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

W. M. LINFIELD, R. E. CASELY, and D. R. NOEL, Soap Research Laboratories, Grocery Products Division, Armour and Company, Chicago, Illinois

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 of 1.0% into a conventional toilet soap base, obtained from the saponification of a blend of 20% coconut oil and 80% inedible tallow. This was accomplished by passing a blend of the dry germicidal ingredient and soap chips (moisture content 11%) repeatedly over a three-roll laboratory soap mill until the blend appeared physically homogeneous. The blended soap chips were then dissolved in water to give a 1.0% soap solution, which corresponds to an 0.01% concentration of germicidal agent. Since some of the latter are not very water-soluble, these soap solutions were shaken before each test to insure a uniform suspension in water.

The bacteriological evaluations were carried out with a modified agar streak method (8). Aliquots of the 1.0% test soap solutions were added to measured amounts of liquid glucose tryptone extract agar, and the resulting mixture was poured into Petri dishes. As soon as the agar had hardened, its surface was streaked with a standard loop containing a 24-hr. culture of *S. aureus* F.D.A. strain No. 209. After an incubation period of 48 hrs. at 37°C. the plates were examined visually, and the amount of growth was recorded.

TABLE I

Antibacterial Effectiveness of Varied Ratios of Hexachlorophene and 3,4,4'-Trichlorocarbanilide (T.C.C.) in Soap (Modified Agar Streak Method)

Ratio	Bacterial growth ratings Total antibacterial agent	
	at 0.04 p.p.m.	at 0.02 p.p.m.
100% Hexachlorophene, 0% T.C.C.	2	3
90% Hexachlorophene, 10% T.C.C.	0	2
70% Hexachlorophene, 30% T.C.C.	0	2
50% Hexachlorophene, 50% T.C.C.	0	1
40% Hexachlorophene, 60% T.C.C.	1	2
30% Hexachlorophene, 70% T.C.C.	1	3
20% Hexachlorophene, 80% T.C.C.	1	3
10% Hexachlorophene, 90% T.C.C.	2	3
0% Hexachlorophene, 100% T.C.C.	3	3

Code:
0–no growth; 1–slight growth; 2–moderate growth; 3–heavy growth.

In this manner a ratio study of the synergistic pair T.C.C. and hexachlorophene was carried out, the results of which are summarized in Table I. The analogous study of the pair T.C.C. and bithionol was carried out by using the same method, and the results of this study are summarized in Table II. The growth was rated and coded in the following manner: 0 = no growth; 1 = slight growth, where only a few sporadic colonies can be seen on the plates; 2 = moderate growth, where growth occurs along the streaks but one-half or more of the surface of the plate still shows little or no growth; and 3 = heavy growth, where there is luxuriant growth over practically the entire surface of the plate.

TABLE II

Antibacterial Effectiveness of Varied Ratios of Bithionol and 3,4,4'-Trichlorocarbanilide (T.C.C.) in Soap (Modified Agar Streak Method)

Ratio	Bacterial growth ratings Total antibacterial agent	
	at 0.1 p.p.m.	at 0.04 p.p.m.
100% T.C.C., 0% Bithionol	1	3
90% T.C.C., 10% Bithionol	0	3
70% T.C.C., 30% Bithionol	0	3
60% T.C.C., 40% Bithionol	0	2
50% T.C.C., 50% Bithionol	0	1
40% T.C.C., 60% Bithionol	0	1
30% T.C.C., 70% Bithionol	0	3
20% T.C.C., 80% Bithionol	1	3
10% T.C.C., 90% Bithionol	3	3
0% T.C.C., 100% Bithionol	3	3

Code:
0–no growth; 1–slight growth; 2–moderate growth; 3–heavy growth.

TABLE III

Potentiating Effect of Various Nonionic Detergents on the Antibacterial Effectiveness of Trichlorocarbanilide in Soap (Ratio of T.C.C. to nonionic 1:10; test organism *S. aureus*, F.D.A. No. 209)

Type of nonionic	Amount of bacterial growth (p.p.m. basis agar)		
	at 0.2 p.p.m.	at 0.1 p.p.m.	at 0.05 p.p.m.
Tetronic 908 [a]	0	0	3+
Tridecyl alcohol + 20 EtO	0	0	3+
Tridecyl alcohol + 15 EtO	0	0	3+
Stearic acid + 15 EtO	1+	3+	3+
Coco fatty acid + 15 EtO	0	2+	3+
Tween 80 [b]	0	1+	3+
Nonylphenol + 9 EtO	0	1+	3+
Nonylphenol + 15 EtO	0	1+	3+
None	1+	3+	3+

[a] Registered trademark of the Wyandotte Chemicals Corporation.
[b] Registered trademark of the Atlas Powder Company.
Code: 0 = no growth; 1+ = slight growth; 2+ = moderate growth; 3+ = heavy growth; EtO = moles of ethylene oxide.

Examination of Table I indicates that a 50/50 ratio of the two components T.C.C./hexachlorophene in a soap medium represents an optimum with respect to bacterial inhibition, and the same phenomenon can be observed with the T.C.C./bithional soap system (Table II).

Effect of Nonionic Surface-Active Agents upon the Activity of Antibacterial Agents. In view of the insolubility in water of the T.C.C. various nonionic surfactants were explored as solvent for the latter. Subsequently it was found that nearly all of these solvents exert a potentiating effect upon the antibacterial activity of T.C.C. Potentiation of either hexachlorophene or bithionol by nonionics is negligible.

The technique used was essentially the same as outlined above for the ratio study. The only deviation was that trichlorocarbanilide was first dissolved in the various nonionics to give a 10% solution. These 10% solutions were then added to soap by milling in, as described above. In all tests the T.C.C. concentration in the soap was 1.0%. The soap chips thus prepared were dissolved in water to give 1.0% soap solutions, and aliquot portions were withdrawn for bacteriological evaluation.

The data in Table III clearly illustrate the potentiating effect of some nonionics. Apparently the monoesters of polyglycols are rather ineffective, particularly in the case of the monostearate. On the other hand, the polyglycol ethers of tridecyl alcohol and nonylphenol are quite effective. Tetronic 908 [1] likewise is an effective potentiating agent. The behavior of Tween 80 [2] should also be noted. This product is widely used as a neutralizing agent for various antibacterial agents, particularly the phenolic types. In view of the potentiating effect observed with Tween 80 and other nonionics in contrast to the neutralizing effect reported in the literature (6), this study was extended to cover a concentration range of the nonionics. The data in Table IV show that the nonionics tested act as neutralizing agents at high levels but act as potentiating agents at low levels.

Antibacterial Spectrum of the Synergist Hexachlorophene-Trichlorocarbanilide. Hitherto this study has covered the evaluation of these antibacterial soaps

[1] Trademark of Wyandotte Chemicals Corporation.
[2] Trademark of the Atlas Powder Company.

TABLE IV
Inhibition or Potentiation of Antibacterial Activity of T.C.C. as a Function of Nonionic/T.C.C. Ratio (in Soap)

Type of nonionic	Ratio Nonionic/ T.C.C.	Amount of growth of *S. aureus* F.D.A. No. 209, at stated concentrations of T.C.C.	
		0.2 p.p.m.	0.1 p.p.m.
Tween 80 [a]	5:1	0	1+
	10:1	0	2+
	20:1	0	3+
	50:1	0	3+
	100:1	3+	3+
Tetronic 908 [b]	5:1	0	1+
	10:1	0	2+
	20:1	1+	3+
	50:1	3+	3+
	100:1	3+	3+

[a] Registered trademark of the Atlas Powder Company.
[b] Registered trademark of the Wyandotte Chemicals Corporation.
Code: 0 = no growth; 1+ = slight growth; 2+ = moderate growth; 3+ = heavy growth.

against but one type of micro-organism, *S. aureus* F.D.A. No. 209. Obviously an effective antibacterial soap must be active against a variety of organisms. Activity against a wide spectrum of micro-organisms has been determined by using soap containing either hexachlorophene or T.C.C., or the synergistic mixture of the two at a 50:50 ratio, which represents the optimum activity as previously shown. The method of evaluation was the same except for the type of test organism employed. A detailed bacteriological study is being published elsewhere. However a brief summary of our findings is given in Table V. The test results show again the rather striking phenomenon of synergism. Incidentally it is clearly indicated that the antibiotic-resistant strains of *S. aureus* are not resistant to the three chemical antibacterial agents tested.

TABLE V
Concentration of Indicated Bacteriostat [a] (in p.p.m.) in Liquid Nutrient Agar Required Completely to Inhibit the Growth of Indicated Organism

Organism	Bacteriostat		
	Hexachlorophene	3,4,4'-Trichlorocarbanilide	Synergistic mixture [b]
Antibiotic-resistant micrococcus			
Strain No. 388010	0.2	0.2	0.1
Strain No. 388014	0.5	0.5	0.25
Strain No. 388062	0.5	0.5	0.25
Strain No. 388115	0.5	0.2	0.1
Strain No. 388128	0.5	0.5	0.1
Gram-negative types			
E. coli	120	120	50
S. typhosa	200	180	90
Pathogenic fungi			
T. interdigitale	2	1	0.5
C. albicans	100	80	40

[a] Bacteriostat in soap vehicle.
[b] Synergistic mixture: 50% hexachlorophene and 50% 3,4,4'-trichlorocarbanilide.

In Vitro versus in Vivo Performance of Bacteriostats. Investigators in the field of bacteriostatic soaps are well aware that *in vitro* activity does not necessarily indicate activity *in vivo*. Less familiar perhaps is the fact that potential deodorant activity is not necessarily related to antibacterial activity *in vivo* as determined by the standard hand washing techniques as set forth by Cade (9).

To determine these relationships a unique subjective method of deodorant evaluation has been developed. In this procedure a panel of 40 to 60 male subjects is selected by screening their ability to distinguish between the effects of a medicated *versus* a nonmedicated soap. Each subject is his own judge of effectiveness, replacing the traditional two or three judges generally used in this work.

Each participating individual uses control soap for all bathing for a period of one week, starting on a Friday. The second Friday the under-arm use of test soaps in a prescribed manner is instituted for a second period of one week. Beginning on Monday of the second week, and daily through Friday, each subject observes and records the odor in each under-arm area of his shirt or Tee shirt. Control soap is continued for bathing all other skin areas during this period. Clean shirts or Tee shirts are worn each day. The usual restriction as to the use of cosmetics in under-arm areas is observed. Reports on odor observations are collected daily, and the results are compiled at the conclusion of the test. It should be emphasized that test bars are assigned to one arm in one-half the panel, and to the other arm in the other half, in a random manner, to eliminate right-arm or left-arm bias.

In the tables the deodorant activity of 2.0% of hexachlorophene in soap is arbitrarily assigned the value of 100, and the activity of other bars is expressed in percentages thereof.

Table VI is illustrative of the fact that deodorant capacity is not necessarily indicated by *in vitro* antibacterial performance. Thus the 0.5% level of brominated salicylanilide and the 1.0% level of tetramethylthiuramdisulfide, while showing comparatively negative antibacterial activity *in vitro*, yield respectable deodorant activity. The skin-substantivity of these two chemicals is not revealed by *in vitro* methods.

TABLE VI
In vitro Activity Compared with Deodorant Effectiveness

Test bar	Amount of growth of *S. aureus* in nutrient agar plates with agar containing equal aliquots of 1.0% solution of test bars: p.p.m. soap basis agar			Deodorant capacity [a]
	20	10	5	
2.0% Hexachlorophene in soap	0	0	0	100
1.0% Synergist hexachlorophene/T.C.C. in soap	0	0	0	92
2.0% T.C.C. in soap	0	0	2+	89
0.5% Tribromosalicylanilide in soap	3+	3+	3+	69
1.0% Tetramethylthiuram-disulfide in soap	3+	3+	3+	67

[a] Hexachlorophene performance rated at 100.

Table VII shows that skin-degerming, as determined by standard handwashing procedures, is not necessarily a precise measure of deodorant activity.

Theoretical

The texture of skin in body areas most productive of body odors is entirely different from that of the palms of the hand. Antibacterial chemicals at the 1.0 or 2.0% level accumulate in relatively large quantity in the larger pore openings of skin areas, such as the under-arm area, as a function of soap retention. Thus the deodorant chemical concentrates where it does the most good.

TABLE VII
Skin Degerming (Cade Technique) Compared with Deodorant Effectiveness

	% Reduction in bacterial counts [a]	Deodorant capacity [b]
2.0% Hexachlorophene in soap	88	100
2.0% T.C.C. in soap	97	89
1.0% Synergist hexachlorophene/T.C.C. in soap	83	92
1.0% Synergist bithionol/T.C.C. in soap	90	87

[a] Fifth basin, 11th day of use.
[b] Hexachlorophene performance rated at 100.

While lesser amounts of skin-substantive agents (0.5%) may give relatively equivalent degerming action on the hands, it is reasonable to suppose that the advantages of skin-substantivity are largely overshadowed by the gross pick-up of soap in the underarm areas. Thus any agent at higher levels (1.0 to 2.0%) in soap yields greater accumulation and greater deodorant activity than at the 0.5% level.

It would therefore appear that the best measure of the deodorant capacity of a soap is a deodorant evaluation study rather than a bacteriological evaluation by means of controlled handwashing.

Toxicology. The complete toxicity picture was developed for the antibacterial synergist system hexachlorophene/T.C.C. in soap.

These studies indicate that soap containing this combination is neither a primary skin irritant nor a skin-sensitizing agent. The acute oral toxicity for dogs lies above 16 g. per kilo which rates as "practically nontoxic."

Summary

It has been shown that mixtures of hexachlorophene and trichlorocarbanilide or bithionol and trichlorocarbanilide in soap show a marked synergism with respect to antibacterial properties. In both cases the approximate ratio of 50/50 of the two components represents an optimum.

Nonionic detergents enhance the antibacterial activity of trichlorocarbanilide *in vitro* when present at relatively low levels. At higher levels of nonionic the antibacterial agent is neutralized and loses its effectiveness.

The antibacterial activity of the pair hexachlorophene-trichlorocarbanilide extends over a wide spectrum of micro-organisms, and synergism is shown with each test organism.

High antibacterial activity of the synergistic pairs in soap is shown both in *in vivo* handwashing studies and in subjective deodorant tests. On the other hand, it is shown by way of several examples that *in vivo* observations do not necessarily correlate with *in vitro* bacteriological screening tests.

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Search for New Industrial Oils. III. Oils from Compositae

F. R. EARLE, I. A. WOLFF, Northern Regional Research Laboratory,[1, 2] Peoria, Illinois; and QUENTIN JONES, Crops Research Division,[2] Beltsville, Maryland

THE COMPOSITAE are the largest family of flowering plants and include about 900 genera and more than 13,000 species. Two species, safflower and sunflower, are grown for oil production on a commercial scale. In addition to these, some two dozen species have been studied sufficiently, with respect to oil composition, to be reported in reference works on oils. Linoleic and oleic acids are the major component acids found in the oils reported, with linoleic acid usually in the greater amount. Linolenic acid is reported in amounts up to 4% in some oils but is absent from others (5). Only *Vernonia anthelmintica* (6, 7), *Calendula officinalis* (9), and *Chrysanthemum coronarium* (10) have been reported to deviate from the pattern.

Among the first 87 oils studied in our extensive search (4) for new industrial oils were 16 from seeds of different species of the *Compositae*. Out of these 16, two are reported in the comprehensive compendia by Eckey (5) and Hilditch (7).

Materials and Methods

The seeds used as sources of oils, like those for Part I (4) of this series, were provided by the New Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Methods used are the same as those described in Part I. Their primary purpose is to permit selection of oils of unusual and potentially useful composition rather than to give positive identification of any acids. They probably give an adequate representation of most of the oils although unsuspected interferences in some crude oils may cause them to give inconclusive or erroneous results.

Results and Discussions

Results of screening analyses are presented in Table I. The range in iodine value from 102 to 147 is typical of the family (5). The low value for *Vernonia anthelmintica* is related to the high proportion of monoene while the low value for *Cosmos bipinnatus* results from the high proportion of saturated acids. The highest iodine value is found in the oil from *Rudbeckia bicolor*, which contains the largest proportion of apparent linoleic acid.

When iodine value is plotted against refractive index, oils of the *Compositae* are in agreement with the regression line calculated for oils of 71 species (4) with four exceptions, those from *Dimorphotheca aurantiaca, Vernonia anthelmintica, Artemisia absinthium,* and *Chrysanthemum leucanthemum.* Data for six additional oils are perhaps far enough from the line to suggest a need for further study. The probable reason for the divergence of *V. anthelmintica* oil is its high content of epoxyoleic acid (6). *D. aurantiaca* and *A. absinthium* oils are shown by tests, discussed later, to contain conjugated unsaturation. The reason for the deviation of *C. leucanthemum* oil is not apparent. The oil has complex absorption in the ultraviolet and an unusual reddish color. It is not suitable for analysis by the A.O.C.S. method for polyunsaturated

[1] This is a laboratory of the Northern Utilization Research and Development Division.
[2] Agricultural Research Service, U. S. Department of Agriculture.