Bicarbonates, sulfates, and chlorides of calcium and magnesium in water are appraised as a source of interference with the germicidal action of quaternary ammonium compounds at various temperatures.

Bactericidal Efficiency of Q.A.C. in Different Waters

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INTERFERENCE of natural tap water with the bactericidal action of quaternary ammonium compounds (Q.A.C.) was first reported by Hanne, referred to by Lawrence (*1*), and has since been confirmed by other investigators: Public Health Service (*2*), Ridenour and Armbruster (*3*), Shere (*4*), Butterfield, Wattie, and Chambers (*5*), Humphreys and Johns (*6*), and others. This study was initiated to further determine and evaluate some of the causes of inhibition of Q.A.C. bactericidal action.

Experimental Methods

One ml. of *Escherichia coli* ATCC–11229 suspension was added to 99 ml. of water-germicide mixture to produce an initial bacterial density of approximately 100,000,000 organisms per ml. Test temperatures were thermostatically controlled at the various levels reported. At specific intervals, aliquots were transferred to Tween 80 Asolectin neutralizer, Weber and Black (*7*), from which duplicate portions were planted for agar plate colony counts. An experiment usually was made with 2 or 3 replicate test flasks for each germicide.

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The method used was similar to the Weber-Black procedure (*7*) but differed from it as follows: (*a*) larger volumes were used to provide material needed for chemical control tests; (*b*) more than one test temperature was used; (*c*) bacterial suspension was prepared in phosphate buffered water instead of in test water; (*d*) suspension was filtered through sterile paper, eliminating most of the "freak jumps"; (*e*) suspension was stored in ice throughout a given experiment (this did not affect the test temperature as the ratio of suspension to test water was 1 ml. to 99 ml.); (*f*) culture was added to test mixture instead of test mixture to culture; (*g*) duplicate plates, using phosphate buffered dilution water, were planted in the range expected to yield 30 to 300 colonies; (*h*) initial and final numbers determinations were based on triplicate plates.

Controls and Compounds

Sterility control was maintained on all water, neutralizer, media, dilution blanks, and equipment used. It was inadvisable, due to the breakdown of bicarbonates, to sterilize raw and tap waters. Controls indicated the presence of extraneous bacteria in some of these waters. Such contaminants, when present, were insufficient in number to affect the initial numbers determination at a dilution of 10^{-6}. These organisms did not result in germicide consumption, and they were killed during the waiting period allowed for the test mixture to come to constant temperature. However, when such contaminants were present on control plates, representative colonies were transferred to lactose broth to prove the absence of coliform organisms. Likewise, representative colonies surviving the longest exposure in germicide tests were transferred to lactose to exclude the possibility of counting extraneous noncoliform contaminants as *E. coli.*

The quaternary ammonium compounds used were: compound A—alkyl dimethyl benzyl ammonium chloride; compound B—para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, and compound C—alkyl tolyl-methyl trimethyl ammonium chloride.

Water Selection, Preparation, Measurement

Norwood, Ohio, well water was used in a considerable portion of this work because: (*a*) it was readily available; (*b*) previous studies (*2*, *5*) had shown that this water interfered with Q.A.C.; (*c*) waters with an equal or higher mineral content are common on many dairy farms; (*d*) numerous municipalities, especially in the west and southwest, use waters with equally high or higher mineral content. The State health departments of Arkansas, Kansas, and Texas provided laboratory facilities, engineering services, and sample collectors, which enabled personnel associated with this project to conduct laboratory investigations in these areas.

Forty-five different types of well, tap, synthetic (distilled water with minerals added), softened, and sequestered waters were investigated. Tap and well waters were used fresh and unaltered, except when chlorine was present a slight excess of sodium sulfite was used for dechlorination. These waters were also investigated after being treated as follows: (*a*) boiled 2 hours, evaporation loss being restored with distilled water; (*b*) boiled, cooled, and recarbonated with CO_2, then decarbonated by shaking to adjust pH to the same level as that of the raw water before boiling; (*c*) stored at room temperature for 3 to 10 days; (*d*) lime softened; (*e*) partially softened by zeolite; (*f*) sequestered with either sodium hexametaphosphate (S.H.M.P.), tetrasodium pyrophosphate (T.S.P.P.), or the tetrasodium salt of ethylenediamine tetra acetic acid (E.D.T.A.).

In all experiments, any precipitate in the water was suspended by agitation while aliquots were being transferred to test flasks. Lime-softened water, in which removal of the sediment is an integral part of the process, was an exception to the preceding statement.

Results

Space limitations prevent the presentation of all data available. Results reported are considered representative of the more significant trends. With the exception of two tables, data obtained with long exposure intervals have been deleted. Findings are reported as percentages of bacteria surviving for a given exposure, based on the initial numbers determination as 100 percent.

A succession of individual experiments, constituting a series, were run with fresh, synthetic, or treated water. In a series using fresh and treated water, aliquots of treated water as well as those used unaltered were drawn from a common sample of fresh water. Most results reported are averages obtained in two or more series of individual experiments. Each series started with a new sample of water of a given type or, in the case of synthetic water, a freshly prepared batch. There was relatively little experimentation with trace elements and exclusion tests.

Calcium and Magnesium

According to Foulk (*8*), calcium and magnesium usually occur as bicarbonates in waters found in strata of the type bearing Norwood water. Therefore, the effect of these bicarbonates was investigated in natural waters and as the pure compounds produced in distilled water.

In tests with compound A in raw Norwood water, with a total hardness of 410 p.p.m. and a combined calcium and magnesium bicarbonate content of about 350–375 p.p.m., marked interference with bactericidal action occurred (table 1). In this and all future references, bicarbonate alkalinities (regardless of attached ion), calcium salts, magnesium salts, and hardness are given as p.p.m. $CaCO_3$ according to reporting conventions of Standard Methods for the Examination of Water and Sewage (*9*). Additional results with raw Norwood water are presented in tables 4 and 5.

When an aliquot of Norwood water was boiled, precipitating the bulk of the bicarbonates as normal carbonates, the bicarbonate and hardness content dropped to 70 p.p.m. and 90 p.p.m., respectively, and interference was reduced from 8- to 25-fold at exposure intervals of 1 and 2 minutes (table 1). Recarbonation of an aliquot of this same water restored the interference. A comparison of results presented in tables 1 and 2 shows that pure calcium and magnesium bicarbonate in distilled water, in amounts equivalent to the total bicarbonate content of Norwood water, interfere to a degree comparable to raw Norwood water. In all instances rapid kills were obtained in distilled water with no hardness added.

The effect of several calcium and magnesium salts in distilled water, at concentrations equivalent to the total calcium and magnesium content of Norwood water, was investigated. These results indicated that, within the limits of the

Table 1. Effect of different waters on the bactericidal efficiency of Q.A.C.

Water source and Q.A.C.	pH	Average percentage of *Escherichia coli* surviving at 22° C. after (minutes)						
		Fresh tap water						
		¼	1	2	5	10	20	30
Compound A,[1] *50 p.p.m.*								
Topeka, Kans	9.8	104	16	4.8	0.015	<0.0001	--------	--------
Dermott, Ark	9.5	0.0003	0	0	--------	--------	--------	--------
Port Isabel, Tex	7.5	--------	--------	--------	110	104	56	6.4
Norwood, Ohio	7.6	96	64	38	12	0.24	0.0036	<0.0001
San Benito, Tex	8.3	--------	--------	71	49	17	0.21	0.0007
Compound B,[2] *200 p.p.m.*								
Topeka, Kans	9.8	6.1	0.076	0.0062	0.0003	--------	--------	--------
Dermott, Ark	9.5	<0.0001	0	0	--------	--------	--------	--------
Port Isabel, Tex	7.5	--------	99	107	88	86	78	67
Norwood, Ohio	7.6	92	20	8.6	2.1	0.78	0.017	0.0026
San Benito, Tex	8.3	54	4.0	0.22	0.015	0.0031	0.0005	0.0001
		Boiled tap water						
Compound A,[1] *50 p.p.m.*								
Topeka, Kans	8.3	89	47	26	2.1	0.030	--------	--------
Dermott, Ark	9.4	0.016	0	0	--------	--------	--------	--------
Port Isabel, Tex	7.9	--------	--------	--------	--------	--------	53	12
Norwood, Ohio	8.7	91	8.3	1.4	0.0003	0	0	--------
San Benito, Tex	8.3	--------	--------	--------	20	3.2	0.0062	--------
Compound B,[2] *200 p.p.m.*								
Topeka, Kans	8.3	11	0.24	0.086	0.0009	--------	--------	--------
Dermott, Ark	9.4	0	0	0	--------	--------	--------	--------
Port Isabel, Tex	7.9	--------	--------	--------	--------	--------	33	27
Norwood, Ohio	8.7	3.2	0.0077	0.0005	<0.0001	--------	--------	--------
San Benito, Tex	8.3	27	3.8	0.57	0.071	0.0064	--------	--------

[1] Alkyl dimethyl benzyl ammonium chloride.
[2] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.

Table 2. The bactericidal efficiency of Q.A.C. in distilled water containing salts of calcium and magnesium [1]

Q.A.C. and salt added	Average pH		Average percentage of *Escherichia coli* surviving at 22° C. after (minutes)						
	Initial	Final	¼	1	2	5	10	20	30
Compound A,[2] 50 p.p.m.									
$MgSO_4$	8.1	7.3	102	86	85	22	0.076	0.0014	------
$MgCl_2$	8.9	8.2	------	------	90	24	0.10	<0.0001	------
$Mg(HCO_3)_2$	8.3	8.3	95	29	11	0.85	0.0003	0	------
$CaSO_4$	8.2	7.5	92	90	81	49	2.3	0.011	------
$CaCl_2$	8.3	8.0	------	103	92	68	6.6	0.022	------
$Ca(HCO_3)_2$	7.4	7.4	92	80	77	48	8.9	0.0015	------
Compound B,[3] 200 p.p.m.									
$MgSO_4$	8.0	7.1	95	70	58	25	7.4	0.86	0.16
$MgCl_2$	8.9	7.7	------	84	64	33	9.1	1.8	0.42
$Mg(HCO_3)_2$	7.8	7.8	88	27	10	1.5	0.28	0.0061	0.0005
$CaSO_4$	8.2	7.2	87	51	22	6.6	2.2	0.40	0.084
$CaCl_2$	8.3	7.4	------	59	31	10	3.2	0.58	0.19
$Ca(HCO_3)_2$	7.4	7.4	78	25	12	3.7	1.2	0.16	0.013

[1] All solutions are equivalent to 375 p.p.m. $CaCO_3$.
[2] Alkyl dimethyl benzyl ammonium chloride.
[3] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.

methods used, there was little difference in the effect of the various salts (table 2). However, the interference of all these salts was significant at the concentrations tested.

Iron

In highly carbonated waters, iron occurs as the bicarbonate according to Foulk (*8*). Mueller and Seely (*10*) demonstrated interference with 10 p.p.m. of iron as Fe^{++} ion at pH 4.0 or lower, but they indicated that above pH 4.0 hydrolysis occurs and interference disappears. However, in many farm dairy uses water is drawn and mixed with germicide before hydrolysis can take place. Therefore, information regarding the effect of iron in the native state should be of value.

Ferrous bicarbonate was produced and maintained in a closed system under nitrogen washed through 5 successive alkaline pyrogallol traps followed by 1 water trap. Experiments were run with 10 p.p.m. of iron as bicarbonate and, also, with a like amount as bicarbonate that was first oxidized by aeration until the test for Fe^{++} was negative and a Fe^{+++} test showed 10 p.p.m. Bactericidal tests in the presence of ferrous bicarbonate were made with oxygen free nitrogen streaming through the flasks,

Table 3. The effect of iron on the bactericidal efficiency of Q.A.C. at pH 6.1–7.0

Q.A.C.	10 p.p.m. iron as	Valence	Average percentage of *Escherichia coli* surviving at 22° C. after (minutes)				
			¼	1	2	5	10
Compound A,[1] 50 p.p.m.	$Fe(HCO_3)_2$	Fe^{++}	26	0.90	0.054	0.010	0.0006
	$Fe(HCO_3)_2$ oxidized	Fe^{+++}	0.0072	0.0095	0.010	0	0
Compound B,[2] 200 p.p.m.	$Fe(HCO_3)_2$	Fe^{++}	0.26	0.0019	0.0007	<0.0001	------
	$Fe(HCO_3)_2$ oxidized	Fe^{+++}	0.10	0	0	0	------

[1] Alkyl dimethyl benzyl ammonium chloride.
[2] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.

above the test mixture, throughout the experiment. With ferrous bicarbonate, initial and final tests from each individual flask showed 10 p.p.m. Fe^{++} and 0 p.p.m. Fe^{+++}. The bicarbonate form was verified by titration. In experiments with ferrous bicarbonate, solutions were clear and colorless at the conclusion of tests. The results show that at pH 6.1 to 7.0 the interference of Fe^{++} is greater than that of Fe^{+++} (table 3).

Effects of Different Waters

Wyoming, Ohio, water originally has a total hardness of 520 p.p.m. and a bicarbonate content of 320 p.p.m. It is softened by lime-soda to a total hardness of 100 p.p.m. and a bicarbonate content of 60 p.p.m. Germicidal interference of this softened water was low, but restoration of the hardness by the addition of calcium bicarbonate resulted in marked interference. Control tests eliminated pH variations as the responsible factor.

Topeka, Kans., tap water was similar to that from Wyoming, Ohio, and likewise caused relatively little interference with compound B (table 1), but it did result in more interference with compound A than was true of Wyoming, Ohio, water. The boiling of Topeka water increased the interference slightly. In this case no mineral was precipitated, but the drop from pH 9.8 to 8.3 was probably a contributing factor.

Table 1 also shows the effect of Dermott, Ark., water (hardness 8 p.p.m. and total mineral content 252 p.p.m.) in which the major part of the mineral content is sodium and potassium bicarbonate (200 p.p.m.) and chloride (27 p.p.m.). Interference was very low in this water. To establish that this lack of interference was not due to the high pH (9.5), tests were run with 375 p.p.m. of sodium bicarbonate in distilled water at pH 7.5 to 7.9 in parallel with tests using raw Norwood water at the same pH. The results clearly indicated that sodium bicarbonate does not interfere under these conditions. These results also showed a marked difference between the interference in Norwood water and that of a synthetic water containing an equivalent concentration of bicarbonate as sodium bicarbonate.

Tests with Cincinnati, Ohio, tap water showed some interference at bicarbonate and total hardness levels of 33 p.p.m. and 94 p.p.m., respectively. Boiling had relatively little effect on this water in which a large percentage of the total hardness is permanent in nature. This is in marked contrast to Norwood water in which 90 percent of the total hardness is present as bicarbonate.

San Benito, Tex., tap water had a total mineral content of 1,150 p.p.m., of which 366 p.p.m. was hardness and a considerable portion of the latter was of the permanent type. Significant interference with germicidal action occurred in this water, and the interference was reduced somewhat by boiling.

Port Isabel, Tex., tap water had a total mineral content of 3,200 p.p.m. and 1,100 p.p.m. total hardness, mostly of the permanent type. This water represents the maximum interference ever encountered in studies with these compounds at the Robert A. Taft Sanitary Engineering Center (table 1). Boiling resulted in very little reduction of interference in this water, as might be expected, since permanent hardness is not affected by boiling.

Exclusion Tests

Norwood water contained a small amount of barium and strontium. No significant interference was noted in tests made with a mixture of barium and strontium bicarbonate in a concentration equivalent to the barium and strontium content of Norwood water.

Norwood water contained about 20 p.p.m. of silicon. Therefore, it was considered desirable to study the effect of this element in distilled water. Foulk (*8*) states that silica in underground waters is generally considered to be in the form of uncombined silicic acid. Accordingly, silicic acid was added to distilled water to produce a concentration of 20 p.p.m. as silica. The solution was initially adjusted to pH 10.0, using sodium hydroxide, in order to facilitate solution of the silicic acid. Storage overnight resulted in solution of the silicic acid while the pH dropped to 7.3. Because sodium hydroxide was used in the preparation of this water, it is probable that the silica was present as sodium silicate at the time the water was used in bacteriological tests. The effect of this form of silica on germicidal activity did not appear to be significant under these test conditions.

Boiled carbon dioxide free distilled water containing suspended calcium and magnesium carbonates, equivalent to the concentration of calcium and magnesium content of Norwood water, did not cause significant germicidal interference.

Softening, Sequestration, and Temperature

Raw Norwood water, fresh and stored, seriously interfered with the bactericidal efficiency of compound A (table 4). This interference was significantly reduced by all water treatment methods used. The same general trend was noted with compound B except that whereas the S.H.M.P. reduced the interference with compound A, its effect was negligible with compound B.

In tests at 10° C., 22° C., and 37° C., with all germicide concentrations at the manufacturers' recommended dosage, 200 p.p.m. of active agent, fresh Norwood water was sequestered or softened to the hardness levels shown in table 5. At 37° C. compound A was effective under all test conditions while compounds B and C were effective in the treated water, but some interference was noted in both the freshly collected and stored raw water. In tests at 22° C. there was little interference with the activity of compound A. There was moderate interference with compounds B and C in all treated waters, T.S.P.P. excepted, and marked interference in the fresh and stored raw water. When studied at 10° C. (table 5) the raw water interfered moderately with the activity of compound A and very severely with the activity of compounds B and C. Treatment prevented most of the interference with compound A. However, with compounds B and C interference was marked in all treated waters except those sequestered with T.S.P.P., in which there was moderate interference. However, an impractical amount, more than 2.0 percent, of T.S.P.P. was necessary to reduce the soap hardness to 75 p.p.m. This led to tests with reduced concentrations of T.S.P.P. (table 6).

These data indicate that in the case of T.S.P.P. there was no correlation between the soap hardness and inactivation of interference. Results show that 0.2 percent of T.S.P.P. is reasonably effective in alleviating the interference of Norwood water under the conditions of these tests except in the case of compound C at 10° C. (table 6). This amount of T.S.P.P. had a negligible softening effect as measured by the soap test. In general these results tend to be similar to those noted by Armbruster and Ridenour (*11*), Elliker (*12*), and others.

Results presented in table 5 show the effect of treatment with sufficient E.D.T.A. to reduce

Table 4. Effect of treatment on the capacity of Norwood water to interfere with the bactericidal efficiency of Q.A.C.

Water treatment	Total hardness in p.p.m.	pH	Average percentage of *Escherichia coli* surviving at 22° C. after (minutes)								
			Compound A,[1] 50 p.p.m.					Compound B,[2] 200 p.p.m.			
			¼	1	2	5	10	¼	1	2	5
Fresh raw	415	7.3–7.9	------	95	78	73	36	73	40	9.3	1.3
Lime softened	73	7.4–7.5	92	46	12	0.12	0.0003	0.88	0.0002	<0.0001	0
Zeolite softened	73	7.2–7.7	86	45	22	0.62	0.0072	0.76	0.0002	<0.0001	----
E.D.T.A. treated[3]	74	8.9–9.1	73	1.8	0.024	<0.0001	-------	0.017	<0.0001	0	----
S.H.M.P. treated[4]	73	7.3–7.8	68	0.37	0.0003	0	-------	83	42	22	2.4
Stored raw	408	7.6–8.2	------	95	90	58	36	83	28	4.5	1.6
Distilled control	0	6.6	0.018	<0.0001	-------	-------	-------	0.0023	0	-------	----

[1] Alkyl dimethyl benzyl ammonium chloride.
[2] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.
[3] Tetrasodium salt of ethylenediamine tetra acetic acid (0.185 percent).
[4] Sodium hexametaphosphate (0.18 percent). Hardness tested with soap—all others tested by Versenate method.

Table 5. Effect of treatment on the capacity of Norwood water to interfere with the bactericidal efficiency of 200 p.p.m. of Q.A.C.

Water treatment	Total hardness p.p.m.	pH	Average percentage of *Escherichia coli* surviving at 10° C. after (minutes)								
			Compound A [1]			Compound B [2]			Compound C [3]		
			¼	1	2	¼	1	2	¼	1	2
Fresh raw	410	7.4–7.8	46	4.1	0.65	97	86	78	92	76	71
Lime softened	75	7.7–8.0	1.1	0.020	0.0002	52	12	4.9	41	35	7.9
Zeolite softened	74	7.7–8.0	0.59	0.0002	0	50	9.9	5.8	34	10	2.6
E.D.T.A. treated [4]	75	8.9–9.2	0.18	<0.0001	0	32	5.2	3.1	31	13	5.6
T.S.P.P. treated [5]	77	9.1–9.3	<0.0001	0	------	0.024	<0.0001	0	2.4	0.0030	0.0018
Stored raw	352	8.0–8.2	42	3.0	0.36	------	------	83	------	------	62
Distilled control	0	6.6	<0.0001	------	------	0.0001	<0.0001	------	0.032	0.0045	0.0003

[1] Alkyl dimethyl benzyl ammonium chloride.
[2] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.
[3] Alkyl tolyl methyl trimethyl ammonium chloride.
[4] Tetrasodium salt of ethylenediamine tetra acetic acid (0.185 percent).
[5] Tetrasodium pyrophosphate (>2.0 percent). Hardness tested with soap—all others tested by Versenate method.

Table 6. The effect of different concentrations of T.S.P.P. on the bactericidal efficiency of 200 p.p.m. of Q.A.C. in Norwood water

Temperature	Percentage of T. S. P. P. in test water	Compound	Average percentage of *Escherichia coli* surviving		
			¼ minute	1 minute	2 minutes
10° C	0.10	[1] A	0.64	0.0003	0
	.15	[1] A	0.0005	<0.0001	0
	.20	[1] A	0.0004	0	------
	.10	[2] B	4.4	0.44	0.10
	.15	[2] B	0.16	0.0010	<0.0001
	.20	[2] B	0.16	0.0008	0
	.10	[3] C	69	55	46
	.15	[3] C	20	21	------
	.20	[3] C	15	10	------
22° C	0.10	[1] A	<0.0001	0	0
	.15	[1] A	0	0	0
	.20	[1] A	0	0	0
	.10	[2] B	0.15	0.0042	<0.0001
	.15	[2] B	0.0023	0.0001	0
	.20	[2] B	0.0003	0	------
	.10	[3] C	2.8	0.0090	0.0028
	.15	[3] C	0.30	0.0004	0
	.20	[3] C	0.067	0	------
37° C	0.10	[1] A	0	0	0
	.15	[1] A	0	0	0
	.20	[1] A	0	0	0
	.10	[2] B	<0.0001	0	------
	.15	[2] B	<0.0001	0	------
	.20	[2] B	0	0	------
	.10	[3] C	0.0007	<0.0001	0
	.15	[3] C	0	0	------
	.20	[3] C	0	0	------

[1] Alkyl dimethyl benzyl ammonium chloride.
[2] Para diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.
[3] Alkyl tolyl methyl trimethyl ammonium chloride.

the hardness to approximately 75 p.p.m. Additional data were obtained using varying amounts of E.D.T.A. without regard for alterations in hardness or pH. The addition of 0.3 percent E.D.T.A. further enhanced the activity of the Q.A.C. However, control tests indicated that 0.3 percent E.D.T.A. exerted a definite germicidal effect within 10 minutes but not within 2 minutes. Similar controls, with 0.2 percent E.D.T.A., established that there was no bacterial toxicity during a 10-minute exposure.

Data obtained indicate that at a concentration of 200 p.p.m. all Q.A.C. tested are reasonably effective at 37° C. in Norwood water containing 0.2 percent E.D.T.A. In similar tests at 22° C. the efficiency of compounds B and C was fair to marginal while that of A remained good. The latter was the only compound which showed good response at 10° C. in Norwood water treated with 0.2 percent E.D.T.A.

Discussion

The present study was concerned solely with the effect of the mineral content of waters on the bactericidal action of Q.A.C. Therefore, important public health considerations such as viruses, fungi, and the effect of factors like proteinaceous material, residual soap, and other foreign materials were not considered.

In the first part of this investigation, Q.A.C. concentrations were set at levels considered best for determining variations due to different types of waters used. Accordingly, results reported were not, in all instances, obtained under conditions best suited to an evaluation of the various germicides (*13*). The concluding work in this study was directly concerned with concentrations recommended for use. However, the scope of this investigation made it necessary to limit tests to three chemical types of Q.A.C.

Interference with germicidal action was generally high in waters having high concentrations of soluble calcium and magnesium salts. Any treatment which tended to precipitate, remove, or inactivate calcium or magnesium reduced the interference. Further evidence of the role of these salts was indicated by increased interference when boiled water was recarbonated or calcium carbonate was added to plant softened water, followed by recarbonation.

Waters in which practically the entire mineral content was bicarbonates of sodium and potassium did not interfere. Boiling, which converted much of the bicarbonate to normal carbonate, did not significantly alter the interference pattern in such a natural water.

High mineral content alone did not necessarily result in high germicidal interference. Bactericidal results with Norwood and San Benito water were similar, although San Benito water has a much higher total solids content. The concentration of calcium and magnesium salts in these two waters is similar in quantity but different in type, occurring mostly as the bicarbonate in Norwood water and as the sulfate in San Benito water. This tends to confirm, in natural waters, the results obtained in synthetic waters, namely, that equivalent concentrations of various soluble calcium and magnesium compounds interfere to approximately the same degree. The nonhardness salts in San Benito water were those of sodium and potassium, and did not appear to have contributed to the interference. This finding coincides with the observations noted in tests with distilled water containing sodium bicarbonate, as well as in Dermott, Ark., water. Still further evidence in this regard was indicated by the significant reduction in interference in Norwood water partially softened by zeolite where sodium was substituted for calcium and magnesium.

The effect of temperature is marked, and the trend is similar to that reported by McCulloch and associates (*14*). At 10° C. all of the compounds at a concentration of 200 p.p.m. showed marked interference in Norwood water.

The analyses of Topeka and Cincinnati water were nearly identical insofar as hardness and alkalinity were concerned. Freshly drawn Cincinnati water interfered with germicidal action somewhat more than Topeka water. The difference in pH could be responsible for the different reactions in these otherwise similar waters. Ridenour and Armbruster (*3*), Mueller and Seeley (*10*), and others have shown that the activity of Q.A.C. is enhanced by high pH values. When Cincinnati and Topeka waters were boiled, the pH of Cincinnati water increased while that of Topeka water decreased. This shift was accompanied by a slight decrease in interference in Cincinnati water while that of Topeka water increased somewhat.

Practical differences in the various methods of treating Norwood water were apparent. There was little difference in either the appearance or interference of the lime or partially softened zeolite-treated water at 75 p.p.m. hardness. Both treatments produced sparkling clear water. However, as a final rinse for utensils, lime or lime-soda softened water, due to the marked reduction in total dissolved solids content, should leave air dried glassware in a much more presentable condition than would be the case with zeolite-softened water.

Water treated with E.D.T.A. was clear while that treated with T.S.P.P. was very turbid. Since both of these additives, even though somewhat more effective than lime or partial zeolite softening in reducing interference, increase the total salt content of a water already high in dissolved solids, the effect on air dried glassware and equipment probably would leave much to be desired.

Summary

Many waters interfere with the bactericidal action of quaternary ammonium compounds. In this study hard waters have been shown to interfere seriously. Bicarbonates, sulfates, and chlorides of calcium and magnesium appear to be the primary cause of interference. Ferrous bicarbonate interfered to a moderate degree, but this interference was minimized when the iron was oxidized.

Natural waters in which the bulk of the mineral content was sodium and potassium bicarbonate did not seriously interfere with the germicidal action of Q.A.C. When such waters were boiled, most of the bicarbonates were converted to normal carbonates with no apparent change in interference.

Lime and partial zeolite softening removed a considerable portion of the interference from a hard water used in tests. Tetrasodium pyrophosphate and the tetrasodium salt of ethylenediamine tetra acetic acid reduced the interference of this water sufficiently to offer promise for use in practical applications.

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