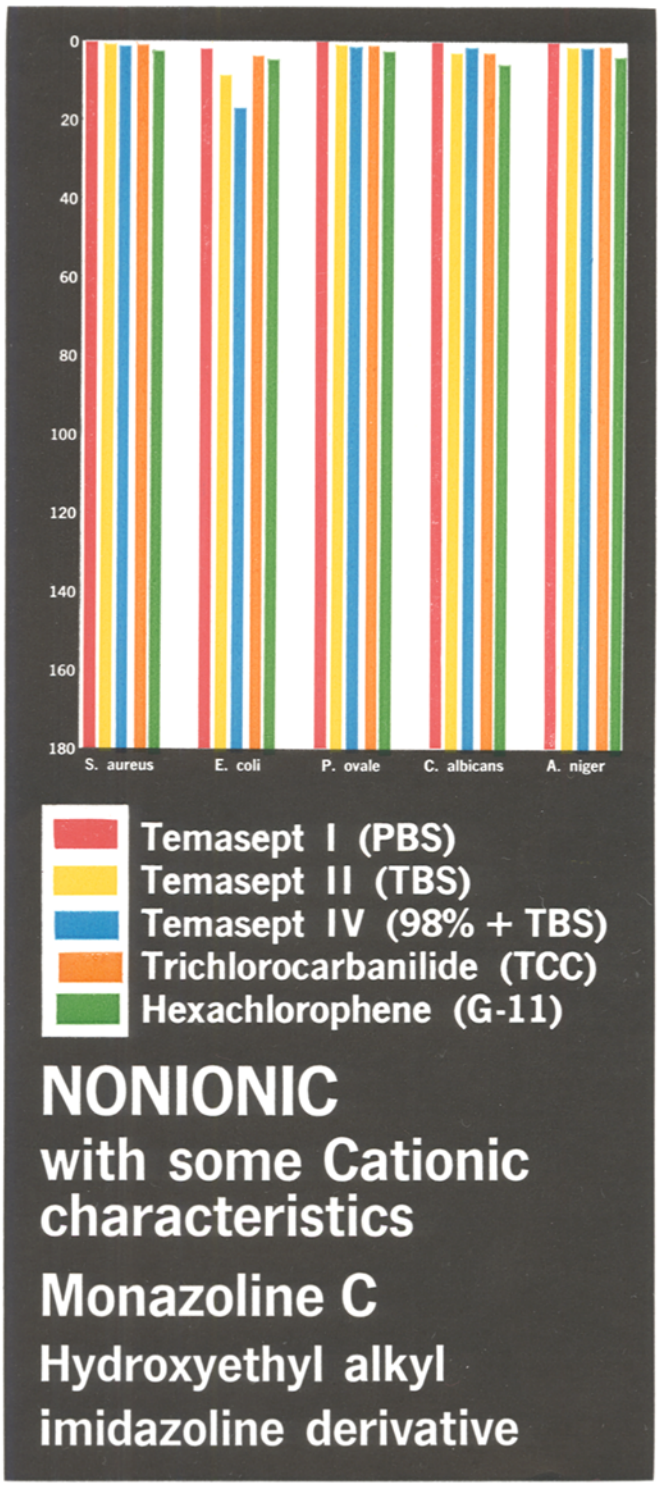

FIG. 8.

on the graph, the lower the activity.

The bars are colored, and there are five for each organism. Temasept I is red, Temasept II yellow, Temasept IV blue, TCC orange, and G-11 green.

It is again stressed that the higher the bar, the better the activity. The lower the bar, the poorer the activity.

The anionics are represented by four compounds, Standapol SHC101, Stepan DS-60, ivory soap, and a neutral bar, the latter two being the most significant of the group.

Standapol SHC101 (Standard Chemical Co., Hoboken, N.J.) (Fig. 1)

This material, an anionic condensate of dibasic half-ester, showed good performance, generally speaking, with all the antimicrobial agents tested. The organisms against which the individual activities are shown are *S. aureus,* representing Gram-positive organisms, *E. coli,* representing Gram-negative organisms, *P. ovale* and *C. albicans,* yeast-like organisms, and *A. niger,* fungi. All of them showed an activity of less than 1 ppm against *S. aureus.* The activity against *E. coli* was poor with all of them, but all of the Temasepts showed an activity superior against *P. ovale, C. albicans,* and *A. niger* to that of G-11 and TCC.

Stepan DS-60 (Stepan Chemical Co., Northfield, Ill.) (Fig. 2)

Stepan DS-60 is a dodecyl benzene sodium sulfonate 50% active. All the products tested with this

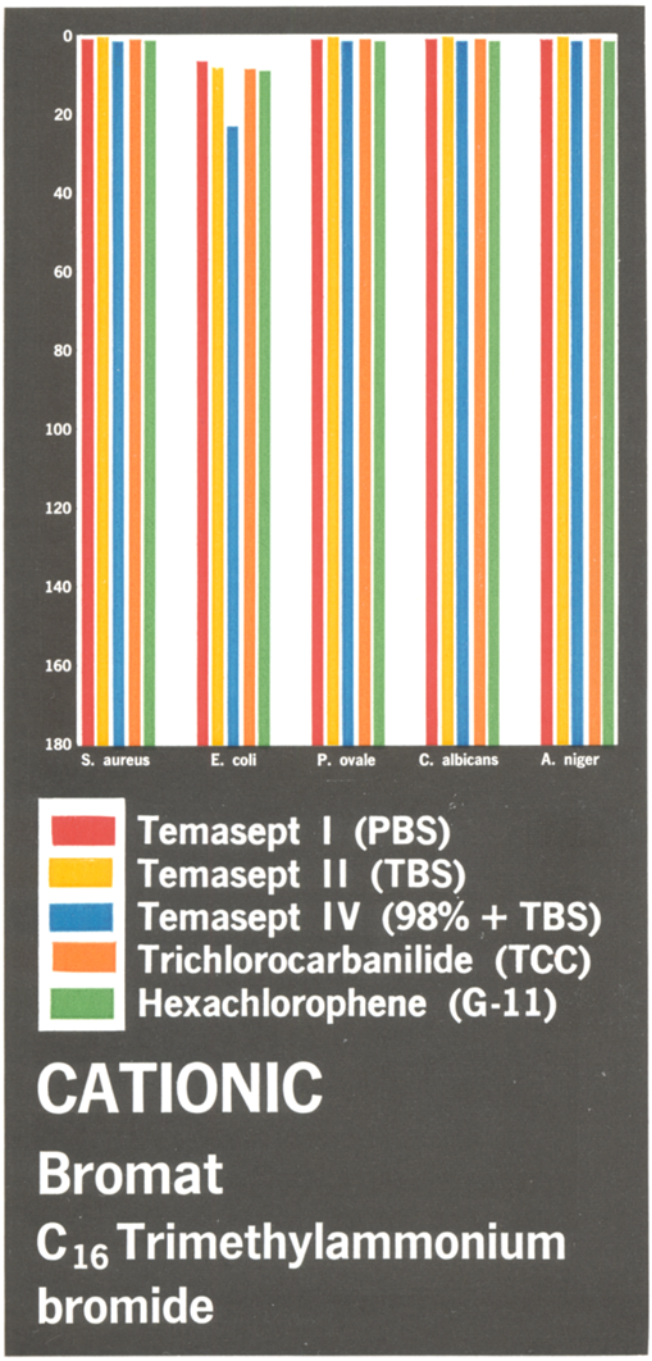

FIG. 9.

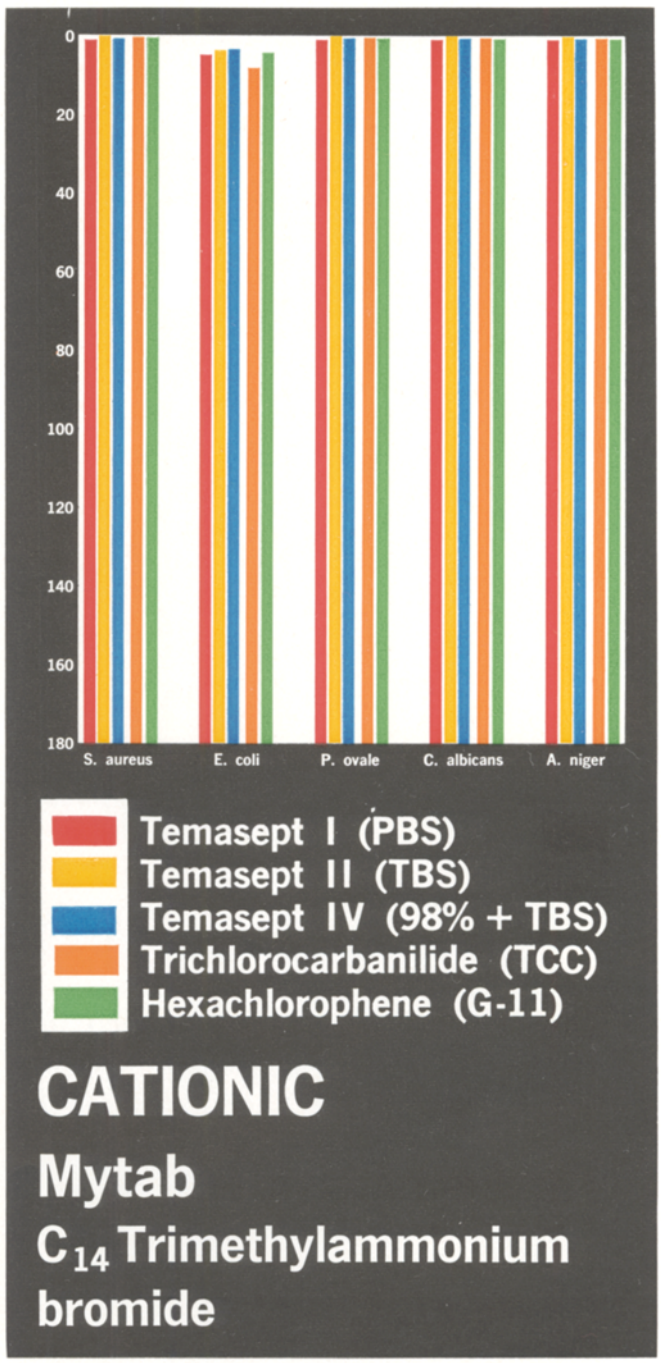

FIG. 10.

anionic were effective at 1 or less than 1 ppm against *S. aureus*. Versus *E. coli*, activity was poor in all instances, as was the activity against *Ps. aeruginosa*. Most of the antimicrobial agents were quite active in this medium against *P. ovale*, *C. albicans*, and *A. niger*, with Temasept II showing an activity of 0.3 ppm against *A. niger*, 3 ppm against *P. ovale*, and 8 ppm against *C. albicans*, whereas G-11 showed better activity against *A. niger* (0.6 ppm) and somewhat poorer activity against *C. albicans* and *P. ovale*, respectively 10 and 8 ppm.

Ivory Soap (Procter & Gamble, Cincinnati, O.) (Fig. 3)

As 80% of the toilet bars on the market are soap-based, the results obtained with Ivory soap are extremely significant in evaluating an antimicrobial agent. This sort of work has already been done by Hurst and co-workers (12) and Bellinger (8). Hurst spoke of the Soap Inactivation Coefficient (SIC). SIC is defined as the concentration of the disinfectant in soap solution divided by its concentration in an equivalently potent soap-free solution. The higher the number, the greater is the loss of efficiency in soap. When the number is below 1, the antimicrobial activity is improved in soap. These workers have found that G-11 gave an SIC of 208, whereas TCC and Tribromsalan were both less than 1. Although we have not in this paper repeated the work of Hurst et al., our work to an appreciable extent confirms the findings of Hurst and coworkers.

All of the antimicrobial agents tested gave good activity against *S. aureus*, as Fig. 3 shows. Temasept I, Temasept II, Temasept IV, TCC, and G-11 all show activity at a level of less than 3 ppm. How-

ever, the activity of Temasepts in Ivory soap against *E. coli* is without question above that of G-11 and TCC. Government Specification P-S-00619 requires effectiveness against both Gram-positive *aureus* and Gram-negative organisms like *E. coli*. Soap with 2% Temasept II passes Specification P-S-00619 against both *S. aureus* and *E. coli*, whereas soap containing G-11 at that level does not pass this test.

Neutral Bar (Fig. 4)

As the other 20% of the toilet bar sales are of neutral synthetic bars (13), the activity of the ring-halogenated aromatics becomes quite significant in this medium as well. The principal ingredient of the particular toilet bar we tested is acyl isethionate and stearic acid.

Some interesting observations can be made, especially in comparing Fig. 3 and 4. All of the antimicrobial agents tested showed good activity against *S. aureus*, although somewhat poorer than the corresponding activity in soap. None of the antimicrobial agents—and again I speak of 160 ppm or less—showed activity with the neutral bar against the Gram-negative *E. coli*, *Ps. aeruginosa*, and *S. typhosa*, whereas conventional soap such as Ivory showed activity against these organisms with the Temasepts.

When the activity of the ring-halogenated aromatics in a neutral toilet bar is compared with that of conventional soap against *P. ovale*, *C. albicans*, and *A. niger*, it can be noted that the Temasepts overall again show better activity. It is noteworthy, however, that in the neutral toilet bar, the activity of G-11 and TCC against these three organisms has also improved considerably in comparison with the activity in soap.

The activity against *C. albicans*, yeast, and fungi in soap and in a neutral bar becomes exceedingly significant in light of the paper of Dr. Palmer (14), who pointed out that yeast and *Candida* infections are very common in underdeveloped countries like Viet Nam, and even in the United States.

Monateric LA (Mona Industries, Paterson, N.J.) (Fig. 5)

Among the amphoterics we have three examples. Monateric LA, which is 2-C_{11}-1-(ethyl-beta-oxipropanoic acid)-imidazoline 100% active, has shown results superior to Ivory soap with all the antimicrobial agents. It should be noted, however, that G-11 gave rather poor results against *C. albicans* in the presence of Monateric L.A.

Miranol CS (Miranol Chemical Co., Irvington, N.J.) (Fig. 6)

This is a sulfonated imidazolinium compound coconut acid derivative 40% active. It is good in the case of all the antimicrobial agents against *S. aureus* and poor against the Gram-negatives tested. Against *P. ovale*, *C. albicans*, and *A. niger*, the activity is quite acceptable with the Temasepts but quite poor with G-11 and TCC.

Miranol HS (Miranol Chemical Co., Irvington, (N.J.) (Fig. 7)

This amphoteric is the same as the Miranol CS, with the exception that the fatty side chain is principally C_{12}. It appreciably improved the activity of G-11 to an MEC of 16 from an MEC of more than 160 as found with Miranol CS against *A. niger*. It proved to be a good choice for the Temasepts, as well as for TCC, showing considerable improvement in comparison to its activity in Miranol CS.

Monazoline C (Mona Industries, Paterson, N.J.) (Fig. 8)

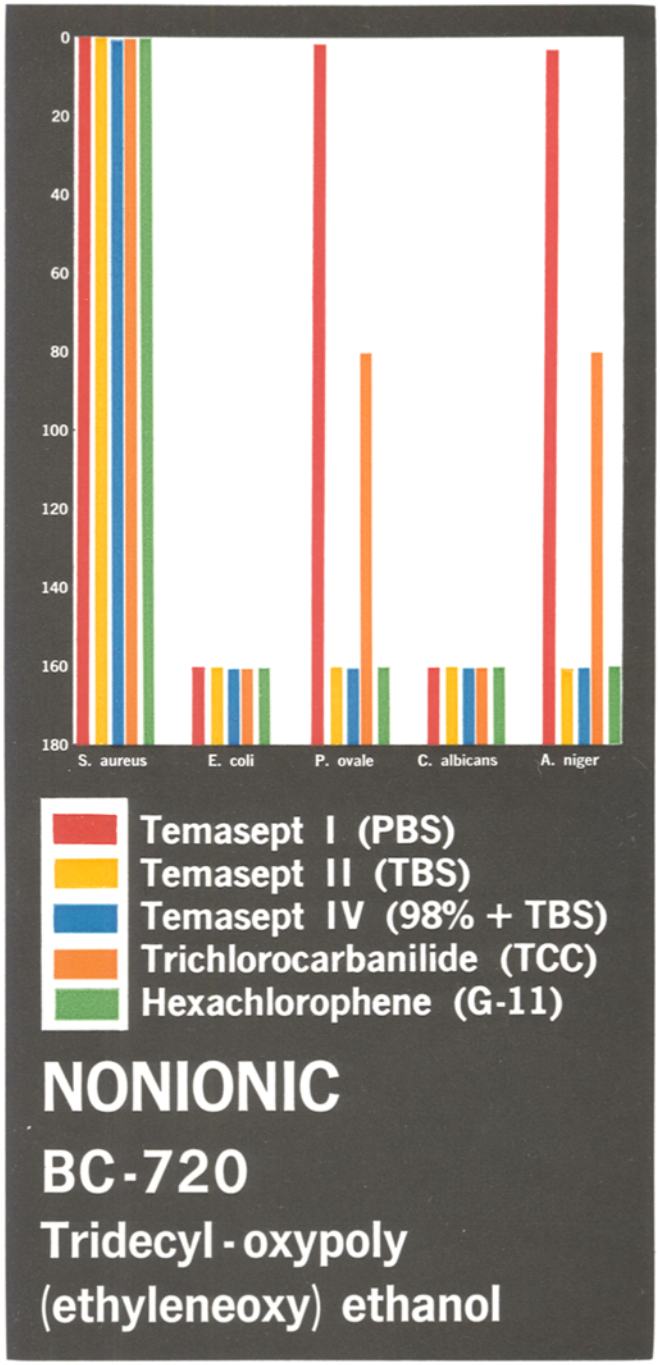

FIG. 11.

This is a nonionic with some cationic characteristics. When it is a free base, it is nonionic. When it is on the acid side, it is cationic. Although mixtures with the various antimicrobial agents exhibited a pH of between 9.9 and 10.0, the culture medium, being close to neutral, renders Monazoline C closer to cationic (pH 8.5). Hence the activity of this material as observed in the test is really due more to its cationic nature.

This material is a good selection for all of the antimicrobial agents tested. It showed activity against Gram-negative organisms far superior to any of the other surfactants in the study. Excellent activity was also noted in the case of *P. ovale*, *C. albicans*, and *A. niger*.

The true cationics were the next under consideration.

TABLE III

Activity of Antimicrobial Agent in Surfactants
(in ppm)

2% Temasept I (PBS)

Surfactant	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhosa*	*P. ovale*	*C. albicans*	*A. niger*
Anionic							
Standapol SHC101	0.3	>160	>160	>160	3	4	3
Stepan DS-60	0.4	>160	>160	>160	3	4	2
Ivory soap	0.3	56	84	47	3	25	4
Neutral toilet bar	0.7	160	>160	>160	3	4	3
Amphoteric							
Monateric LA	0.3	10	>160	11	1	3	1
Miranol CS	0.3	>160	>160	>160	2	3	2
Miranol HS	0.3	>160	>160	>160	1	4	2
Nonionic with some cationic characteristics							
Monazoline C	<0.1	2	>160	1	<0.1	0.3	0.2
Cationics							
Bromat	<0.1	6	38	8	<0.1	<0.1	<0.1
Mytab	<0.1	4	29	4	<0.1	<0.1	<0.1
Nonionic							
Emulphogene BC720	0.2	>160	>160	>160	2	>160	3

TABLE IV

Activity of Antimicrobial Agent in Surfactants
(in ppm)

2% Temasept II (TBS)

Surfactant	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhosa*	*P. ovale*	*C. albicans*	*A. niger*
Anionic							
Standapol SHC101	0.4	>160	>160	>160	21	36	4
Stepan DS-60	0.4	>160	>160	>160	3	8	0.3
Ivory soap	0.3	80	>160	80	4	27	12
Neutral toilet bar	2	>160	>160	>160	4	6	3
Amphoteric							
Monateric LA	2.2	9	>160	14	3	5	3
Miranol CS	0.4	>160	>160	>160	4	5	4
Miranol HS	0.4	>160	>160	>160	4	8	4
Nonionic with some cationic characteristics							
Monazoline C	0.3	8	21	2	0.3	3	0.5
Cationics							
Bromat	<0.1	8	43	8	<0.1	1	0.3
Mytab	<0.1	4	28	4	<0.1	0.4	0.3
Nonionic							
Emulphogene BC720	0.4	>160	>160	>160	>160	>160	>160

TABLE V

Activity of Antimicrobial Agent in Surfactants
(in ppm)

2% Temasept IV

Surfactant	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhosa*	*P. ovale*	*C. albicans*	*A. niger*
Anionic							
Standapol SHC101	0.4	>160	>160	>160	3	24	3
Stepan DS-60	1.0	>160	>160	>160	3	5	3
Ivory soap	3.0	80	87	37	4	30	12
Neutral toilet bar	2	>160	>160	>160	4	6	4
Amphoteric							
Monateric LA	0.5	8	>160	8	2	3	1
Miranol CS	0.5	>160	>160	>160	3	4	2
Miranol HS	1	>160	>160	>160	3	4	3
Nonionic with some cationic characteristics							
Monazoline C	0.2	16	>160	2	0.2	0.3	0.3
Cationic							
Bromat	<0.1	22	51	4	<0.1	0.3	<0.1
Mytab	<0.1	3	28	3	<0.1	0.1	<0.1
Nonionic							
Emulphogene BC720	0.6	>160	>160	>160	>160	>160	>160

TABLE VI

Activity of Antimicrobial Agent in Surfactants (in ppm)

2% Trichlorocarbanilide

Surfactant	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhosa*	*P. ovale*	*C. albicans*	*A. niger*
Anionic							
Standapol SHC101	0.2	>160	>160	>160	29	80	33
Stepan DS-60	0.2	>160	>160	>160	24	8	4
Ivory soap	0.2	>160	>160	>160	37	80	27
Neutral toilet bar	0.3	>160	>160	>160	8	42	0.1
Amphoteric							
Monateric LA	0.1	8	>160	8	2	2	2
Miranol CS	0.2	>160	>160	>160	>160	>160	80
Miranol HS	0.2	>160	>160	>160	8	22	8
Nonionic with some cationic characteristics							
Monazoline C	0.5	3	>160	0.5	0.6	2	0.4
Cationic							
Bromat	<0.1	8	40	7	<0.1	0.2	<0.1
Mytab	<0.1	8	36	3	<0.1	0.3	0.1
Nonionic							
Emulphogene BC720	0.1	>160	>160	>160	80	>160	80

Bromat (Fine Organics, Inc., Lodi, N.J.) (Fig. 9)

This quaternary is chemically Cetyl (C_{16}) trimethyl ammonium bromide. When tested at an 8% concentration level containing 2% halogenated ring compound on the basis of 100% of the surfactant, there was activity at less than 0.1 against *S. aureus.* Against *E. coli,* Temasept I, Temasept II, TCC, and G-11 were approximately equal in activity, Temasept I being slightly better and Temasept IV showing only 22 ppm activity. (On Fig. 10, it is shown that when tested in the Mytab system, this activity has improved to 3 ppm.)

Against *C. albicans,* excellent activity was shown, being less than 0.1 ppm for Temasept I and the others running approximately equal in activity, either 1 or less than 1. Against *P. ovale* all the antimicrobial agents showed activity in combination with Bromat at less than 0.1 ppm. *A. niger* also showed the same excellent activity of less than 1, most being less than 0.1 ppm.

Mytab (Fine Organics, Inc., Lodi, N.J.) (Fig. 10)

Mytab is a homolog of Bromat but is somewhat more water-soluble by virtue of being myristyl (i.e., the myristyl C_{14} trimethyl ammonium bromide).

With Bromat, Temasept IV showed only 22 ppm activity against *E. coli.* A mere change of two carbons in the length of the chain has improved the activity of Temasept IV against *E. coli* from 22 to 3. The improvement can also be noted in the case of *Ps. aeruginosa,* which went from 51 to 28 ppm in Temasept IV. Similar improvement can be noted with G-11 and TCC.

With *C. albicans,* all the antimicrobial agents were well below 1 ppm in activity. Against *P. ovale* all showed less than 0.1 ppm. Against *A. niger* they again were all below 1 ppm.

The nonionic was the final surfactant tested.

Emulphogene BC720 (General Aniline & Film Corp., New York, N.Y.) (Fig. 11)

BC720, also known as Emulphogene, is chemically tridecyloxypoly(ethyleneoxy)ethanol 100% active. All of the compounds under test with BC720 showed activity at less than 1 ppm against *S. aureus.* At that level, if any distinction is possible, G-11 appeared to be the best at less than 0.1 ppm, followed by TCC, which had an activity of 0.1 ppm. However, the Temasepts were also considerably below 1 ppm in activity. Against *E. coli* there was no activity demonstrated with any of the antimicrobial agents at the highest concentration level of 160 ppm. Hence it can be said that they were all inactive at 160 ppm.

In testing *P. ovale,* Temasept I was the best, with 2 ppm. The next was TCC with 80 ppm. Temasept II, Temasept IV, and G-11 all showed no activity at 160 ppm.

Against *A. niger* activity was shown only with Temasept I and TCC, respectively 3 and 80 ppm. For the others an MEC of apparently above 160 ppm was found.

The actual activity in parts per million of the various antimicrobial agents tested in the surfactants discussed is shown in Tables III, IV, V, VI, and VIII.

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