Adsorption of Available Chlorine and Quaternary by Cotton and Wool Fabrics from Disinfecting Solutions

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Disinfection of sickroom clothing and bedding for which high temperatures are unavailable or impractical because of damage to the fabric is frequently a perplexing problem. Woolen garments and blankets are especially troublesome.

The need for information on the value of quaternary ammonium germicides now available prompted studies on the bacteriostatic, sanitizing, and disinfecting properties of these agents (Latlief *et al.*, 1951, 1952, 1952a). The results of these investigations showed quite clearly that germicides of this class, when applied at proper concentrations on cotton fabric, would provide certain practical residual bacteriostatic benefits, pronounced sanitizing activities and disinfection. Considerably higher concentrations were necessary to disinfect than to sanitize or to provide bacteriostatic effects. Moreover, the efficacy of the germicides depended on the bacterial population of the initial contamination, fabric inoculated with large numbers of organisms requiring more concentrated solutions.

The hypochlorites of sodium and calcium are frequently recommended for their disinfecting as well as bleaching properties. Direct evidence on the germicidal value of both hypochlorites and quaternary ammonium germicides for fabrics of different composition is meager.

The purpose of these investigations was to determine the effective concentrations of a sodium hypochlorite and of a selected quaternary ammonium germicide which would disinfect a given weight of cotton and wool fabrics immersed in a standard volume of the germicide. The procedures employed also offered an opportunity to ascertain the degree of adsorption of these chemicals from solutions by fabrics of different composition, and to bring into focus some of the basic problems concerned in the use of disinfectants on cellulosic and proteinaceous fabrics.

Procedures

Sodium hypochlorite and alkyl (C_8-C_{18}) dimethyl benzyl ammonium chloride were selected as the disinfecting agents for use in these studies. Three grams of fabric to 100 ml of disinfecting solution were chosen as a realistic fabric-water ratio and one which would be feasible to apply in the disinfection of clothing.

Three grams of cotton gauze fabric were cut, for ease in aseptic handling, into two 4 by 5-inch half samples. Three grams of wool flannel yielded two 3¼ by 3½-inch half samples. The cotton samples were steam sterilized in Petri dishes for 20 minutes at 115 C and dried overnight at 105 C. Just before using, the wool samples were autoclaved for 11 minutes only, the minimum time determined experimentally to yield sterile samples. On cooling, the damp wool samples absorbed the inoculum readily.

The test organisms, *Escherichia coli* and *Micrococcus pyogenes* var. *aureus* 209, were cultured and stored on medium specified by the Association of Official Agricultural Chemists (1950) containing per liter, beef extract, 5 g; sodium chloride, 5 g; Armour's peptone, 10 g; Bacto-agar, 15 g; pH 7.2 to 7.4. Daily transfers in nutrient broth (same as above without agar, pH 6.8) provided cultures to seed Kolle slants of Bacto Tryptone Glucose Extract Agar, composed per liter of beef extract, 3 g; tryptone, 5 g; glucose, 1 g; agar, 15 g; pH 7.0. The inocula, obtained by washing the 24-hour cultures from the surface of the Kolle slants and filtering, were standardized from readings on the Klett-Summerson colorimeter and diluted to contain approximately 10 million organisms per ml. Phenol coefficients and resistance to Hyamine 1622[1] were established weekly for *M. pyogenes* var. *aureus*, according to the procedures of the A.O.A.C. (1950).

Five 3-gram samples of fabric were each inoculated with 4 ml of the specific bacterial suspension, and introduced into pint jars containing 96 ml of the germicide. The jars were agitated in a launderometer at the testing temperature and for the appropriate time. With sterile forceps the fabric was removed from the germicide, wrung out by twisting and then placed in the neutralizer. From the hypochlorite disinfectant, each half sample was introduced into a 1-inch test tube containing 20 ml of fluid thioglycollate broth, obtained from the Baltimore Biological Laboratory with the following formula per liter, trypticase, 15 g; *l*-cystine, 0.75 g; dextrose, 5 g; yeast extract, 5 g; sodium chloride, 2.5 g; sodium thioglycollate, 0.5 g; resazurin, 0.001 g; agar, 0.75 g. This medium neutralized the residual available chlorine and served as substrate for growth of the

[1] Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, monohydrate.

surviving organisms. For the quaternary, 20 ml of 5 per cent Tamol N[2] solution were used for each half sample (Goetchius, 1949). After 90 minutes the half samples were again wrung aseptically and subcultured in 20 ml of tryptone glucose extract broth, (T.G.E.).

All tubes were incubated at 37 C for 48 hours. As controls, samples of the cloth inoculated with the test organisms were immersed in distilled water and subjected to agitation. Estimation of numbers of organisms removed from the fabric by this treatment was made by plating aliquots of the rinse water. Autoclaved test samples subcultured in T.G.E. broth also served as controls.

Effective germicidal treatment was indicated by lack of growth in all ten replicates of subcultured half samples at the end of the incubation period.

To detect the survival of the test organism, *E. coli*, all turbid subculture tubes were streaked on Eosin Methylene Blue plates containing per liter, Bactopeptone, 10 g; lactose, 5 g; saccharose, 5 g; dipotassium phosphate, 2 g; agar, 13.5 g; eosin Y, 0.4 g; methylene blue, 0.065 g. With the studies using *M. pyogenes* var. *aureus*, all tubes showing growth were streak-cultured on tryptone glucose extract agar and the development of the test organisms denoting ineffective treatment, was confirmed by examination for typical pigmented colonies and by subsequent gram staining.

In order to estimate how much of the disinfectant was adsorbed by the cotton and wool fabrics, the concentrations of available chlorine and quaternary were determined in the original germicidal solutions and after removal of the fabrics from test solutions. Available chlorine was estimated by the method outlined by Theroux *et al.* (1936), substituting concentrated HCl for glacial acetic acid. For the quaternary, the Auerbach (1943, 1944) colorimetric assay method was employed. Solutions were diluted to contain 25 to 100 micrograms of quaternary, thus giving on the Klett-Summerson colorimeter with red filter #60, readings from 50 to 220, a range of low error (personal communication).

Results and Discussion

The results of the disinfection studies are recorded in table 1, the adsorption data in figures 1, 2, 3, and 4, and the combined data in table 3.

Examination of the results in table 1 shows that both cotton and wool fabric may be disinfected by sodium hypochlorite and by alkyl (C_8-C_{18}) dimethyl benzyl ammonium chloride. With the hypochlorite and cotton, a 10-minute exposure period was more effective than a 5-minute treatment, but no increase in effectiveness was gained by elevating the temperature. Thus, the use of a hypochlorite in sufficient concentration should eliminate effectively from cotton fabric, micoorganisms of both the coliform and gram positive pyogenic varieties.

Wool fabric requires more available chlorine for disinfection than cotton, but is more easily disinfected if inoculated with *E. coli* than with *M. pyogenes* var. *aureus*. The concentrations, 300 to 800 ppm, yellowed the wool undesirably and with the alteration in appearance, a reduction of the initial hypochlorite solutions from pH 9–9.4 to 5.6–5.9 occurred. Increasing the temperature definitely increased the efficiency of the hypochlorite and although not of great magnitude, the temperature effect was measurable whereas no measurable effect was found with cotton. The exception to be noted is that in which *M. pyogenes* var. *aureus* required 600 ppm available chlorine at both 20 and 45 C. Also, a 10-minute exposure period required less available chlorine than a 5-minute in all instances except for *M. pyogenes* var. *aureus*.

The data obtained in using the quaternary reveal that neither time nor temperature increases promote the efficacy of the germicide when cotton is inoculated with *E. coli*. Moreover, twice as much of the germicide is required to disinfect cotton inoculated with *M. pyogenes* var. *aureus* than with *E. coli* under all conditions except at 45 C for 10 minutes. That a 10-minute exposure period and an increase in temperature of 25 C brought about a tenfold increase in germicidal efficiency for *M. pyogenes* var. *aureus*, as judged by concentration, should be noted.

The concentrations of quaternary which will disinfect wool inoculated with the two organisms are considerably less than those effective for cotton. Increases in time and temperature promote the germicidal action

Table 1. *Disinfection of inoculated cotton and wool fabrics with two germicides*

Temp	Time	Minimum Disinfecting Concentration			
		Sodium hypochlorite		Alkyl (C_8–C_{18}) dimethyl benzyl ammonium chloride	
		Escherichia coli	*Micrococcus pyogenes* var. *aureus*	*Escherichia coli*	*Micrococcus pyogenes* var. *aureus*
		Cotton			
C	*min.*	*ppm*	*ppm*	*ppm*	*ppm*
20	5	20	10	1000	2000
	10	15	8	1000	2000
45	5	20	18	1000	2000
	10	19	10	1000	200
		Wool			
20	5	64	800	250	666
	10	60	600	200	500
45	5	57	300	145	500
	10	41	600	100	250

[2] Rohm and Haas Company, Philadelphia, Penna.

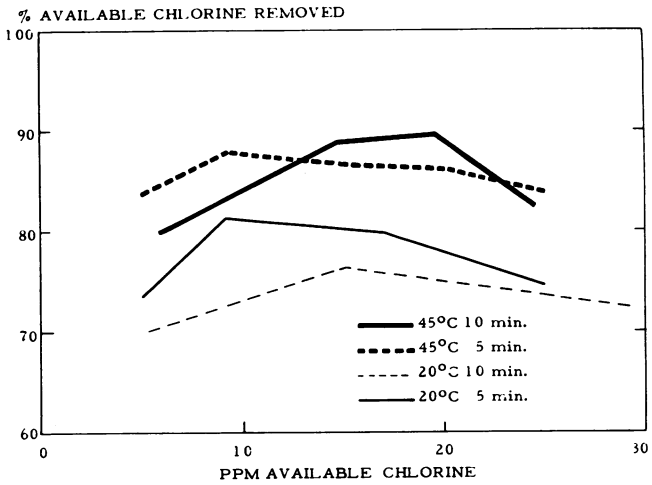

FIG. 1. Per cent available chlorine removed by 3 grams cotton fabric from 100-ml disinfecting solutions.

of this germicide for both organisms. Although the gram positive cocci tend to be more sensitive to the quaternaries than the gram negative rods when tested according to the phenol coefficient or dilution tube methods, in these studies using cellulosic and keratinous carriers, the susceptibility has been reversed, showing that less quaternary is required to eliminate *E. coli* than is needed for *M. pyogenes* var. *aureus*.

In figure 1 are presented graphically the data which show that cotton removes 70 to 90 per cent from solutions which contain 10 to 30 ppm available chlorine. The apparent difference in adsorption between 5- and 10-minute exposure periods may be within the range of experimental error at the low concentrations studied. The average of two determinations was used in plotting the curves shown in this figure. It should be noted that the general character of the four curves is the same. These curves suggest that the percentage adsorption may increase slightly as the concentration is increased from 5 to 15 ppm with a gradual decrease thereafter.

That wool reacts chemically with available chlorine has long been recognized (figure 2). If treated with available chlorine under carefully controlled conditions, however, wool may be made resistant to shrinkage. A review of the literature concerning such treatments as well as some of the factors affecting the reaction between available chlorine and wool are reported by Frishman *et al.* (1948).

The adsorption curves indicate that this reaction may be constant in low concentrations of available chlorine, such as 25 to 50 ppm, and the constancy is exhibited by an initial drop in the percentage removal curve. Above 100 ppm, however, irrespective of time and temperature, the capacity of the wool to react with available chlorine increases until almost complete disappearance of the chlorine from solution occurs. Attention should be called to the fact that fewer than 100 ppm of available chlorine were found adequate to disinfect wool inoculated with *E. coli* whereas for *M. pyogenes* var. *aureus* up to 800 ppm were required. The results with *M. pyogenes* var. *aureus* emphasize the undesirability of using a hypochlorite as a disinfectant for wool fabric since the high concentrations found effective also damage the fabric, and in these studies such treatments were carried out only to determine into what concentrations the competitive action of the keratin would continue.

To aid in the discussion of the results with the quaternary germicide, some of the concentrations used in this study are listed together with the equivalent mg per 100 ml of disinfectant as well as the corresponding dilution (table 2).

It can be seen in figure 3 that when the concentration

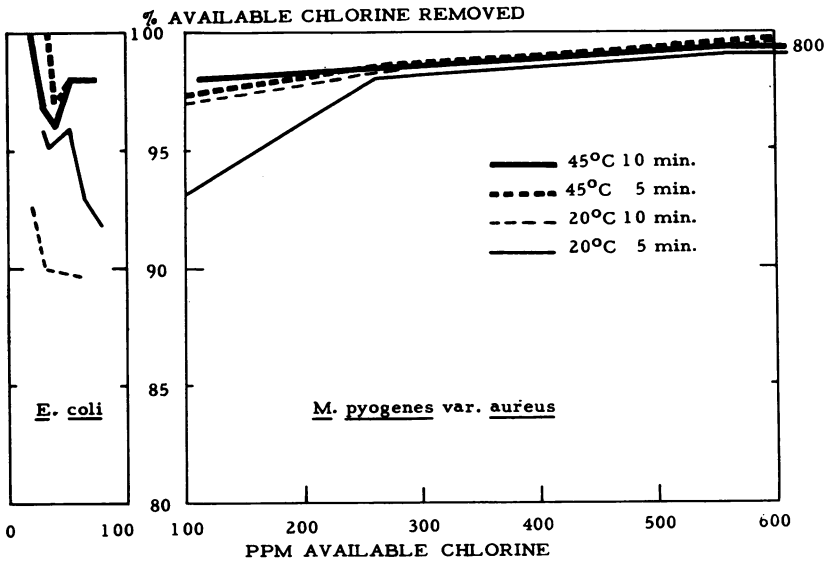

FIG. 2. Per cent available chlorine removed by 3 grams wool fabric from 100-ml disinfecting solutions

TABLE 2. *Equivalent concentrations of alkyl (C_8–C_{18}) dimethyl benzyl ammonium chloride*

Ppm	Mg per 100 ml	Dilution
25	2.5	1:40,000
50	5	1:20,000
100	10	1:10,000
200	20	1:5,000
250	25	1:4,000
500	50	1:2,000
666	66.6	1:1,500
1000	100	1:1,000
2000	200	1:500

of the germicide is low, the per cent adsorption is high but as the concentration increases, the per cent removed from the solutions by the cotton fabric decreases rapidly. With cotton the adsorption is only slightly affected by time and temperature changes. In contrast, with wool (figure 4) as concentrations increase, the relative amounts removed by the fabric decrease gradually. Also, increases in time and temperature promote the adsorption of the quaternary by the keratinous protein.

In order to facilitate the comparison of information developed concerning adsorption with that of disinfection, these data have been combined in table 3. The results of these studies suggest that in the disinfection of cotton fabric, sufficient quaternary must be present to satisfy the adsorptive capacity of the cellulosic material first and to provide, in addition, an adequate concentration to eliminate the microorganisms as well. For example, no disinfection of cotton inoculated with *E. coli* was obtained until an initial concentration of 100 mg of quaternary was used. After adsorption, 72 to 82 mg remained in solution, providing 720 to 820 ppm.

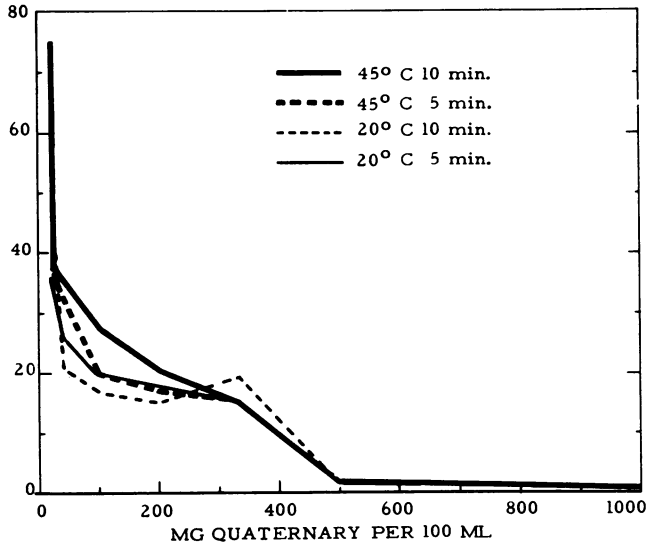

FIG. 3. Per cent quaternary removed by 3 grams cotton fabric from 100-ml disinfecting solutions.

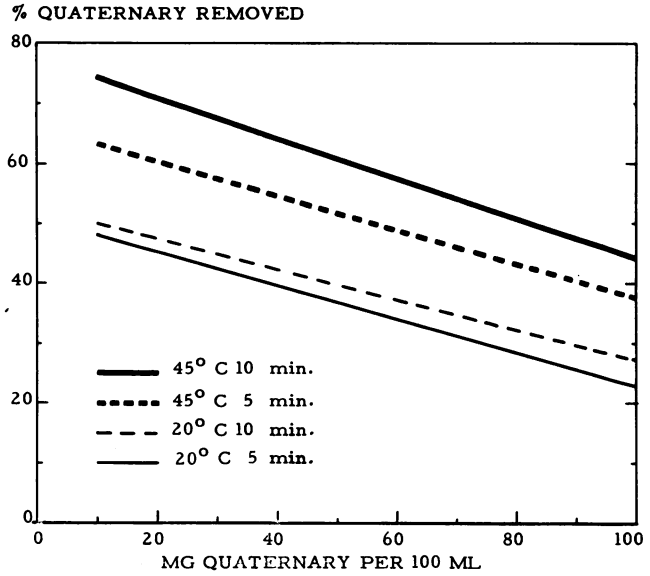

FIG. 4. Per cent quaternary removed by 3 grams wool fabric from 100-ml disinfecting solutions.

That the factors involved in disinfecting wool are different from those of cotton may be concluded from the conditions under which *E. coli* was eliminated from this fabric. Here the original concentrations up to 25 mg, depending on time and temperature, are lethal for this organism, and under these conditions only slightly more than 100 ppm were left unadsorbed.

If the adsorption data for the two fabrics with an initial concentration of 100 mg are compared, it is apparent that cotton adsorbs 6 to 9 mg per gram of fabric while wool adsorbs 8 to 15 mg per gram. The explanation of the affinity of the quaternary for the different fabrics undoubtedly is due to the physical and chemical

TABLE 3. *Adsorption of quaternary* by 3 g of fabric from 100-ml solutions and disinfecting concentrations*

Initial Concentration	Adsorbed	Final Concentration	*Escherichia coli*	*Micrococcus pyogenes* var. *aureus*
		Cotton		
mg/100 ml	*mg/100 ml*	*mg/100 ml*		
20	15	5	No disinfection	45 C–10 min.
50	10–17.5	32.5–40	No disinfection	No disinfection
100	18–28	72–82	All times and temp	
200	30–40	160–170		All times and temp
		Wool		
10	5–7.5	2.5–5	All times and temp	No disinfection
20	9–14	6–11		
50	18–30	20–32		All times and temp
100	23–45	55–77		

* Alkyl (C_8–C_{18}) dimethyl benzyl ammonium chloride.

characteristics of the cellulosic and keratinous materials which determine the nature and degree of adsorption. The data tend to support the assumption that cotton may inactivate some of the quaternary while with wool the quaternary is not inactivated, but on the contrary, by attraction to the fabric, may undergo local accumulation. To illustrate, in an initial concentration of 50 mg, cotton inoculated with *E. coli* adsorbs up to 17.5 mg of quaternary but the organism survives. Inoculated on wool, this same organism is killed concurrently with 14 mg or less of the quaternary, adsorbed from an initial concentration of 20 mg.

If the residual unadsorbed concentrations of the quaternary are considered, it is apparent that in most cases the concentrations are higher than are necessary to kill the unprotected cells of the test organism in the phenol coefficient method of the Association of Official Agricultural Chemists. Studies of bacterial populations in germicidal solutions and of treated cotton fabric suggest that cells do survive on the fabric, but at the same time cannot be demonstrated in the original or Tamol N solutions in which the inoculated fabric has been treated.

The explanation for the fact that more quaternary is required to disinfect both cotton and wool fabric inoculated with *M. pyogenes* var. *aureus* than is required for *E. coli* is not apparent. The physical and chemical properties of the fabrics controlling the adsorption of chemicals and the factors governing the reaction of cells under such conditions would have to be more fully known to account for the reversal of sensitivity exhibited in these studies. The results indicate, however, that the adsorptive characteristics of the fiber can be the controlling factor in determining the effectiveness of any given concentration of a germicidal quaternary as a disinfectant for fabrics.

In these studies using *E. coli* and *M. pyogenes* var. *aureus*, the data suggest that the disinfectant of choice for wool garments would be a selected quaternary ammonium compound, thus avoiding the undesirable effects produced by heat and the damage which would be expected in the use of chlorine. On the other hand, a germicide providing available chlorine appears desirable for undyed cotton fabric.

Summary

The concentrations of available chlorine from sodium hypochlorite and of alkyl (C_8-C_{18}) dimethyl benzyl ammonium chloride which disinfect 3 grams of cotton and wool fabrics, inoculated with *Escherichia coli* and *Micrococcus pyogenes* var. *aureus* 209 and treated in 100 ml of the germicides, have been determined under various conditions of time and temperature.

Cotton requires 8 to 20 ppm and wool 41 to 800 ppm of available chlorine. The reaction of the wool with high concentrations of chlorine damages the wool fabric. With the quaternary 200 to 2000 ppm disinfected cotton whereas only 100 to 666 ppm were required for wool.

Cotton adsorbs from 70 to 90 per cent when available chlorine concentrations are as low as 10 to 30 ppm. As much as 98 per cent disappears from solution containing 800 ppm when wool is treated with hypochlorite.

In low concentrations of quaternary, cotton fabric adsorbs as much as 75 per cent but in high concentrations relatively little is removed. Wool removes 25 to 75 per cent of quaternary from solutions with initial concentrations ranging from 10 to 100 mg of germicide. In low concentrations the loss to the fabric is high.

In most instances both cotton and wool inoculated with *M. pyogenes* var. *aureus* require a higher concentration of both of these germicides than is needed for *E. coli*.

The factors controlling the efficacy of germicides for fabrics of different composition are discussed.

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