Some Problems Involved in the Use of Quaternary Ammonium Compounds as Fabric Disinfectants

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The current awareness of problems concerned with drug resistant strains of *Staphylococcus* and other bacteria includes an interest in the role of bedding, nightclothing, and other garments as means of transmission of these bacteria. Duguid and Wallace (1948), Ridenour (1950), Church and Loosli (1953), Blowers and Wallace (1955), and Ravenholt and La Veck (1956) are among those who have reported on the dangers of this type of transmission. Engley (1958) emphasized that *Staphylococcus* has remained viable on surfaces (including clothes) for several months. It is obvious, then, that proposed means of control of the spread of infection should include studies on both direct and residual disinfection of fabrics. Fabric disinfection in turn, involves a knowledge not only of the properties of the disinfectant but also of the physicochemical relationship between fabric and disinfectant.

The quaternary ammonium compounds are among the chemicals frequently used in fabric disinfection. Goldsmith *et al.* (1956) reported on the importance of the weight-volume-concentration relationship in the adsorption of quaternary compounds on cotton gauze and on wool. To remove the starch and other foreign material from the fibers they desized the gauze with acid using the Textile Test Methods, Federal Specification 2610.2 (Federal Specification, 1953). Because of the possibility that the acid may have modified the adsorptive characteristics of cotton for the quaternary, it seemed advisable to investigate adsorption under other conditions. The purpose of this paper is: (a) to compare the amount of alkyldimethylbenzyl ammonium chloride adsorbed on undesized, acid desized, and enzyme desized gauze and muslin sheeting; and (b) to evaluate the bactericidal activity of the fabrics treated with quaternary.

MATERIALS AND METHODS

Three-gram samples of gauze (Curity[2] cloth) and bleached muslin sheeting (at least 70 threads lengthwise and 60 crosswise) were used as standard test units. Samples of this weight were provided by four 4 by 5 in. pieces of gauze and two 4 by 5 in. pieces of sheeting. Before acid and enzyme treatment the fabrics were extracted in chloroform for 16 hr to remove fat soluble material. Acid desizing was carried out according to Textile Test Methods, Federal Specification 2610.2. The fabric was boiled in an 0.5 per cent hydrochloric acid solution (hydrochloric acid 1.19 sp gr) for 30 min. Enzyme desizing was carried out according to a slight modification of Textile Test Methods, Federal Specification 2611.1 (Federal Specifications, 1953), using a combination of proteolytic and amylolytic enzymes in the amount of 3 to 5 per cent (by weight) of the fabric. After enzyme treatment the fabric was washed in hot distilled water, then rinsed in water adjusted to pH 6.8 to 7.0. After desizing, the samples were dried at room temperature, placed in Petri dishes, autoclaved at 15 lb pressure for 15 min, and dried at 70 C.

To simulate in-use conditions, three amounts of quaternary were used (10, 50, and 100 mg), each in four different volumes (25, 50, 75, and 100 ml). The solutions were prepared in sterile glassware, and aliquots were assayed before use (Auerbach, 1943).

For the adsorption tests, sterile 3-g fabric samples, in replicates of five, were placed aseptically in pint jars containing the quaternary, and agitated in a Launder-Ometer[3] for 10 min at 20 C. The samples were then wrung out with sterile forceps, placed in sterile Petri dishes, and dried at 70 C. The quaternary remaining in solution in the pint jars was then assayed. Any test was repeated if there was a significant difference between any two of the five results within a replication as analyzed by the Duncan and Bonner test (1954). As stated by Goldsmith *et al.* (1956), the amount adsorbed on the fabric is calculated by subtracting from the original amount the quantity in solution after exposure to the fabric. A certain additional amount is present in the liquid retained by the fabric, and must be accounted for. This is determined by obtaining the average weight of liquid per 3-g sample (by weighing both wet and dried samples) and calculating, from the Auerbach assay, the amount of quaternary in that weight. This amount was designated by Goldsmith *et al.* (1956) as "absorbed." To avoid confusion, since absorption usually implies penetration, we propose the term "carry-over" for this amount.

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Using 1.5-g treated fabric samples, the bactericidal activity against *Staphylococcus aureus*, *Brevibacterium ammoniagenes*, *Bacillus cereus*, and *Proteus mirabilis* was determined as follows. An inoculum of approximately 13 million bacteria in 2 ml of tryptone glucose broth was placed in 125-ml sterile Erlenmeyer flasks. A 1.5-g fabric sample was then placed in each flask and paddled until moistened by the inoculum to simulate moistening of clothes by perspiration. The flasks were then incubated for 4 hr. At that time, 25 ml of thioglycollate broth, which supports good growth of a wide variety of bacteria, were added to each flask. The flasks were then incubated for 18 hr. After incubation the flasks were examined for turbidity and the results recorded. Broth from each flask was inoculated on tryptone glucose extract agar with the exception of *Proteus*, which was inoculated on desoxycholate agar. *Brevibacterium ammoniagenes* was incubated at room temperature and the other three species at 37 C. The number of sterile plates was correlated with the total amount of quaternary compound on the fabric (table 1).

Results and Discussion

Adsorption. In all cases the amount of quaternary adsorbed on acid desized and enzyme desized fabric was less than that adsorbed on undesized fabric (table 1). The method of desizing did not greatly affect the amount adsorbed. At the 10- and 50-mg levels, more quaternary was adsorbed on untreated gauze than on muslin, but at the 100-mg level, slightly more was adsorbed on muslin than on gauze. At the 10-mg level, differences between the amounts of quaternary adsorbed on acid desized and enzyme desized gauze and muslin were very small. At the 50-mg level, more was adsorbed on desized gauze than on desized muslin. At the 100-mg level, more was adsorbed on desized muslin

TABLE 1

Adsorption of quaternary by 3 g of fabric from solutions differing in volume and concentration

Treatment and Type of Fabric	Milligrams of Quaternary Adsorbed from Specified Volume											
	25 ml			50 ml			75 ml			100 ml		
	CO*	Ad*	T*	CO	Ad	T	CO	Ad	T	CO	Ad	T
10 mg Quaternary												
Not desized:												
Gauze	0.005	9.99	10	0.004	9.99	10	0.007	9.99	10	0.004	9.99	10
Muslin	0.6	6.9	8	0.3	6.4	7	0.2	6.6	7	0.2	6.1	6
Acid desized:												
Gauze	1.5	3.6	5	0.8	3.4	4	0.5	3.9	4	0.5	2.3	3
Muslin	1.2	3.4	5	0.6	3.0	4	0.4	3.1	4	0.4	2.6	3
Enzyme desized:												
Gauze	1.6	3.3	5	0.8	3.5	4	0.5	3.0	4	0.5	2.6	3
Muslin	1.1	3.9	5	0.6	3.7	4	0.4	3.7	4	0.3	2.8	3
50 mg Quaternary												
Not desized:												
Gauze	5.5	27.3	33	5.5	25.7	31	1.9	25.7	28	1.7	21.6	23
Muslin	6.4	14.5	21	2.6	11.5	14	2.4	9.8	12	2.5	8.2	11
Acid desized:												
Gauze	9.0	12.5	22	5.3	6.0	11	2.6	5.3	8	2.3	5.8	8
Muslin	7.7	6.9	15	4.0	5.3	9	2.7	4.3	7	2.2	3.0	5
Enzyme desized:												
Gauze	8.8	13.4	22	5.0	9.1	14	3.2	8.0	11	2.5	7.5	10
Muslin	7.4	8.8	16	2.1	5.9	8	2.7	4.3	7	2.2	3.9	6
100 mg Quaternary												
Not desized:												
Gauze	17.6	26.3	44	9.0	26.1	35	6.3	20.0	26	4.9	18.5	23
Muslin	13.4	29.3	43	6.4	28.2	35	4.5	26.4	31	3.7	20.7	24
Acid desized:												
Gauze	21.0	19.0	40	10.0	15.0	25	7.0	10.0	17	5.6	7.0	13
Muslin	13.8	23.5	37	7.4	17.5	25	4.9	19.8	24	3.9	15.9	19
Enzyme desized:												
Gauze	20.0	15.0	35	10.0	13.0	23	7.7	6.0	14	5.6	6.3	12
Muslin	13.3	25.8	39	7.0	22.0	29	4.9	17.9	23	4.0	14.8	19

* CO, carry-over; Ad, adsorbed; T, total.

than on desized gauze. At the 50- and 100-mg levels, more was adsorbed from 25 ml than from 100 ml. This may be a result of greater availability of quaternary molecules in the smaller volume.

Gauze is a loosely woven fabric with approximately 36 x 40 threads per sq. in. as contrasted with 60 x 70 in the muslin sheeting. It seemed possible that a greater surface area would be available for adsorption in the loosely woven fabric than in the tightly woven one. To explore this hypothesis, yarns were unraveled from 3-g samples of undesized gauze and muslin and given the same quaternary treatment as the fabrics. At the level of 50 mg in 100 ml, almost identical amounts of quaternary were adsorbed on the yarns, 21.1 and 20.8 mg (table 2). When these were woven into fabric the amounts were 21.6 and 8.2 mg for gauze and muslin, respectively. The 100-mg level is apparently close to the maximal adsorption point for both yarns. In all cases more was adsorbed on the yarn than on the fabric.

The fact that less quaternary was adsorbed on desized than on undersized fabric, but that the method of desizing did not greatly affect the amount adsorbed, poses some interesting questions. Since it is known that quaternary compounds can be adsorbed on starch, and since starch is used in sizing of fabrics, the question arose as to whether the differences in adsorption were due simply to removal of starch. The muslin sheeting is guaranteed to contain less than 3 per cent sizing (or less than 90 mg per 3-g sample). Curity cloth and other absorbent gauze contain much less starch than sheeting. No exact figures are available, but an estimated 1 per cent would be 30 mg per 3-g sample. Known amounts of potato starch were added to quaternary solutions and agitated in the Launder-Ometer for 10 min at 20 C. The starch was allowed to settle and the supernatant fluid was assayed. The results indicated that the removal of starch did not in itself explain the differences in adsorbability between undesized and desized fabric. Dextrins are formed in the hydrolysis of starch, and since it is very difficult to remove all dextrins from fabric, no conclusions as to their role in adsorption or desorption can be drawn from these experiments.

The possibility that the cellulose molecules themselves were altered by the desizing process cannot be overlooked. The amorphous portion of the molecule is more readily affected by chemical agents than the crystalline portion. If the molecules were shortened or otherwise altered there might be fewer areas of attachment for the quaternary. Certainly there is need for more basic research, both chemical and bacteriological, before these questions can be fully answered. The answers would be helpful from a practical standpoint, because under in-use conditions fabrics are subjected to many types of treatment.

Bactericidal action. All inoculated flasks were compared visually with the controls at the end of 22 hr of incubation. It was not possible to use turbidity as a criterion for bacterial growth because of the cloudiness caused by fabric particles. This was especially evident in the fabrics which had been impregnated in 25-ml volumes. The fiber particles were most numerous in the acid desized material.

The data on the relationship between amount of available quaternary on the fabric and its bactericidal activity were studied in several ways. The amounts of quaternary on acid desized, enzyme desized, and undesized swatches were tabulated separately and compared as to bactericidal action. The amount of quaternary available, not the method of treatment, was found to be the important factor.

The figures indicated as "adsorption" were tabulated alone and studied as to bactericidal action. This was unrealistic, since the "carry-over" plus the amount designated as "adsorbed" represents the amount im-

TABLE 2

Absorption of quaternary by yarn and by fabric

Quaternary Solution	Milligrams of Quaternary Adsorbed by 3 Grams of:			
	Yarn		Fabric	
	Gauze	Muslin	Gauze	Muslin
50 mg in 100 ml	21.1	20.8	21.6	8.2
100 mg in 25 ml	32.7	29.6	26.3	29.3
100 mg in 100 ml	32.0	28.0	18.5	20.7

TABLE 3

Proportion of samples sterilized by fabric impregnated with quaternary

Milligrams Quaternary per 1.5 g Fabric	Gauze				Muslin			
	Staphylococcus aureus	*Brevibacterium ammoniagenes*	*Bacillus cereus*	*Proteus mirabilis*	*Staphylococcus aureus*	*Brevibacterium ammoniagenes*	*Bacillus cereus*	*Proteus mirabilis*
2–4	28/40	30/36	20/20	20/20	49/56	31/38	32/34	0/37
5–7	39/44	28/30	22/22	0/22	13/16	10/10	10/10	0/13
8–10	3/4	2/2	2/2	0/2	10/10	6/6	6/6	0/8
11–13	22/24	12/12	12/12	5/22	18/19	10/10	10/10	1/15
14–16	14/17	6/6	6/6	1/10	9/9	4/4	4/4	0/8
17–19	8/8	4/4	4/4	3/8	5/5	2/2	2/2	0/5
20–22	8/8	4/4	4/4	10/18	17/17	6/6	6/6	8/38

mediately available to inhibit bacterial growth. In dilutions representing figures below the saturation point, some of the quaternary labeled "carry-over" was undoubtedly adsorbed after the fabric was removed from the jar.

The total amounts of available quaternary were compared as to bactericidal action. It was concluded that this figure, whether on desized or undesized fabric, was the most reliable and realistic figure for evaluating the antibacterial action of the treated fabric (table 3). According to Harris (1948) the monomolecular layer of quaternary is most tightly bound because the bond is between like ends of different molecules (hydrophobic end to hydrophobic surface). The overlayers will not be as tightly bound because the bonding is between like ends of the same molecule. The availability (or desorption rate) of the "carry-over," therefore, should be dependent on whether the total amount adsorbed is greater than or less than the amount required for a monomolecular layer.

Table 3 is a summary of efficiency of bactericidal action of the treated fabric. The vegetative form of *B. cereus* was sensitive to very small amounts of quaternary (2 to 4 mg), and *B. ammoniagenes* was resistant to only slightly larger amounts (5 to 7 mg). *Staphylococcus aureus* was somewhat more erratic, with 100 per cent bactericidal activity at the level of 17 to 19 mg. A fairly high percentage of treated fabric samples sterilized the cultures at much lower concentrations. *Proteus* (the gram-negative bacterium) was the most resistant, and even at the level of 20 to 22 mg, only 50 per cent of the inoculated flasks were sterilized.

Similar or identical amounts of quaternary can be made available on the 1.5-g fabric samples under a variety of conditions. For example, under the conditions of these experiments one can obtain a 14- to 16-mg level in several ways (table 1): 100 mg of quaternary in 50 ml with enzyme desized muslin; 100 mg in 75 ml with undesized muslin; 50 mg in 50 ml or 75 ml with undesized gauze. The 11- to 13-mg level can be obtained by using: 100 mg in 75 ml with acid or enzyme desized muslin and in 100 ml with undesized muslin; 100 mg in 50 ml with acid or enzyme desized gauze or in 100 ml with undesized gauze; 50 mg in 25 ml with undesized muslin; 50 mg in 25 ml with acid or enzyme desized gauze or in 100 ml with undesized gauze. If the time or temperature is changed the amount of adsorption may also be changed (Goldsmith *et al.*, 1954). Such information is of value from an in-use standpoint.

Summary

The adsorbability of a quaternary ammonium compound on desized cotton gauze and muslin fabric samples was compared with that on undesized samples. The bactericidal activity of the quaternary treated fabrics was evaluated by a simulated in-use method.

Undesized gauze and muslin adsorbed more quaternary compound than acid or enzyme desized gauze and muslin.

Gauze adsorbed more quaternary at the lower concentrations than muslin, but at the highest concentration (1:250) muslin adsorbed slightly more than gauze.

Comparison of the adsorbability of the fabrics and their yarn components indicated that tightness of weave influenced the amount of quaternary adsorbed on the fabric.

The large differences in adsorbability between desized and undesized fabrics could not be explained solely by removal of starch.

Vegetative cells of *Bacillus cereus* were most sensitive to the antibacterial activity of the treated fabric, followed closely by *Brevibacterium ammoniagenes*. *Staphylococcus aureus* was more variable in its response and required fairly large amounts of quaternary for sterilization. *Proteus* was the most resistant, and even at the 20- to 22-mg level only 50 per cent of the inoculated flasks were sterilized by gauze and 23 per cent by muslin.

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