The Effect of Quaternary Treatment under Varied Ratios of Weight-Volume-Concentration on the Bacteriostatic Property of Fabrics

M. T. GOLDSMITH, M. A. LATLIEF, J. L. FRIEDL AND L. S. STUART

Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

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Quaternary nitrogen compounds are being widely used as sanitizers and to impart bacteriostatic properties to fabrics. When properly applied, the residual quaternary may assist materially in clinical problems associated with the activities of microorganisms in infants' garments, bedding, and similar articles.

Directions commonly furnished for the use of these agents specify a unit weight of product to a given weight of fabric with no limitations on the volume of water to be used. It seemed important, therefore, to determine whether such directions are adequate or whether there are certain limitations with regard to concentrations and volumes. This study was undertaken to determine the relationship between the amount of quaternary removed by cotton, wool, and nylon fabrics with the quaternary-fabric weight ratios kept at constant values and the volumes of solution varied. The amounts of quaternary adsorbed and absorbed under each condition were determined and these values correlated with those of bacteriostatic studies. Certain variations in technique were made in order to evaluate the procedure as a standard test method.

MATERIALS AND METHODS

Three-gram samples of desized cotton (Federal Specifications, 1953), wool, and nylon[1] in replicates of five were steam sterilized, dried and then agitated at 20 C for 10 minutes in volumes of germicide from 25 through 200 ml, containing 5 through 100 mg of the quaternary, alkyl (C_9-C_{15}) tolyl methyl trimethyl ammonium chlorides. The samples were wrung aseptically and dried in Petri dishes. Each half sample was subcultured in 0.01 buffered urease test medium of Stuart *et al.* (1945) and incubated the first 24 hours at 37 C and thereafter at room temperature. Two tubes contained 15 ml and 8 tubes contained 7 ml of the medium. Before the fabric was introduced, the medium was inoculated with one ml of a saline suspension of *Proteus mirabilis*, obtained by washing the surface growth from a 24-hour tryptone glucose extract agar (Difco) culture and filtering. The suspensions were diluted to give a reading of 230 on the Klett-Summerson photoelectric colorimeter. Suspensions with this reading gave plate counts of approximately 1.5 billion cells per ml and changed 0.01 buffered medium to pH 7.4 or above within an hour. Thus, by using a controlled inoculum with a reproducible urease activity, culturing in a lightly buffered medium, and selecting a 7-hour delay over the control as the criterion for bacteriostatic action of treated fabric, inhibition results could be obtained within an 8-hour period, rather than 16 hours, as with the procedure previously described (Latlief *et al.*, 1951).

Readings of the tubes were made hourly by comparison with phenol red color standards. Lack of bacteriostatic activity was indicated by a rise to pH 7.4 or above. The quaternary selectively adsorbed was calculated by determining the change in concentration in the bath after the treatment (Auerbach, 1943, 1944). By removal of the fabric, additional quaternary was carried over and is designated in this report as absorbed. The total quaternary removed is the sum of these amounts. The amounts of quaternary absorbed by cotton were contained in 8.1 ml, by wool in 7.8 ml, and by nylon in 5.1 ml. These volumes were derived from mean values of weight differences between dry fabric samples and those wrung with forceps. Thus, the absorbed quaternary is a multiple of the concentration per ml after treatment of the fabric and the volume removed by the specific fabric.

RESULTS AND DISCUSSION

The data obtained in determining the bacteriostatic action of treated fabric are summarized in table 1. For purposes of discussion, dilutions are also included. The results show that reliable bacteriostatic properties may be more readily imparted to cotton than to wool or nylon. For this reason, the major portion of the discussion will be confined to the results for cotton.

When cotton fabric was treated in all volumes from 25 through 200 ml containing only 5 mg of the quaternary, no inhibition of *P. mirabilis* was obtained on subculturing the samples. With 10 mg of quaternary in 25 and 75 ml, the urease activity of the test organism was inhibited seven hours, but in 50 ml some, but not all, the samples showed bacteriostatic action.

[1] Submerged in CCl_4 at room temperature for 15 minutes with occasional stirring. Air dried. Repeated in C_2H_5OH and air dried.

TABLE 1. *Bacteriostatic property of fabrics treated with quaternary in various concentrations and volumes*

Quaternary in Bath	Volume														
	25 ml			50 ml			75 ml			100 ml			200 ml		
	Cotton	Wool	Nylon	Cotton	Wool	Nylon	Cotton	Wool	Nylon	Cotton	Wool	Nylon	Cotton	Wool	Nylon
5 mg	N*	P†	N	N	P	N	N	P	N	N	N	N	N	P	N
Dilution	1:5,000			1:10,000			1:15,000			1:20,000			1:40,000		
10 mg	I‡	P	N	P	P	N	I	P	N	N	N	N	N	P	N
Dilution	1:2,500			1:5,000			1:7,500			1:10,000			1:20,000		
20 mg	I	P	N	I	P	N	I	P	N	P	P	N	P	P	N
Dilution	1:1,250			1:2,500			1:3,750			1:5,000			1:10,000		
50 mg	I	P	P	I	P	N	I	P	N	I	P	N	I	P	N
Dilution	1:500			1:1,000			1:1,500			1:2,000			1:4,000		
100 mg	I	P	I	I	P	P	I	P	P	I	P	N	I	N	N
Dilution	1:250			1:500			1:750			1:1,000			1:2,000		

* N, no inhibition.
† P, partial inhibition. Some tubes contained growth.
‡ I, inhibition.

With 20 mg of quaternary in 25, 50, and 75 ml, bacteriostatic activity was imparted to the fabric, but in 100 and 200 ml, only part of the subcultured samples inhibited *P. mirabilis*. Fifty and 100 mg in all volumes used repressed the growth of the test organism.

If the dilutions are now observed, it may be seen that when 10 mg was used, although a dilution of 1:7500 appeared to be effective, a dilution of 1:5000 showed only partial inhibition. With 20 mg present the highest effective dilution was 1:3750 whereas with 50 mg 1:4000 showed complete inhibition. If these dilutions are compared with the effective dilutions of several quaternary compounds reported earlier (Latlief *et al.*, 1951), it will be noted that the dilutions 1:3750 to 1:7500 compare not too unfavorably with 1:5000 to 1:10,000. Among the factors to be borne in mind in making these comparisons are the "static" property of the germicide, the tenacity with which fabric retains the quaternary, and finally the effect of the concentration of the buffer on the speed of the appearance of the ammonia. Wide variations exhibited by different quaternaries in "static" properties for a given test organism, and desorbing levels from cotton were reported by Goldsmith *et al.* (1955).

With respect to the effects of the concentration of the buffer, it can be stated from data developed in this laboratory that for a given concentration of cells, the change of pH in Stuart's 0.01 buffered medium occurred in one hour, in 0.1 buffered medium in three hours, and in highly buffered medium in 10 hours. That a treatment with a somewhat more concentrated solution of a germicide would be required to suppress the appearance of ammonia in 0.01 buffered medium would be a normal expectation. This factor should not be overlooked in interpreting the results obtained with 0.01 buffered medium and in comparing these with effective treatments obtained by subculturing in a highly buffered medium.

The results with wool show that quaternary in the amounts used only partially inhibited *P. mirabilis* regardless of ratio of quaternary to volume. Of the fabric samples treated, about 200 half samples allowed the test organism to change the pH within seven hours, whereas in the presence of 100 half samples, the test organism was inhibited.

Only with 100 mg of the quaternary in 25 ml did the nylon fabric remove sufficient quaternary to inhibit *P. mirabilis*. Partial inhibition was obtained with 50 mg in 25 ml and with 100 mg in volumes of 50, 75, and 100 ml.

Some of the data obtained in determining the adsorption and absorption of the quaternary by cotton and wool are summarized in table 2. Omitted from the table are data obtained in using 5 mg of the quaternary.

Absorption of quaternary by cotton decreases as volumes increase. For a constant weight of quaternary, the absorption reflects the reduced concentration due to volume increase as well as a reduction of concentration due to adsorption.

As would be expected, with a constant volume, ad-

TABLE 2. *Quaternary removed by 3 grams fabric*

Quaternary in Bath	Milligrams of Quaternary Removed from Specified Volume														
	25 ml			50 ml			75 ml			100 ml			200 ml		
	Ab*	Ad*	T*	Ab	Ad	T	Ab	Ad	T	Ab	Ad	T	Ab	Ad	T
Cotton															
10 mg	2	3	5	<1	3	4	<1	4	5	<1	4	4	<1	4	4
20 mg	5	5	10	3	4	7	2	5	7	1	4	5	<1	2	3
50 mg	12	14	26	7	6	13	5	5	10	4	10	14	2	8	10
100 mg	28	13	41	13	22	35	10	14	24	7	7	14	3	5	8
Wool															
10 mg	<1	8	8	<1	5	6	<1	4	5	<1	4	4	<1	5	5
20 mg	2	13	15	2	9	11	1	7	8	<1	6	7	<1	7	8
50 mg	9	21	30	5	17	22	3	18	21	2	18	20	2	15	17
100 mg	21	25	46	10	29	39	7	18	25	6	14	20	3	10	13

* Ab, absorbed; Ad, adsorbed; T, total.

sorption increases with increasing concentration in most instances. With a constant weight of quaternary in increasing volumes, however, a tendency for decreased adsorption is exhibited. Exceptions may be noted. Adsorption continues at the same level when 10 mg are used in 100 and 200 ml; with 20 mg adsorption decreases only in 200 ml and with 50 mg adsorption from 100 and 200 ml increases over that from 50 and 75 ml.

With 100 mg quaternary in volumes of 50 ml and more, the adsorption decreases markedly with increased volumes. The reduced adsorption with 25 ml may be attributed to low water-fabric ratio, resulting in insufficient contact between the cloth and the quaternary during agitation.

That equal quantities of quaternary are selectively adsorbed from solution by cotton fabric regardless of the volume, is borne out almost consistently when 10 and 20 mgs were used in the five volumes. When 50 and 100 mgs were used evidence for selective adsorption was not obtained.

If the data for total removal of quaternary are compared with those for inhibition, it may be seen that the minimum amount of quaternary removed and sufficient to impart bacteriostatic properties to three grams of cotton, under the varied conditions of these experiments, was 5 mg. However, this minimum amount was not sufficient to prevent the development of ammonia under all conditions studied. Thus, the dilutions in which total removal amounted to 7 to 10 mg would appear to be a more reliable minimum figure. The quaternary absorbed from these effective dilutions amounted to a considerable fraction of the total.

Although the capacity for wool to remove quaternary from solution exceeds that of cotton in almost all concentrations and volumes used, the bacteriostatic property imparted to wool for *P. mirabilis* was inferior in nature. That a reversible reaction between the wool and the quaternary when immersed in the subculture medium is responsible for this behavior is possible.

An evaluation of the results of using 7- and 15-ml volumes for subculturing the samples revealed no advantages in either case. Although the desorption of quaternary from fabric may produce a higher concentration in 7 than in 15 ml, the number of cells per ml of medium in 7 ml was double those in 15 ml, and their urease activity in the smaller volume was reflected by a more rapid change of pH. In those tubes which showed no inhibition in the first six hours, the average delay in 7 ml containing cotton and nylon was 1.8 hours and in 15 ml, 2.5 hours. With wool the average delay in 7 ml was 2.7 hours and in 15 ml 2.1 hours. The tubes containing 15 ml were easier to read. In view of these results, subculturing 1.5 g fabric in a 10-ml volume might be preferred to 7 or 15 ml.

In high concentrations of the germicide, that is, those containing more than 1:1000, the phenol red indicator reacted with the quaternary and precipitated on the cloth. That the concentrations of quaternary prevented change of pH could be demonstrated by the use of a pH meter. On removal of the fabric, the pH could also be confirmed by restoring the color to the medium by the addition of phenol red.

SUMMARY

Fabrics of different composition were treated in various concentrations of the quaternary germicide, alkyl (C_9-C_{15}) tolyl methyl trimethyl ammonium chlorides, contained in five volumes ranging from 25 through 200 ml. The amounts of quaternary removed by the fabric were determined chemically by methods described.

Bacteriostatic properties were imparted to cotton more readily than to wool or nylon. Although wool removed from solution more quaternary than cotton, the bacteriostatic property was inferior. Nylon removed relatively little quaternary from solution.

Evidence for selective adsorption by cotton was obtained only when 3 g fabric was treated with 10 or 20 mg of quaternary contained in the different volumes. With larger quantity of quaternary, selective adsorption was not observed. In solutions which imparted bacteriostatic property to cotton, the absorbed quaternary amounted to a considerable portion of the total quaternary removed.

The concentration of the treating solution appears to be the controlling factor in imparting practical bacteriostatic property to cotton fabric, rather than the ratio of the quaternary-fabric weights.

Subculturing treated samples in a 10-ml volume of 0.01 buffered urease test medium inoculated with 1.5 billion cells makes results available in eight hours, an advantage for a test method. It must be acknowledged, however, that such a test procedure may be more critical, requiring a somewhat higher concentration of quaternary, than when the highly buffered medium is employed.

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