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Factors Affecting Germicidal Efficiency of Chlorine and Chloramine*

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REPORTS in the literature disclose a marked divergence of opinion on the relative germicidal efficiency of chlorine and chloramine. Rideal (1910) observed that when an equivalent of NH_3 was added to sodium hypochlorite (1 per cent available chlorine) made by electrolysis, the phenol coefficient rose from approximately 2 for the hypochlorite to 6 for the ammoniated solution (chloramine). It should be noted that the solutions employed were distinctly alkaline.

During World War I difficulties in obtaining an adequate supply of chlorine led Race (1918) to utilize ammonia in conjunction with chlorine for sterilization of the water supply of Ottawa, Canada, with a view to conserving chlorine. He reported experiments in which it was observed that ammoniated bleach solution containing 0.2 p.p.m. available chlorine and 0.1 p.p.m. ammonia was as effective a germicide as was 0.6 p.p.m. available chlorine when employing bleaching powder alone.

Tilley (1920) observed that addition of ammonia to Dakin's solution resulted in an increased germicidal efficiency whereas when ammonia was added to chlorinated water a drop in germicidal properties was effected.

Holwerda (1928) and Gerstein (1931) studying the germicidal efficiency of chlorine-water (with and without addition of ammonia) reported that the germicidal properties were greater when ammonia was not present.

Charlton and Levine (1937) suggested that the conflicting reports in the literature on the germicidal efficiencies of chlorine and ammonia-chlorine (chloramine) solutions might be reconciled if influence of reaction (pH) on the disinfecting properties of these two types of chlorine compounds was taken into consideration.

The objectives of the following brief report are to present data on the relative effects of such factors as (1) presence of ammonia, (2) reaction (pH), (3) concentration, and (4) temperature, on the germicidal efficiencies of chlorine and chloramine, in the absence of organic matter.

TECHNIC

The test organism employed was a suspension of *Bacillus metiens* spores prepared in the following manner: The growth from 20 day–30° C. agar slant cultures was washed off in Butterfield Formula C water and filtered through Whatman No. 2 filter paper to remove clumps. The filtrate was then heated

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at 80° C. for 10 minutes to destroy vegetative cells. This spore suspension, stored at 5° to 10° C., did not disclose any appreciable change in resistance to chlorine for a period of over a year and therefore lends itself particularly well to comparative studies over a long period of time. Just prior to use a portion of the suspension was diluted to approximately 20 million viable spores per ml.

Previous observations had disclosed that the reaction of alkaline unbuffered chlorine solutions tends to become more acid on exposure to air and stirring. All experiments reported herein were therefore carried out in M/20 concentration of appropriate buffers at desired reactions (pH), which were determined with the glass electrode. All water employed in the following experiments was ammonia-free when tested with Nessler's reagent.

Chlorine solutions were prepared by diluting a saturated stock solution made by passing gaseous chlorine into ammonia-free water. This saturated chlorine was kept in a glass stoppered bottle painted black on the outside and stored in a refrigerator at approximately 10° C. Under these conditions the chlorine content was found to remain quite constant. Ammonium sulfate was the source of ammonia, but all computations are made as NH_3.

The procedure for determining germicidal efficiency was briefly as follows:

To 75 ml. of an appropriately buffered solution, containing a desired concentration of NH_3, was added 25 ml. of chlorinated water containing the desired concentration of chlorine, and the flask containing the mixture was placed in a water bath. One ml. of the spore suspension (diluted so as to contain about 20 million viable cells per ml.) was placed into 100 ml. of the test solution which was being constantly stirred. (This would yield an initial inoculum of one million viable cells per 5.0 ml. of the test solution. The number of viable spores was determined by plating a portion of the spore suspension on nutrient agar.) At the time that the spore suspension was introduced into the disinfecting mixture, the concentration of available chlorine was determined for a duplicate sample by titration with sodium thiosulfate in acid solution.

At desired time intervals, 5 ml. portions were withdrawn and placed in 45 ml. of sterile water containing sufficient sodium-thiosulfate to neutralize any residual chlorine, and the number of surviving bacteria was ascertained by plating on nutrient agar (24 hour 30° C.).

Curves were then made by plotting the logarithms of the percentage of survivors against the period of exposure in minutes, and the time for effecting a reduction of 99 per cent of the exposed spores was read from the curves and designated as the killing time (K.T.).

The question might readily be raised as to why spores were used and whether the results are applicable to water sterilization. With reference to the first question, it might be pointed out that chlorine is so effective a germicide that when employing vegetative cells the rate of death is so high (and the concentrations of chlorine employed necessarily being very low and therefore subject to wide errors in determination as well as to combination with traces of such compounds of ammonia which might inadvertedly be present), that observations on rates of death and the effects of variations in temperature and reaction or concentration on these rates would be extremely difficult to carry out.

In contrast to this, by utilizing spores, it is feasible to employ high concentrations of chlorine which would not be affected appreciably by traces of impurities; studies over a wide range

of reaction, concentration, and temperature, are possible and the rates of death are sufficiently low so that patterns of the survivor curves may be readily observed. Furthermore, it is very difficult to obtain vegetative cells of uniform resistance; whereas the spore suspension employed was found to be strikingly uniform in resistance to chlorination and could therefore be employed over a long period of time for comparative studies.

As to the question whether the results with spores would be indicative of what might be expected with vegetative cells, it might be pointed out that survivor curves obtained by the authors for spores exposed to chlorine and chloramine were later confirmed by Schmelkes for *Escherichia coli* and that the relative efficiency of chlorine and chloramine reported for spores was recently found to hold for vegetative cells. Thus, Weber, Bender, and Levine (1940) reported that *B. metiens* spores exposed to approximately 22 p.p.m. available chlorine (as hypochlorite) were killed in 3 minutes, whereas it required about 28 times as long (approximately 85 minutes) when the same concentration of available chlorine was employed as chloramine. Streeter (1943) reports a killing time of 2–3 minutes for *E. coli* when employing 0.05 to 0.1 p.p.m. chlorine as compared with 40 to 90 minutes for the same concentration of available chlorine as chloramine. The relative killing times of chlorine as compared with chloramines for spores and vegetative cells, respectively, were therefore quite similar.

SURVIVOR CURVES FOR DISINFECTION WITH CHLORINE AND CHLORAMINE

In Table 1 and Figures 1 and 2 are shown results illustrative of the rates of death of spores exposed to chlorine and chloramine. In survivor curves for disinfection with chlorine, there are exhibited distinct periods of lag in rates of death—very prolonged when employing high alkaline solutions, low temperatures or low concentrations—followed by progressively increasing death rates. Thus, with chlorine at pH 7, 24 per cent of the exposed spores died in the 1st minute, 49 per cent of

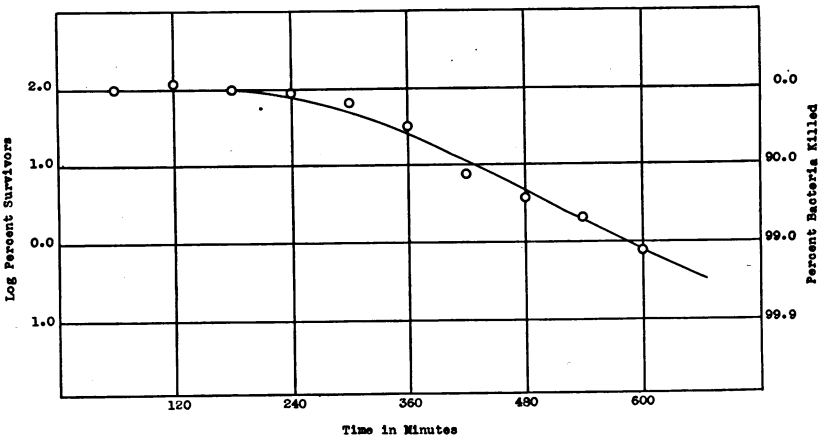
TABLE 1

Showing Per cent Reduction of Surviving Bacillus metiens Spores in Consecutive Equal Periods of Exposure to Chlorine and Chloramine at 20° C.

Chlorine			Chloramine		
*Time in Min.**	*Per cent Surv.†*	*Per cent Red.‡*	*Time in Min.**	*Per cent Surv.†*	*Per cent Red.‡*
		pH 10			
0	100	..	0	100	..
60	100	0	30	53	47
120	100	0	60	23	57
180	94	6	90	12	47
240	78	17	120	5.4	58
300	60	23	150	2.9	60
360	29	52	180	1.0	50
420	10	66	210	0.5	50
		pH 7			
0	100	..	0	100	..
1	76	24	20	72	28
2	39	49	40	28	61
3	1.1	97	60	9.8	65
			80	1.7	83
			100	0.6	65

* Period of Exposure (in minutes) to germicide
† Per cent survivors after stipulated periods of exposure
‡ Per cent reduction of survivors in consecutive equal periods of exposure

Fig. 1
RESISTANCE OF B. METIENS SPORES TO CHLORINE AT pH 10.0
(25 p.p.m. Av. Cl.; 20° C.)



those which survived 1 minute died in the 2nd minute, and 97 per cent of the then remaining cells died in the 3rd minute of exposure. In the series with chlorine at pH 10, there was no decrease in bacterial count for 2 hours. This was followed by a progressively increasing rate of death. Thus, during the 3rd hour, only 6 per cent of the survivors after 2 hours were killed; 24 per cent of the spores which survived for 4 hours were killed in the 5th hour; whereas of those which survived 6 hours, 66 per cent were killed during the 7th hour of exposure.

In contrast to these curves of increasing death rates observed with chlorine (Figure 1), disinfection with chloramines discloses but short lags followed by quite constant death rates throughout the period of disinfection, so that the survivor curves are practically straight lines (Figure 2).

EFFECT OF AMMONIA ON CHLORINE RESIDUAL

In Table 2 are shown the residuals remaining 60 minutes after addition of approximately 25 p.p.m. available chlorine to solutions containing various

Fig. 2
RESISTANCE OF B. METIENS SPORES TO CHLORINE AND AMMONIA AT pH 10.0
(25 p.p.m. Av. Cl.; 6 p.p.m. NH_3; 20° C.)

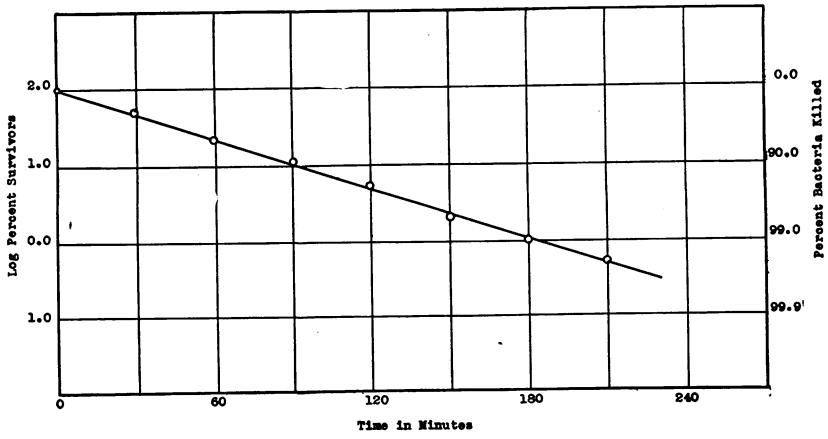


TABLE 2

Effect of Presence of Ammonia on Residual Available Chlorine

Reaction			pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
			Residual Available Chlorine (p.p.m.) 60 Minutes after Admixture					
Series	NH_3 Added (p.p.m.)	Cl/NH_3*						
A	0.0	....	24.2	23.4	23.2	22.0	24.2	24.2
B	0.5	50/1	21.3	19.8	19.4	18.6	20.4	21.1
C	2.0	12.5/1	10.2	10.8	7.3	7.7	10.9	11.7
D	6.0	4.2/1	22.1	21.8	21.1	19.6	22.6	23.2
E	18.0	1.4/1	23.4	23.4	23.0	21.6	22.9	23.1
			Decrease in Residual Av. Cl. (p.p.m.) Over Series Without Added Ammonia †					
B	0.5	50/1	2.9	3.6	3.8	3.4	3.8	3.1
C	2.0	12.5/1	14.0	12.6	15.9	14.3	13.3	12.5
D	6.0	4.2/1	2.1	1.6	2.1	2.4	1.6	1.0
E	18.0	1.4/1	0.8	0.0	0.2	0.4	1.3	1.1
			Decrease in Residual Av. Cl. (p.p.m.) per p.p.m. Ammonia Added †					
B	0.5	50/1	5.8	7.2	7.6	6.8	7.6	6.2
C	2.0	12.5/1	7.0	6.3	7.9	7.2	6.7	6.3
D	6.0	4.2/1	0.4	0.3	0.4	0.4	0.3	0.2
E	18.0	1.4/1	0.0	0.0	0.0	0.0	0.1	0.1

* Approximate ratio of available chlorine to ammonia on basis that 25 p.p.m. available chlorine had been added

† To nearest 0.1 p.p.m.

concentrations of ammonia (added as ammonium sulfate) and buffered at reactions pH 5 to pH 10. The losses in residual chlorine per p.p.m. ammonia added are also indicated. It will be noted that when the ratio of chlorine to ammonia was high (12.5/1 and 50/1) there was considerable loss of chlorine (approaching about 7.5 p.p.m. chlorine lost per p.p.m. ammonia added) and that this loss was greatest in solutions near the neutral point; but, as the relative concentration of ammonia increased (ratio of chlorine/ammonia = 4.2/1 or less) the loss of chlorine became negligible. In general, if the concentration of NH_3 is less than about one-eighth that of the available chlorine, NH_3 will be oxidized and the chlorine residual (which will be reduced in proportion to the quantity of ammonia present) will consist of hypochlorite. The effect is therefore merely to reduce the concentration of the active germicide but its nature is unaltered. If, however, the concentration of ammonia is more than one-quarter that of available chlorine, then chloramines will be formed and the effect is to change the nature of the germicide, whereas the concentration of available chlorine is only slightly altered. With a chlorine/ammonia ratio of 4.2/1 the available chlorine is presumed to exist as chloramine, the relative concentrations of the mono and dichloramine being a function of the reaction.

EFFECT OF REACTION ON GERMICIDAL EFFICIENCY OF CHLORINE, CHLORAMINE, AND CHLORAMINE WITH EXCESS AMMONIA

Observations were made at reactions of pH 5 to pH 10, employing the following solutions to which were added chlorinated water to yield approximately 25 p.p.m. available chlorine.

Series I. Buffered water (chlorine as hypochlorite)

Series II. Buffered water containing 6 p.p.m. NH_3 (chlorine as chloramine)

Series III. Buffered water containing 18 p.p.m. NH_3 (chloramine with excess NH_3)

The test organism was introduced into the disinfecting solution 15 minutes after addition of chlorine and the temperature was maintained at 20° C.

TABLE 3

Effect of Reaction (pH) on Germicidal Efficiency of Chlorine, Chloramine, Chloramine with Excess Ammonia

(*Bacillus metiens* spores; app. 25 p.p.m. av. cl.; 20° C.)

Reaction			pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Series	NH_3 Added (p.p.m.)	Cl/NH_3*	Killing Time (in minutes)					
I	0	....	2.1	2.3	3.0	7.6	58.0	570.0
II	6	4.2/1	168.0	85.0	89.0	83.0	182.0	186.0
III	18	1.4/1	99.0	59.0	84.0	107.0	263.0	456.0
Series	NH_3 Added (p.p.m.)	Cl/NH_3	Initial Residual Chlorine †					
I	0	....	24.2	23.4	23.0	22.0	24.2	24.2
II	6	4.2/1	22.6	21.8	23.6	22.0	23.0	23.0
III	18	1.4/1	23.3	23.1	23.4	22.8	22.6	23.0

* See Table 2

† At time of addition of test organism (15 minutes after addition of chlorine)

In Table 3 are shown the initial residual chlorine concentrations (at time of introduction of test organisms) and the killing times (time to kill 99 per cent of the exposed spores).

Comparing Series I (chlorine) with Series II (chloramine), several things are strikingly evident. For chlorine, germicidal efficiency was a direct function of reaction. The killing time varied slightly, from 2.1 minutes at pH 5, to 7.6 minutes at pH 8, thereafter increased very rapidly to 58 minutes at pH 9, and 570 minutes at pH 10.

In marked contrast to this, there was no significant difference in the killing times (83 and 89 minutes) for chloramine (Series II) in the range pH 6 to pH 8, whereas for solutions more acid (pH 5) and more alkaline (pH 9 and pH 10) the killing times (168 and 186 minutes) were approximately double those in the range pH 6 to 8.

The effect of presence of a large excess of ammonia on germicidal efficiency of chloramine may be noted by comparison of Series II and III. At pH 7 the killing times (89 and 84 minutes) were the same within limits of experimental error of determination. Chloramine in the presence of a large excess of ammonia (Series III) was more effective at pH 5 and pH 6 but distinctly less effective as a germicide at pH 9 and pH 10 than was chloramine solution in which there was not present any excess of ammonia.

The data for chlorine (Series I) and the chloramine (Series II and III) are shown graphically in Figure 3 where the logarithms of the killing times are plotted against the reactions (pH). The curves for chlorine and chloramine cross at pH 9.4. At reactions more acid than pH 9.4 chlorine is markedly more effective, whereas at more alkaline reactions chloramine is the more efficient. In this connection it might be pointed out that the increased germicidal effect produced by addition of an equivalent of ammonia to electrolyzed hypochlorite solution, first noted by Rideal (1910), was probably associated with the fact that he was working with sodium hypochlorite which was quite alkaline.

The greater germicidal efficiency of chloramine as compared with hypochlorite at reactions more alkaline than pH 9.4 may be particularly significant in conjunction with chlorination of softened water and water treated by the excess lime process, if reactions of about pH 10 are maintained.

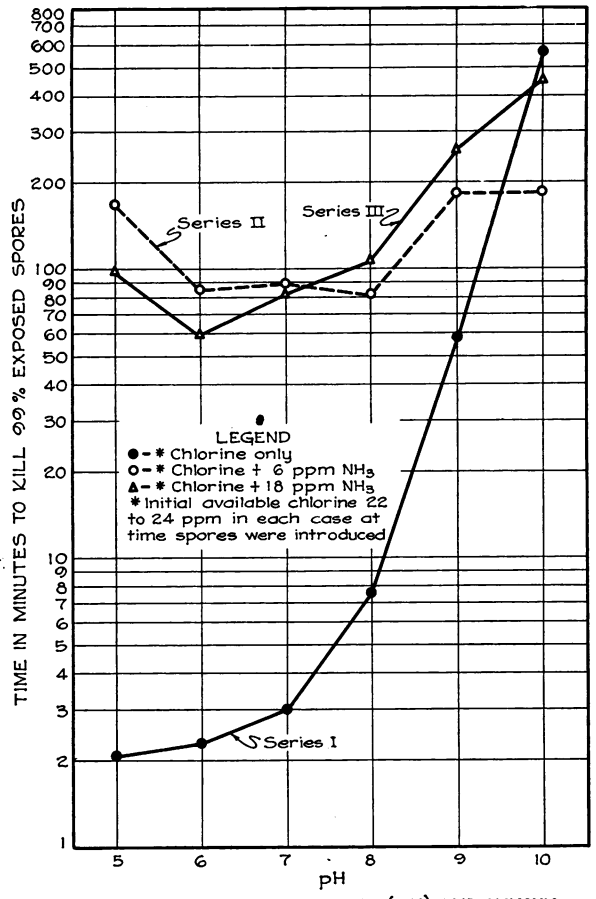


FIG. 3 EFFECT OF REACTION (pH) AND AMMONIA ON GERMICIDAL EFFICIENCY OF CHLORINE (20°C)

EFFECT OF CONCENTRATION ON GERMICIDAL EFFICIENCY OF CHLORINE AND CHLORAMINE AT VARIOUS REACTIONS

Killing times for each of four concentrations of chlorine and chloramine were determined for reactions pH 10, pH 7, and pH 5 at a temperature of 20° C. The data are shown in Table 4.

With chlorine, the results were very consistent. They indicated that by doubling the concentration, the time required to kill 99 per cent of the exposed spores was reduced by 45 to 50 per cent or, to put it conversely, as the concentration of available chlorine was cut in half the period of exposure (or killing time) was increased 1.8 to 2.0 fold. This seemed to hold true for each of the reactions under observation.

The results were more erratic with chloramine but indications were that the effect of varying concentration was somewhat less marked than was observed with chlorine and that a reduction of 35 to 40 per cent may be expected on doubling the concentration, i.e., the period of exposure would have to be increased 1.6 to 1.7 fold if the concentration of chloramine were reduced by 50 per cent.

TABLE 4

Relation of Concentration to Germicidal Efficiency of Chlorine and Chloramine

(*Bacillus metiens* spores; app. 25 p.p.m. av. cl.; 20° C.; pH 5, 7 and 10)

Chlorine				*Chloramine*			
Av. Cl.	*"KT" (Min.)* ‡	*Per cent Red.* "KT"*	*Relative† Increase "KT"*	*Av. Cl.*	*"KT" (Min.)* ‡	*Per cent Red.* "KT"*	*Relative† Increase "KT"*
	pH 10				*pH 10*		
24.2	570	..	...	23.0	186	..	...
47.0	291	49	2.0	41.5	95	49	2.0
94.3	170	41	1.7	84.6	60	37	1.6
188.0	98	43	1.8	140.0	51	15	1.2
Average		44	1.8	Average		33	1.6
	pH 7				*pH 7*		
6.3	11.7	..	...	11.3	110	..	...
12.4	5.4	54	2.2	23.6	89	19	1.2
23.0	3.0	45	1.8	44.7	41	53	2.2
48.6	1.4	53	2.1	86.4	27	34	1.5
Average		51	2.0	Average		35	1.6
	pH 5				*pH 5*		
6.3	8.5	..	...	11.4	251	..	...
12.5	3.8	55	2.2	22.6	168	33	1.5
24.2	2.1	45	1.8	44.4	77	53	2.1
49.3	1.2	43	1.8	87.0	47	39	1.6
Average		48	1.9	Average		42	1.7

* Per cent reduction in "KT" effected by approximately doubling concentration av. cl.
† The number of fold increase in "KT" if concentration of available chlorine is approximately halved
‡ "KT" = Killing time, i.e., period of exposure to effect destruction of 99 per cent exposed spores

TABLE 5

Relation of Temperature to Germicidal Efficiency of Chlorine and Chloramine

(*Bacillus metiens* spores; app. 25 p.p.m. av. cl.; pH 5, 7 and 10)

Chlorine					*Chloramine*				
Temp. ° C.	*Av. Cl. (p.p.m.)*	*"KT" Min.*‡	*Per cent Red. "KT"**	*Relative Increase "KT" (Q 10)*†	*Temp. ° C.*	*Av. Cl. (p.p.m.)*	*"KT" Min.*‡	*Per cent Red. "KT"**	*Relative Increase "KT" (Q 10)*†
	pH 10					*pH 10*			
20	24.2	570	..	...	20	23.1	186	..	...
30	23.3	240	58	2.4	30	21.7	46	75	4.2
40	23.2	100	58	2.4	40	22.3	11.2	76	4.2
50	22.8	46	54	2.1	50	20.7	3.3	71	3.4
Average			57	2.3	Average			74	3.9
	pH 7					*pH 7*			
0	24.5	12.9	..	...	20	23.6	89.0	..	...
10	24.2	6.2	52	2.1	30	22.1	29.5	67	3.0
20	23.0	3.0	51	2.1	40	21.3	8.6	71	3.4
30	24.1	1.4	53	2.1	50	19.3	2.9	66	3.0
Average			52	2.1	Average			68	3.1
	pH 5					*pH 5*			
0	24.8	10.0	..	...	20	22.6	168	..	...
10	24.5	4.3	57	2.3	30	21.5	38	77	4.3
20	24.2	2.1	51	2.1	40	20.6	11.3	70	3.4
30	24.1	1.2	43	1.8	50	20.7	3.8	66	3.0
Average			50	2.1	Average			71	3.6

* Per cent reduction in "KT" effected by a rise of 10° C.
† The number of fold increase in "KT" effected by a drop of 10° C.
‡ See Table 4.

EFFECT OF TEMPERATURE ON GERMICIDAL EFFICIENCY OF CHLORINE AND CHLORAMINE AT VARIOUS REACTIONS

Observations on the effect of temperature were made employing approximately 25 p.p.m. available chlorine at reactions pH 10, pH 7, and pH 5. Temperatures of 20°, 30°, 40°, 50° C. were employed for all observations with chloramine and for the chlorine solution at pH 10, but at the more acid reactions, temperatures of 0°, 10°, 20°, and 30° C. were employed for the chlorine series. The data are detailed in Table 5.

Generally speaking, the temperature coefficients seem to be relatively slightly influenced by reaction, but they are significantly different for the two compounds. Thus, with chlorine, a rise of 10° C. effected a reduction of 50 to 60 per cent in the killing time so that for a drop of 10° C. the period of exposure would have to be increased 2.1 to 2.3 fold. With chloramine, on the other hand, a rise of 10° C. is accompanied by a reduction of approximately 68 to 74 per cent in killing time; i.e., a drop of 10° C. entails a 3.1 to 3.8 fold increase in the period of exposure to effect the same bacterial reduction. The practical significance of the foregoing observations might be illustrated by the following example: If a contact period of 30 minutes at 20° C. is considered adequate for safety for a stipulated residual chlorine, the contact period would have to be raised to 60 to 70 minutes if the residual were chlorine (hypochlorite) or to 95 to 115 minutes for a chloramine residual, should the temperature of the water fall to 10° C. If the temperature of the water approached 0° C. then the period of contact for a stipulated chlorine (hypochlorite) residual would have to be increased to 130 to 160 minutes, whereas for a chloramine residual a contact period of 290 to 430 minutes would have to be employed to effect an equivalent germicidal action.

SUMMARY

A technic is described for determining the nature of survivor curves and the time required to effect a reduction of 99 per cent of *Bacillus metiens* spores exposed to chlorine and chloramine at various reactions and temperatures.

On the basis of previous findings and reports in the literature, results obtained with spores are considered applicable to those which might be anticipated for vegetative cells exposed to chlorination.

In disinfection with chlorine there was exhibited a marked lag followed by progressively increasing death rates; whereas with chloramine the rates of death were (except for a short lag) quite constant throughout the period of disinfection.

With concentrations of ammonia less than one-eighth that of available chlorine (hypochlorite) the chlorine residuals were reduced in proportion to the ammonia present but the nature of the germicide remained unchanged (hypochlorite). With concentrations of ammonia greater than one-fourth that of the available chlorine, the concentration of available chlorine was but slightly altered, but the nature of the germicide was changed from hypochlorite to chloramine.

Doubling the concentration of available chlorine reduced the killing time by approximately 50 per cent and 40 per cent for chlorine and chloramine, respectively.

A drop of 10° C. resulted in a two-fold increase in the period of exposure when employing chlorine and a 3 to 4 fold increase for chloramine, to effect equivalent germicidal action.

The germicidal efficiency of chloramine was not appreciably affected by the presence of a large excess of ammonia at pH 7, but at more acid reac-

tions its efficiency was greater, whereas at more alkaline reactions the killing power was markedly less than was observed for chloramine alone.

At reactions more acid than pH 9.4 chlorine (hypochlorite) was by far the more efficient germicide, but at more alkaline reactions chloramine was better than chlorine.

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Prescriptions for Heavy Cream to be Approved by Health Officers

The War Food Administration has announced the adoption of measures to prevent the abuse of certain provisions of War Food Order 13 with regard to the prescription of heavy cream for use in the treatment of the sick. On June 2, an amendment to the Order was issued which requires that prescriptions for heavy cream be approved " by the public health officer, or the secretary of the county medical society, of the municipality or county " where the patient or hospital desiring the cream is situated.