1930

Effect of salts on alkali disinfection

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EFFECT OF SALTS ON ALKALI [illegible]

BY

Omar Edwin Lowman

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Food Chemistry

Approved

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor J. H. Buchanan and Dr. Max Levine, both for the suggestion of the problem and for their most helpful advice and criticism throughout the progress of the work.

EFFECT OF SALTS ON ALKALI DISINFECTION

INTRODUCTION

The mechanical washing of bottles is a universally adopted practice in the beverage, dairy, and other industries. The modern bottle-washing machine combines the processes of cleansing and sterilizing in a single operation. Nearly all of the commercial washing compounds used in these machines as detergents and germicides consist of sodium hydroxide as a base, to which has been added some other mild alkali such as sodium carbonate or trisodium phosphate.

There is considerable disagreement concerning the theory of alkali disinfection, although it has been rather generally accepted that the concentration of free hydroxyl ions in solution is the important factor. However, many investigators have pointed out that other factors have an important bearing on the germicidal action of the alkali.

A few years ago, the chemical and bacteriological laboratories at Iowa State College became interested in the germicidal properties of commercial washing compounds and the data secured from various experiments seemed to indicate that the undissociated molecule of the alkali was an important factor in alkali disinfection. The present investigation was undertaken to determine the effect of the addition of various sodium halides on the germicidal efficiency of sodium hydroxide. Also, it was hoped, from the data obtained and from other

available data, that some information might be secured which would assist in determining the primary factor or factors in alkali disinfection.

HISTORICAL

Until several years ago, there was a great dearth of information concerning the relative efficiency of these agents as detergents or germicides. Probably the first work done on the germicidal efficiency of alkalies was by Paul and Krönig in 1896 (1). They worked with ammonium, lithium, sodium, and potassium hydroxides. They found the last three mentioned to be powerful germicides while ammonium hydroxide had practically no germicidal value. They found potassium hydroxide most effective with sodium and lithium hydroxides following closely in the order mentioned. From their data, they concluded that the disinfecting efficiency of these hydroxides was proportional to their degree of electrolytic dissociation and that the concentration of the hydroxyl ions was responsible for the disinfecting action.

In 1921, Weiss (2), working on the thermal resistance of Clostridium botulinum, found that the death rate increased with the concentration of the hydroxyl ion. Also, he found that as the hydrogen ion increased the thermal death rate increased.

In 1926, the chemical and bacteriological laboratories of Iowa State College, due to their connection with the beverage industry, became interested in the relative germicidal efficiency of sodium hydroxide at different concentrations and temperatures.

Levine, Buchanan, and Lease (3) began a series of experiments to secure data and the results have been reported showing that sodium hydroxide was a more effective germicide in the higher concentrations at the same temperatures. They found that the velocity coefficients of the rate of death were not constant but increased with the time of exposure.

Another series of experiments with sodium hydroxide, sodium carbonate, and trisodium phosphate of the same H-ion concentration but of different molar concentrations as determined by titrations, demonstrated that the killing times varied greatly with the different alkalies (4). However, for each of the alkalies considered individually, the germicidal efficiency was a direct function of the H-ion concentration. Also, by another series of experiments, it was shown that at a constant temperature and H-ion concentration, sodium hydroxide and sodium hydroxide-carbonate mixtures were not equally efficient germicides (5). The addition sodium carbonate to sodium hydroxide had little influence on the H-ion concentration but increased greatly the germicidal efficiency.

Sherman (6), as well as Mudge and Lawler (7), has reported that the germicidal efficiency of alkali solutions is directly correlated with the alkalinity of the solution as measured by the pH. They note that the lethal effect of a solution of lower pH may be made equal to one of higher pH by raising the temperature.

Myers (8) observed that different buffer mixtures of approx-

imately the same pH value exert very different germicidal effects on Bacterium coli. An increase in pH on the alkaline side of neutrality increased the lethal power of a given solution at a given temperature. In another work on washing powders (9), he found that the pH was the controlling factor in the germicidal power but that buffer index and osmotic pressure were other factors as well.

The addition of neutral salts and mild alkalies has been shown to increase the germicidal efficiency of sodium hydroxide (10) (11). Equal amounts of sodium chloride and sodium carbonate increase the efficiency of the hydroxide approximately the same, whereas trisodium phosphate was less effective. When the concentrations of salts added to the hydroxide were increased, the killing time was decreased but at a decreasing rate.

A resume of the literature shows that it has been generally accepted that the concentration of free hydroxyl ions is largely responsible for the disinfecting action of alkalies. Some investigators have suggested that other factors, such as undissociated molecules and the physical forces of surface tension, adsorption, swelling, and osmotic pressure, must be considered (12).

It is the belief of the investigators in these laboratories (5) (10) (11) that the undissociated sodium hydroxide as well as the hydroxyl-ion concentration may be the controlling factor in alkali disinfection. The present investigation was undertaken to present a series of data from which an adequate theory of the chemistry of alkali disinfection might be offered.

THE TEST ORGANISM AND TECHNIQUE EMPLOYED

Characteristics of Test Organism.

In this work it was thought best to use a spore form of the organism since its resistivity to disinfection would more nearly approach the type likely to be found in dirty milk bottles, beverage bottles, and other containers cleansed and disinfected by the use of sodium hydroxide. The organism used was originally isolated from a sample of spoiled ginger ale and is known as No. 25. It has been described as follows: "It was a gram positive rod (about 1.0 u by 2.0 to 4.0 u), facultative, motile, with central spores equal to or slightly less than the diameter of the cells. The vegative cells occurred singly, in pairs, and occasionally in short chains. Gelatin was liquefied, milk slowly curdled (rennet), and peptonized, nitrates were reduced to nitrites not gas, and indol was formed. Acid was formed from glucose but not from lactose nor sucrose and starch was hydrolyzed. Colonies on agar were strikingly similar to those of B. subtilis (5)."

This organism was well adapted to plate cultures since it grew well on nutrient agar, forming distinct colonies in about 2 days with little tendency to confluence. Solutions just alkaline to methyl orange did not inhibit its growth in any manner.

Method of Preparing Test Organism.

The original test organisms were prepared from a 24 hour broth culture. This was smeared over the surface of nutrient

agar (Difco) in 9 Holle flasks and allowed to incubate at 27°C. for 19 days. At this time practically all the organisms had passed into the spore stage. The surface growth was scraped off into a sterile dish and dried over sulfuric acid in a partial vacuum. After drying for several days, the mass was ground very thoroughly in a sterile agate mortar and very thoroughly mixed with sterile powdered cane sugar. The mixture was placed in a sterile weighing bottle and kept in a desiccator over sulfuric acid. The number of viable organism per unit weight was ascertained from time to time by removing small portions from the bottle. The original organisms were prepared in 1926 and these were still quite viable in 1928 when the supply became exhausted. At that time a new supply was prepared according to the method described above.

Technique Employed in Disinfection Tests.

Approximately 0.02 gram of the spore-sugar mixture was placed in 10 cc. of sterile tap water. After thorough shaking the suspension was filtered through a sterile filter of fine grade filter paper to remove clumps. The filtrate constituted the bacterial suspension used in the disinfection tests.

The test alkali solution (100 cc.) was placed in a 200 cc. round-bottomed Woulff flask provided with three necks. A glass stirrer was fitted through a stopper in the middle neck. The other two openings were stoppered with cotton and the whole sterilized in an autoclave at 15 pounds for 15 minutes. After cooling, the flask containing the test solution was placed in a

DeKhotinsky water bath which had been brought to the proper temperature and the stirrer set in motion. The stirrer was run at such a speed so that no bubbles or foaming developed.

When the test solution had attained and remained at the desired temperature (in about 10 minutes), 1.0 cc. of the bacterial suspension was introduced under the surface of the test alkali by means of a carefully calibrated capillary pipette. At desired time intervals, 5 cc. portions were removed and introduced into 150 cc. sterile Erlenmeyer flasks containing 45 cc. of sulfuric acid (with methyl orange indicator) of sufficient strength to just neutralize the 5.0 cc. of alkali added. In this manner, the effects of alkali and temperature were simultaneously stopped. 1.0 cc. portions were plated on nutrient agar (Difco) and the number of surviving bacteria determined. All bacterial counts were calculated on the basis of 5.0 cc. of disinfecting mixture. It was aimed to employ a suspension giving an initial count of about 1,000,000 per unit volume (5.0 cc.). In several experiments, the above procedure was varied by subjecting the test-organisms to a preliminary soaking of one hour in various halide solutions before disinfection.

The sodium hydroxide used for test solutions in these experiments was prepared by making a saturated solution from C.P. stick sodium hydroxide. After standing for some time, the saturated solution was decanted and filtered through glass wool to remove any carbonate which had accumulated. This was made up to 1 normal by titration against a standard acid (phenol-

phthalein indicator) and kept in stoppered pyrex flasks as a stock solution. The solutions used in the germicidal tests were made up as needed from this solution.

In the tests where sodium halides were used, the dry C.P. salt was weighed and added to the sodium hydroxide test solution before sterilization.

The killing time was taken to be the time in minutes required to reduce the number of viable bacterial cells 99.9 percent. Further reductions would be subject to considerable error due to the small number of colonies developing per plate.

All plates were incubated for 48 hours at 27°C.

EXPERIMENTAL

I. Germicidal Effect of Distilled Water and Certain Halides on Bacteria (No. 25) at 60°C.

In view of the work previously done in these laboratories and the work about to be attempted, it was thought that it would be of interest to know what the germicidal effect on the test organisms of distilled water and of certain sodium halides at 60°C. would be. Accordingly, 0.342 M solutions were selected. Previous work had been done using 2 percent (0.342 M) NaCl and in order for the work to be comparative, the same molar concentrations were employed. Sodium chloride and sodium bromide test solutions were not considered necessary because of some previous work on sodium chloride (11) and from previous experiments it was certain that sodium iodide would be at least as effective as either of the above. Sodium fluoride was used because it

was found that a 0.342 M solution gave an alkalinity of 0.04 N as determined by titration with a standard acid (phenolphthalein indicator) although the pH of this solution was only 6.7. When sodium iodide was used, it was necessary to make the test solution just alkaline by adding a few drops of sodium hydroxide to prevent the liberation of free iodine.

Using the technique described above, the bacterial spores were inoculated in test solutions of distilled water (control), sodium iodide, and sodium fluoride, respectively, at 60°C. Portions were withdrawn at different time intervals up to 50 minutes. The germicidal effects of these tests are given in Table I and the logarithm percent of survivors is plotted against time in minutes in Fig. 1. According to the technique employed, each colony developed on the plates to which had been added 1.0 cc. of the neutralized test solution from the Erlenmeyer flasks represented 50 bacteria per unit volume (5.0 cc.). When the number of counts per plate become less than 15 or 20, the results are subject to considerable error and counts of 700 to 1000 per unit volume are unreliable. Therefore, with an initial counts of about 1,000,000 reductions of 99.9% can be readily and reliably determined.

Table I.

Germicidal Effect of Distilled Water, 0.342 M NaI, and 0.342 M NaF at 60°C.

Time in Minutes	Surviving Bacteria in 5.0 cc.			Percent Surviving Bacteria			Log. percent Survivors		
	Distilled Water	NaI	NaF	Distilled Water	NaI	NaF	Distilled Water	NaI	NaF
0	660,000	660,000	745,000	100.0	100.0	100.0	2.000	2.000	2.000
10	460,000	455,000	439,000	69.7	69.0	59.0	1.84323	1.83885	1.77085
15	390,000	320,000	419,000	59.1	48.5	56.2	1.77159	1.68574	1.74974
20	615,000	430,000	407,000	93.3	65.1	54.7	1.96988	1.81358	1.73799
25	605,000	-	400,000	91.7	-	53.7	1.96237	-	1.72997
30	500,000	315,000	242,000	75.9	47.7	32.5	1.88024	1.67897	1.51188
35	435,000	355,000	271,000	65.9	53.8	36.4	1.81889	1.73078	1.56110
40	-	300,000	235,000	-	45.0	31.6	-	1.65369	1.49969
45	475,000	385,000	111,000	72.0	58.4	14.9	1.85733	1.76641	1.17319
50	920,000	225,000	219,500	113.94	34.1	29.4	2.05652	1.53275	1.46835

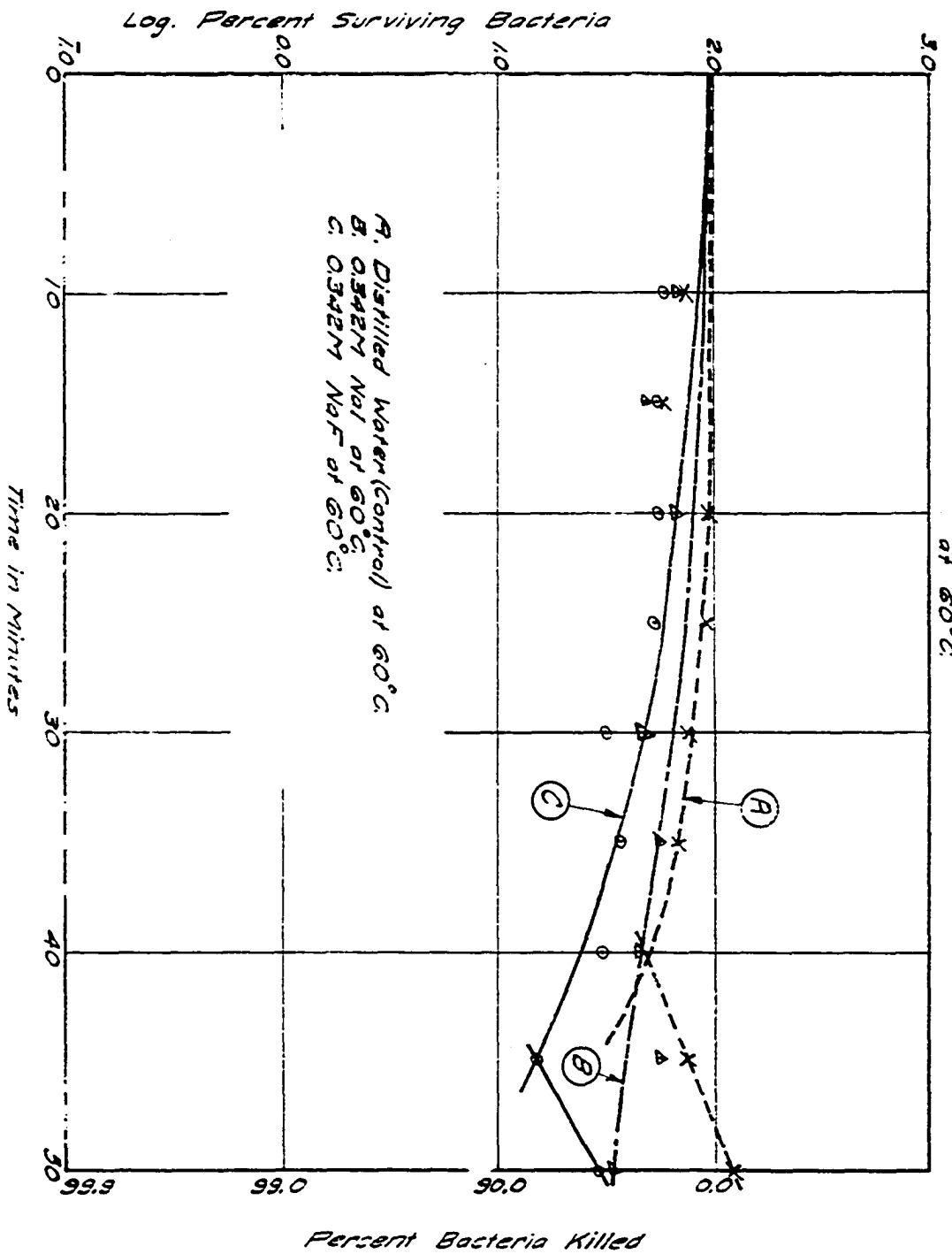

Fig. 1. Germicidal Efficiency of Distilled Water and 0.342N Sodium Halides at 60°C.

Discussion of Data

Examination of Curves A, B, and C in Fig. 1 show that there is lowering of the bacterial count in all the test solutions during the first 20 minutes of the test periods. This would indicate that the weaker bacteria were unable to withstand the effects of temperature and the halides of the test solutions. It should be noted that between 42 and 45 minutes there is a reduction of about 31, 40, and 74 percents, respectively, for the distilled water, sodium iodide, and sodium fluoride test solutions at this temperature. After this time, the counts begin to increase, indicating that the bacteria were passing into a different life phase of growth (13) or it may be that minute clumps have broken up. With distilled water (Curve A) at 50 minutes, the count runs considerably above the initial count. In the case of sodium fluoride (Curve C) attention is called to the fact, that the counts run consistently lower. This is probably caused by the greater alkalinity of this solution due to the hydrolysis effect of this salt.

Myers (9) found that a 0.5 M sodium chloride solution at 60°C. reduced a spore culture of No. 25 99.9 percent in 5500 minutes.

The data gained from these tests offer adequate proof that the greatly increased germicidal action presented later, when a halide-hydroxide mixture is used, is not to any great extent alone the result of either temperature or the sodium halides.

II. Effect of Addition of Sodium Halides on the Germicidal Efficiency of 0.25 M Sodium Hydroxide at 60°C.

From some previous work done in our laboratories, it was known that the addition of a neutral salt (sodium chloride, 2 percent or 0.342 M) to a 0.25 M sodium hydroxide test solution greatly increased its germicidal efficiency at 60°C. (10) (11). It was thought that it would be of interest to present data to show the comparative effects of the other sodium halides (sodium bromide, sodium iodide, and sodium fluoride) with that of the chloride-hydroxide mixture at the same concentration and temperature. Accordingly, the dry C.P. salts, equivalent to 0.342 M, were weighed and added, respectively, to each of the 100 cc. portions of 0.25 M sodium hydroxide test solutions in the disinfection flasks. The whole was then sterilized, placed in the constant temperature bath, and tests run according to the technique described. A control of 0.25 M alkali was run with each individual test. Duplicate tests were run in each instance.

In the sodium iodide-hydroxide experiments it was found necessary to vary the technique slightly. When the 5 cc. portions of bacterial suspension withdrawn from the disinfecting flasks were made just neutral or slightly alkaline to methyl orange, the killing time was reduced to about 8 minutes and free iodine was present (starch test). This was undoubtedly due to the formation hydroiodic acid which on oxidation by

air, liberated free iodine. However, when these portions were made quite alkaline to methyl orange but just acid to phenolphthalein, no free iodine was liberated even after standing several days.

In Table II, Series A, B, C, and D, the data from this series of experiments are shown. In Figs. 2, 3, 4, and 5, the logarithm percents of survivors are plotted as ordinates with the time intervals as abscissas.

Table II

Showing Effect of Added Salts on Surviving Bacteria (No. 25) in 0.25 M. Sodium Hydroxide at 60°C.

Series A.

Material	0.25 M. NaOH					
Dates	8-20-27	2-11-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	935,000	60,000	100.00	100.00	2.000	2.000
5	-	-	-	-	-	-
10	586,000	26,900	62.9	44.80	1.799	1.651
15	510,000	12,400	54.7	20.70	1.734	1.316
20	318,000	5,250	33.9	8.75	1.531	0.942
25	123,000	1,400	13.2	2.33	1.122	0.367
30	57,500	650	6.16	1.08	0.791	0.033
35	14,200	250	1.52	0.42	0.183	1.623
40	2,400	-	.257	-	1.411	-
45	750	-	.081	-	2.909	-

Material	0.25 M. NaOH + 0.342 M. NaCl					
Dates	8-20-27	2-11-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	933,000	60,000	100.00	100.00	2.000	2.000
5	-	40,000	-	66.60	-	1.823
10	210,000	10,300	22.50	17.20	1.353	1.236
15	71,000	2,000	7.61	3.34	0.882	0.524
20	7,850	270	0.842	0.45	1.926	1.653
25	275	-	0.030	-	2.478	-
30	-	-	-	-	-	-

Table II (ontinued)

eries B.

Material	0.25 M NaOH					
Dates	4-7-28:	4-14-28:				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	720,000	1,050,000	100.00	100.00	2.000	2.000
5	-	-	-	-	-	-
10	194,500	268,000	26.98	25.5	1.431	1.406
15	170,000	178,500	23.60	17.0	1.373	1.230
20	115,500	162,500	16.05	15.47	1.205	1.189
25	82,500	80,500	11.45	7.66	1.059	0.884
30	50,500	57,100	7.02	5.45	0.846	0.736
35	20,300	11,500	2.82	1.095	0.450	0.039
40	6,400	2,800	0.89	0.267	$\bar{1}$.944	$\bar{1}$.426
45	825	250	0.114	0.024	$\bar{1}$.057	$\bar{2}$.376

Material	0.25 M NaOH + 0.542 M NaBr					
Dates	4-7-28	4-14-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	720,000	1,050,000	100.00	100.00	2.000	2.000
5	187,500	214,000	26.10	20.40	1.417	1.309
10	66,500	32,500	9.24	3.095	0.966	0.491
15	43,850	21,700	6.09	2.065	0.785	0.315
20	6,000	4,600	0.855	0.438	$\bar{1}$.922	$\bar{1}$.641
25	400	550	0.056	0.053	$\bar{2}$.745	2.724
30	-	-	-	-	-	-

Table II (Continued)

Series C.

Material	0.25 M. NaOH					
Dates	6-13-28	6-16-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	4,000,000	390,000	100.00	100.00	2.000	2.000
2	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
8	-	-	-	-	-	-
10	1,152,000	123,000	28.35	31.60	1.453	1.499
12	-	-	-	-	-	-
14	-	-	-	-	-	-
15	1,071,500	77,500	26.90	19.90	1.431	1.299
16	-	-	-	-	-	-
18	-	-	-	-	-	-
20	726,000	37,000	18.15	9.50	1.259	0.978
25	197,000	16,400	4.93	4.20	0.693	0.623
30	-	5,600	-	1.44	-	0.158
35	82,950	1,150	2.07	0.295	0.316	1.469
40	20,200	-	0.505	-	$\bar{1}$.703	-
45	2,650	-	0.066	-	$\bar{2}$.823	-

Material	0.25 M. NaOH + 0.542 M. NaI					
Dates	6-13-28	6-16-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	4,000,000	390,000	100.00	100.00	2.000	2.000
2	674,500	-	16.85	-	1.227	-
4	592,500	-	14.80	-	1.170	-
5	-	58,000	-	14.90	-	1.173
8	187,750	-	4.70	-	0.672	-
10	224,500	4,500	5.60	1.15	0.748	0.061
12	-	-	-	-	-	-
14	37,000	-	0.925	-	$\bar{1}$.971	-
15	-	800	-	0.205	-	$\bar{1}$.312
16	15,600	-	0.390	-	$\bar{1}$.561	-
18	4,450	-	0.114	-	1.057	-
20	-	-	-	-	-	-

Table II (Continued)

Series D.

Material	0.25 M. NaOH					
Dates	7-10-28	7-11-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	780,000	280,500	100.00	100.00	2.000	2.000
5	-	-	-	-	-	-
6	-	-	-	-	-	-
8	-	-	-	-	-	-
10	317,500	163,500	40.60	58.20	1.609	1.765
12	-	-	-	-	-	-
14	-	-	-	-	-	-
15	244,500	-	31.35	-	1.496	-
16	-	-	-	-	-	-
20	158,500	73,000	20.30	25.90	1.308	1.413
25	78,500	55,250	10.05	19.65	1.002	1.293
30	24,750	28,100	3.17	10.00	0.501	1.000
35	4,500	12,550	0.58	4.47	$\bar{1}$.763	0.650
40	900	2,400	0.115	0.855	$\bar{1}$.061	$\bar{1}$.932
45	-	500	-	0.178	-	$\bar{1}$.250

Material	0.25 M. NaOH + 0.342 M. NaF					
Dates	7-10-28	7-11-28				
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Log. percent Survivors	
0	780,000	280,500	100.00	100.00	2.000	2.000
5	184,000	-	23.60	-	1.373	-
6	-	78,000	-	27.80	-	1.444
8	-	47,000	-	16.70	-	1.223
10	73,000	31,250	9.35	11.10	0.971	1.045
12	-	13,000	-	4.63	-	0.666
14	-	7,150	-	2.54	-	0.405
15	17,450	-	2.24	-	0.350	-
16	-	4,450	-	1.58	-	0.199
20	700	800	0.09	0.285	$\bar{2}$.954	$\bar{1}$.455
25	-	-	-	-	-	-

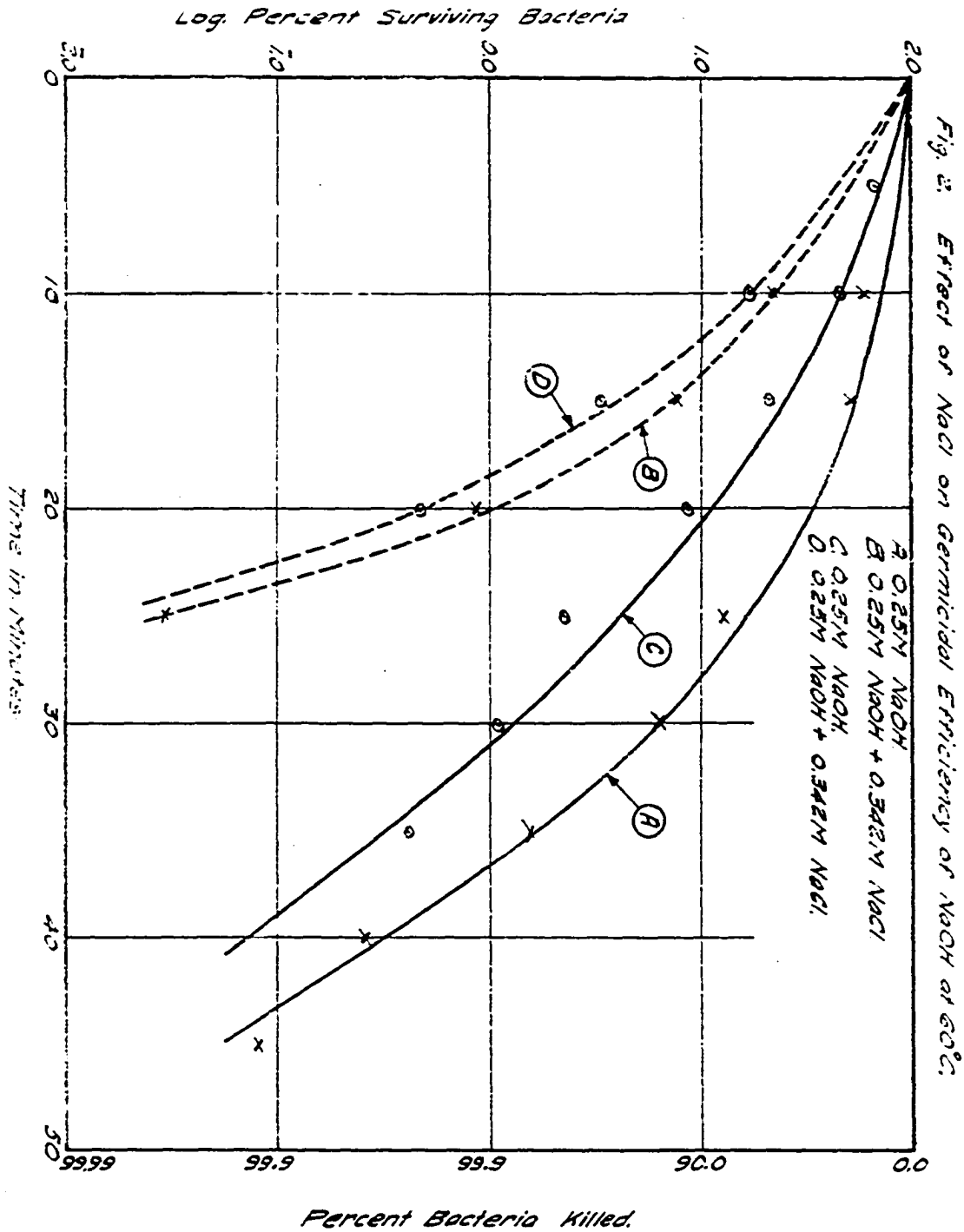

Fig. 2. Effect of NaCl on Germicidal Efficiency of NaOH at 60°C.

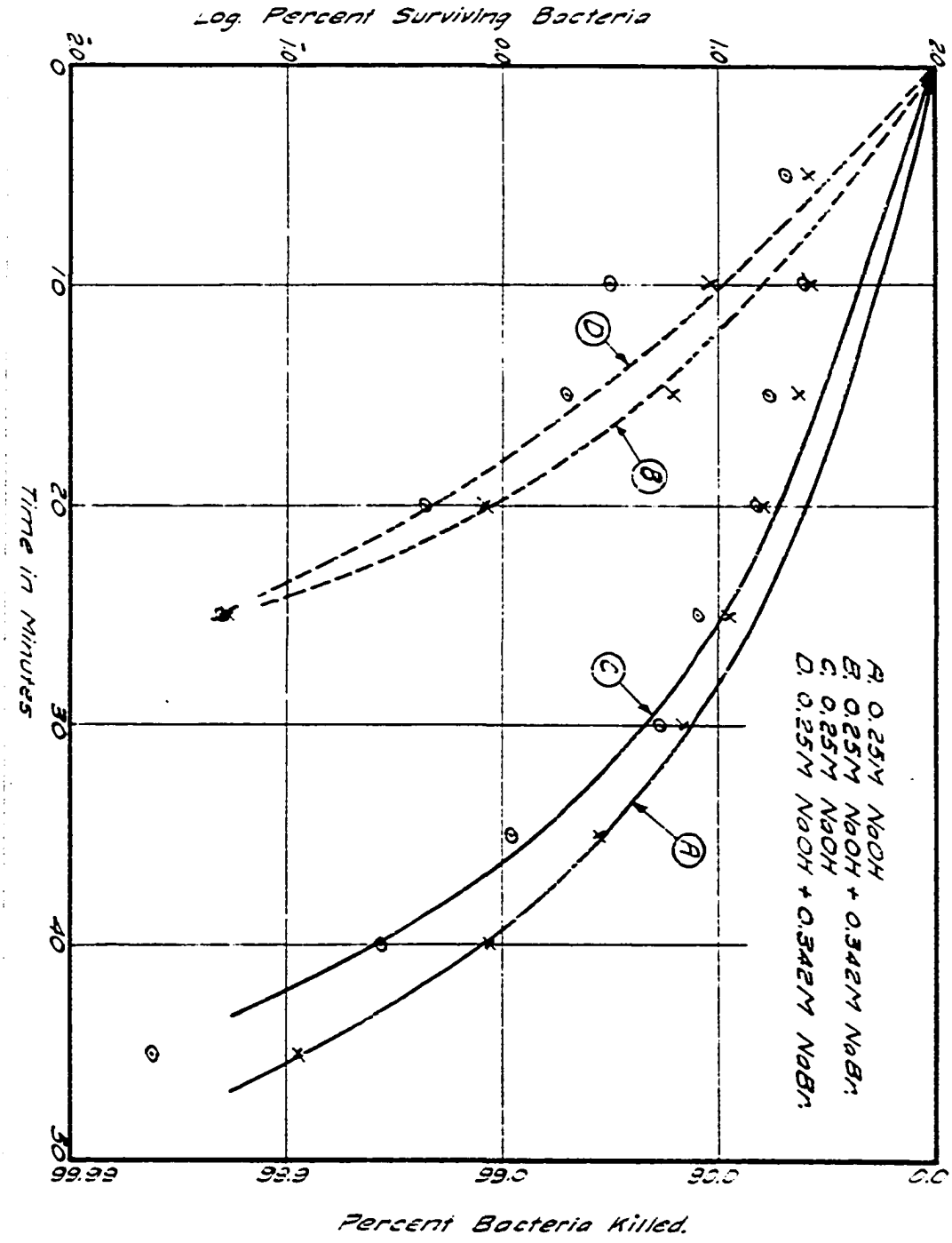

Fig. 3. Effect of NaBr on Germicidal Efficiency of NaOH at 60°

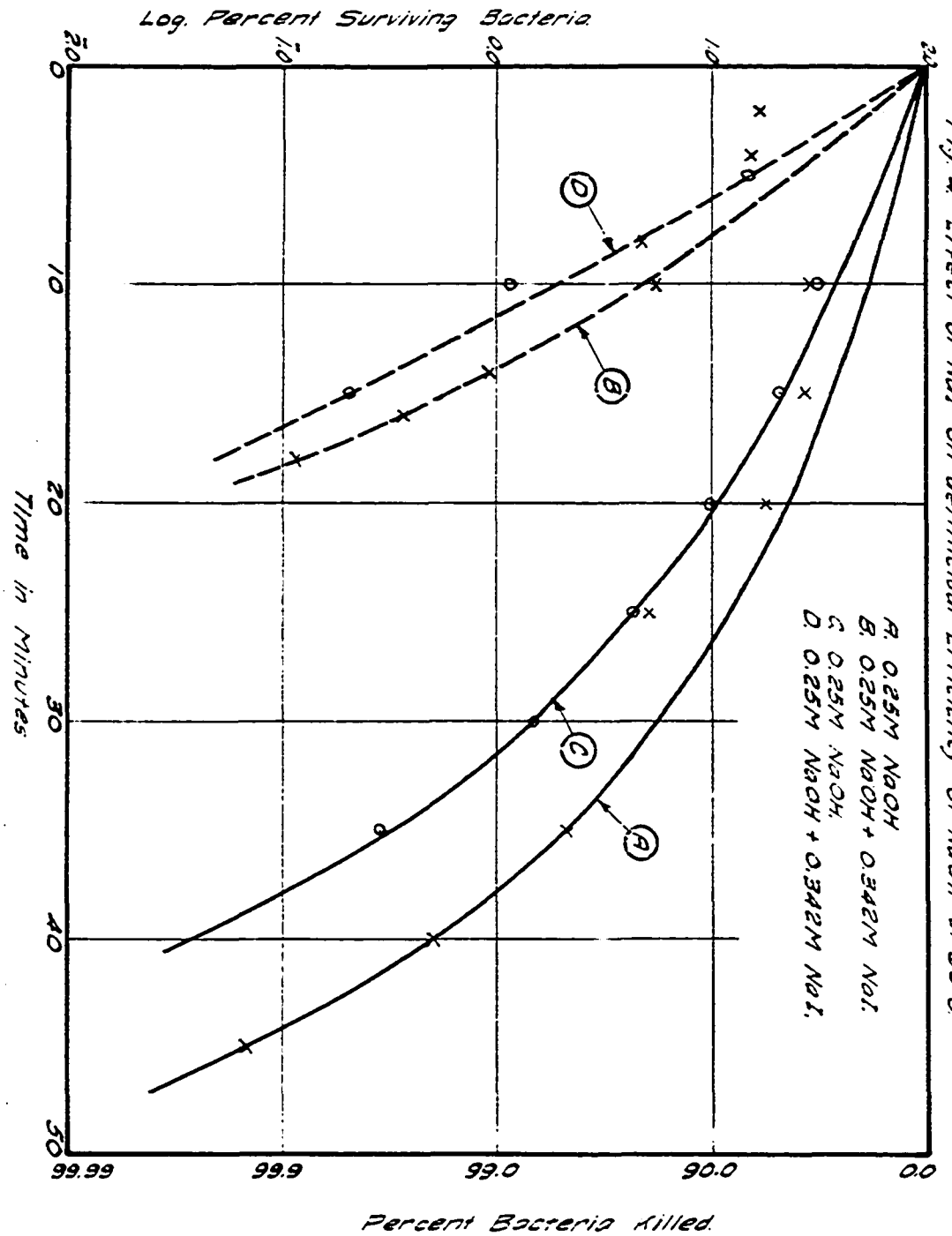

Fig. 4. Effect of NaI on Germicidal Efficiency of NaOH at 60°C.

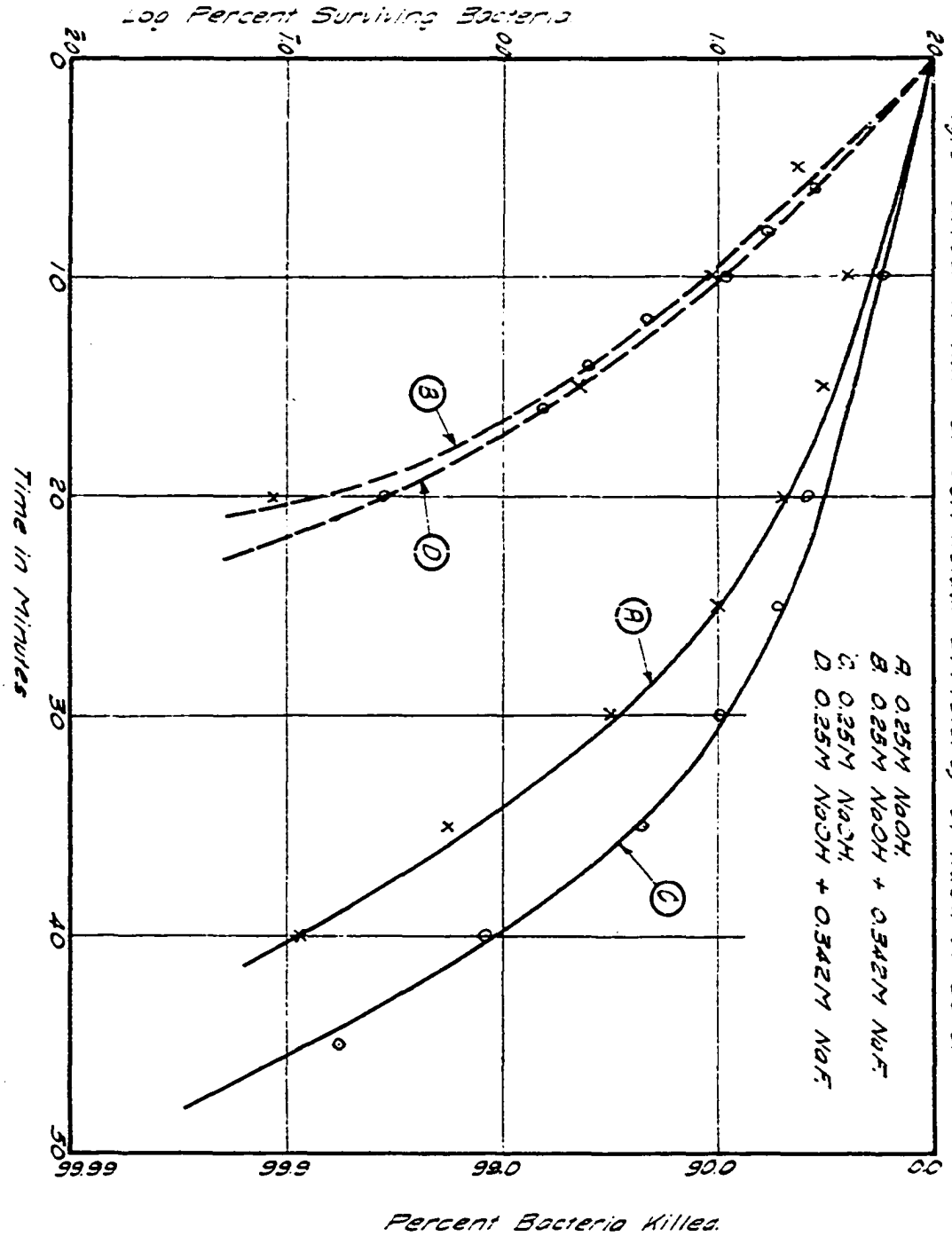

Fig. 5. Effect of NaF on Germicidal Efficiency of NaOH at 60°C.

Discussion of Data

Examination of the curves plotted from the data compiled in Table II, Series A, B, C, and D, is interesting. In the case of sodium chloride-hydroxide mixture (Fig. 2), the two controls (Curves A and C) of 0.25 M sodium hydroxide reduced the bacterial count 99.9% in 43 and 39 minutes, respectively. The first time is considered as more representative since it agrees more nearly with other killing times of the same concentration and, also, since the initial count of the second run (Curve C) was quite low (60,000). However, the differences of time are within 10% of each other which may be considered as within the limit of bacteriological error. The addition 0.342 M sodium chloride to the alkali test solutions reduced the killing times to 23.5 and 22.5 minutes, respectively (Curves B and D). Taking 23 minutes as the average killing time, the time is 53.5% of the time required by the control alkali test solution or a reduction of 46.5%. The pH of this chloride-hydroxide test solution was 12.88 at 25°C. while the pH of the alkali control test solution was 12.86 at the same temperature.

The effect of sodium bromide on the alkali test solution is shown in Fig. 3. The alkali controls (Curves C and A) show killing times of 42 and 45.5 minutes, respectively, or an average of 43.75 minutes. The mixtures (Curves D and B) killed in 23.5 and 24 minutes, or an average time of 23.75 minutes.

In this case, the average time for the mixtures is 14.3% that of the average control time or a reduction of 85.7%. This compares well with the chloride-hydroxide test solution. The pH of this bromide-hydroxide test solution was 12.84 at 25°C.

The alkali controls in the case of the iodide-hydroxide mixture (Fig. 4, Curves C and A) show considerable variation in killing times, 38.75 and 44 minutes, respectively. In the first instance, greater sterilization was employed (20 pounds for 20 minutes). This was necessitated by the fact that a new bottle of nutrient agar was employed which showed considerable contamination and hence the greater sterilization. It is believed that the higher sterilization concentrated the test solution somewhat as will be shown later and hence lowered the killing time abnormally. However, an average of 41.4 minutes agrees well with the other average control times. The iodide-hydroxide test solutions (Curves D and B) show killing times of 16.5 and 18.25 minutes, respectively, or an average of 17.4 minutes. This lowered time is 42.0% that of the average alkali control time or a reduction of 58%. The pH value of the iodide-hydroxide mixture was 12.83 at 25°C. In the second and all future experiments the agar was sterilized separately at the higher temperatures while the test solutions were subjected to the usual 15 pounds for 15 minutes.

It should be recalled at this time that the iodide-hydroxide disinfection tests necessitated a slight variation in the technique but this in no manner accounts for the greatly reduced

killing time as compared to the other halide mixtures. An explanation will be offered later when the theory of alkali disinfection is discussed.

When the fluoride-hydroxide test solutions were used (Fig. 5), the alkali controls (Curves A and C) reduced the bacteria 99.9 percent in 40.25 and 45.25 minutes, respectively, or an average of 43.25 minutes. The fluoride-hydroxide mixtures (Curves B and D) gave killing times of 20.25 and 21.75 minutes, respectively, or an average of 21 minutes. This is 48.6% of the average time required by the controls or a reduction of 51.4%. It is to be noted that in this case, the fluoride-hydroxide mixture became more alkaline, the pH value being 12.92 as compared with 12.86 of the alkali control solution. By actual titration against a standard acid (phenolphthalein indicator), the mixture was 0.29 M while the alkali control was 0.25 M. This increased alkalinity, as will be shown later, undoubtedly accounts for the slightly less killing time compared to the chloride and bromide-hydroxide test solutions.

III. Effect of Preliminary Soaking on Bacterial Spores (No. 25) in Various Sodium Halide Solutions Before Disinfection Tests.

It is common practice to soften and remove dried albuminous material from pipettes by soaking in a saturated sodium chloride solution. Also, it has been observed by one investigator (14) that sodium chloride decreases the solubility of protein and at the same time increases the lowering of the surface tension. It was thought it would be valuable information to know the effect of a preliminary soaking of the bacterial spores in different halide solutions before disinfection. Accordingly, a series of experiments were arranged whereby the spore suspension was soaked for one hour in a 0.342 M concentration of the sodium halide to be used before inoculation into the 0.25 M sodium hydroxide test solution. In the case of sodium iodide, it was necessary to add a few drops of alkali to the preliminary soaking solution to prevent the liberation of free iodine. At the same time a control of 0.25 M sodium hydroxide was inoculated with a bacterial spore suspension which had not been subjected to a preliminary halide soaking. In order to make the salt effect comparable in the two tests, 1 cc. of the 0.342 M halide solution used was added to the control alkali test solutions, respectively, since when 1 cc. of the soaked bacterial suspension was inoculated into the alkali test solutions, approximately 1 cc. of the salt solution was added with it.

Experiments showing the effect of a preliminary soaking in sodium fluoride were not made. It was thought that little information would be gained since the hydrolysis effect of the salt showed an alkalinity of 0.05 N by titration with a standard acid (methyl orange indicator).

The data obtained from these experiments is compiled in Table III, Series A, B, and C. In this table, an average of the percent surviving bacteria is compiled for the duplicate runs in each series and the logarithm of average percent survivors is plotted against time in Curves A and B, Figs. 6, 7, 8, respectively.

Table XII.

Effect of Preliminary Soaking for One Hour in Halide Solutions on Bacterial Spores (No. 25) in 0.25 N Sodium Hydroxide at 60°C.

Series .

Preliminary Treatment in NaCl Solution

Material	0.25 N NaOH + 1 cc. [illegible] Halide Solution					
Date	8-20-27	8-11-28				
Preliminary Treatment	Not Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. per-cent Survivors	Log. av. percent Survivors
0	988,000	60,000	100.00	100.00	100.00	2.000
10	491,000	46,250	52.70	77.00	66.35	1.822
15	430,000	13,500	46.60	22.50	34.40	1.537
20	258,000	4,000	27.65	6.67	17.16	1.235
25	126,000	-	13.50	-	13.50	1.130
30	47,700	475	5.12	0.79	2.96	0.471
35	12,450	-	1.36	-	1.36	0.134
40	3,700	-	0.396	-	0.396	1.598
45	-	-	-	-	-	-

Material	0.25 N NaOH					
Date	8-20-27	8-11-28				
Preliminary Treatment	Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. per-cent Survivors	Log. av. percent Survivors
0	1,440,000	54,000	100.00	100.00	100.00	2.000
10	1,115,000	29,000	77.10	53.75	65.43	1.816
15	780,500	13,100	54.80	24.20	39.50	1.597
20	540,500	8,600	37.60	15.90	26.75	1.427
25	320,500	2,150	22.15	3.90	13.03	1.115
30	75,500	825	5.25	1.50	3.78	0.577
35	18,300	100	1.27	0.185	0.727	1.862
40 45	3,500	=	0.24	=	0.24	1.380

Table III (Continued)

Series D.

Preliminary Treatment in NaBr Solution

Material	0.25 M NaOH + 1 cc. 0.342 M NaBr Solution					
Date	4-14-28	4-7-28				
Preliminary Treatment	Not Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. percent Survivors	Log. av. percent Survivors
0	1,050,000	720,000	100.00	100.00	100.00	2.000
10	621,500	246,000	59.20	34.20	46.70	1.669
15	446,000	216,000	42.50	30.00	36.25	1.559
20	331,500	114,500	31.50	15.90	23.70	1.375
25	245,500	87,500	23.40	12.15	17.77	1.249
30	129,150	53,600	12.50	7.45	9.87	0.994
35	55,000	25,200	5.24	3.50	4.37	0.640
40	4,100	4,600	0.39	0.64	0.515	1.712
45	-	-	-	-	-	-

Material	0.25 M NaOH					
Date	4-14-28	4-7-28				
Preliminary Treatment	Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. percent Survivors	Log. av. percent Survivors
0	1,800,000	675,000	100.00	100.00	100.00	2.000
10	459,000	221,500	25.50	32.80	29.15	1.465
15	334,500	175,500	18.60	26.00	22.30	1.348
20	149,500	111,500	8.32	16.55	12.43	1.094
25	107,000	66,000	5.95	9.78	7.86	0.895
30	35,950	24,950	1.995	3.69	2.84	0.453
35	9,100	14,400	0.505	2.14	1.32	0.121
40	350	1,750	0.020	0.259	0.139	1.143
45	-	-	-	-	-	-

Table III (Continued)

Series C.

Preliminary Treatment in NaI Solution

Material	0.25 M NaOH + 1 cc. 0.342 M NaI Solution					
Date	4-21-28	5-5-28				
Preliminary Treatment	Not Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. percent Survivors	Log. av. percent Survivors
0	395,000	1,282,000	100.00	100.00	100.00	2.000
10	93,000	240,500	23.50	19.00	21.25	1.527
15	36,000	152,500	9.10	12.10	10.06	1.003
20	18,250	19,500	4.63	1.54	3.09	0.490
25	5,000	-	1.27	-	1.27	0.104
30	2,250	5,500	0.57	0.435	0.503	$\bar{1}$.702
35	250	2,200	0.064	0.174	0.119	$\bar{1}$.076
40	-	-	-	-	-	-
45	-	-	-	-	-	-

Material	0.25 M NaOH					
Date	4-21-28	6-29-28				
Preliminary Treatment	Treated in Halide Solution					
Time in Minutes	Surviving Bacteria in 5.0 cc.		Percent Survivors		Av. percent Survivors	Log. av. percent Survivors
0	455,000	300,000	100.00	100.00	100.00	2.000
10	138,000	180,000	30.40	60.00	45.20	1.655
15	91,000	157,250	20.00	52.50	36.25	1.559
20	32,000	56,000	7.05	18.65	12.85	1.109
25	12,500	32,000	2.75	10.07	6.41	0.807
30	1,750	4,250	0.385	1.42	0.903	$\bar{1}$.956
35	375	500	0.083	0.166	0.124	$\bar{1}$.093
40	-	-	-	-	-	-
45	-	-	-	-	-	-

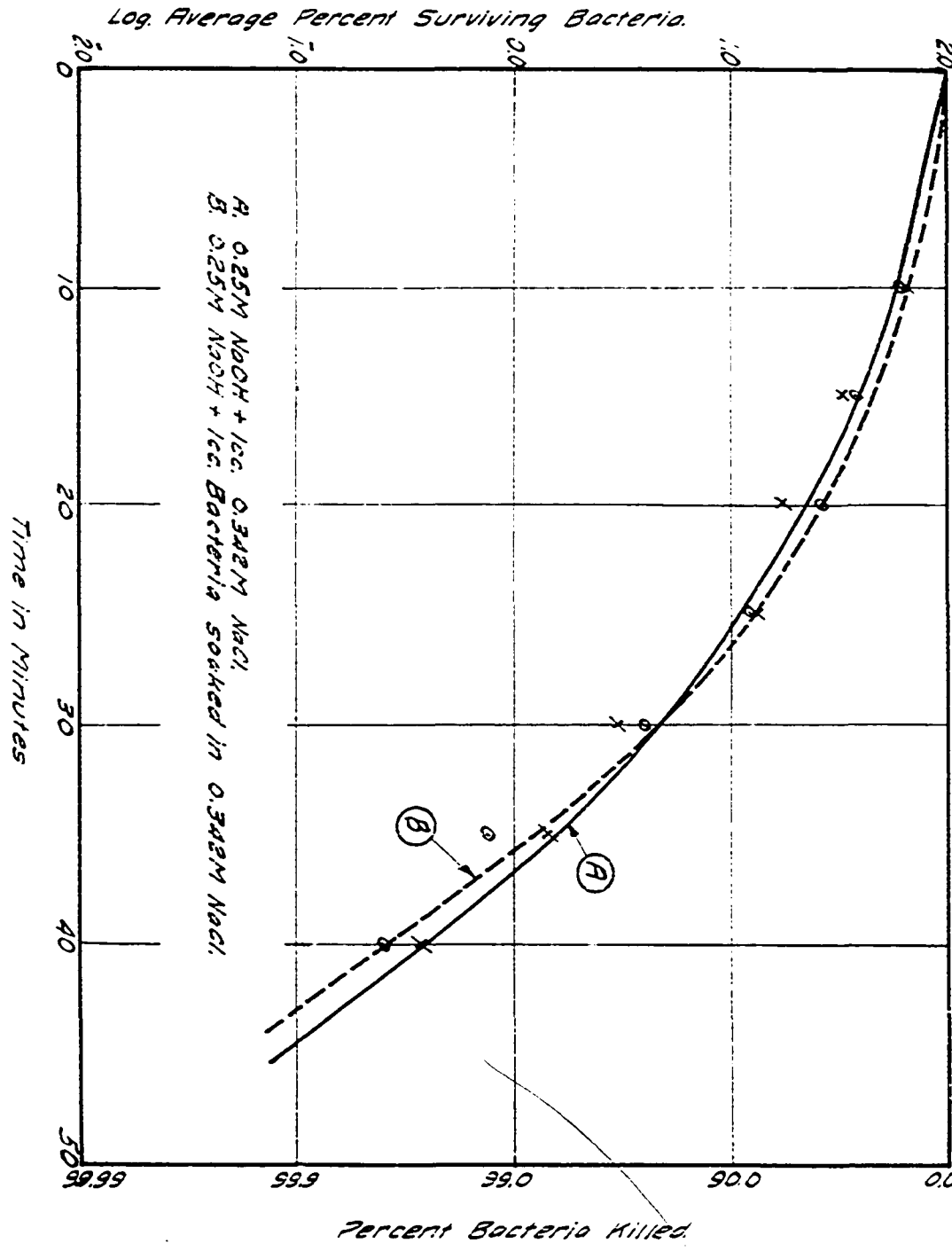

Fig. 6. Effect of Preliminary Soaking in NaCl solution on Bacteria (No. 25) in NaOH at 60°C.

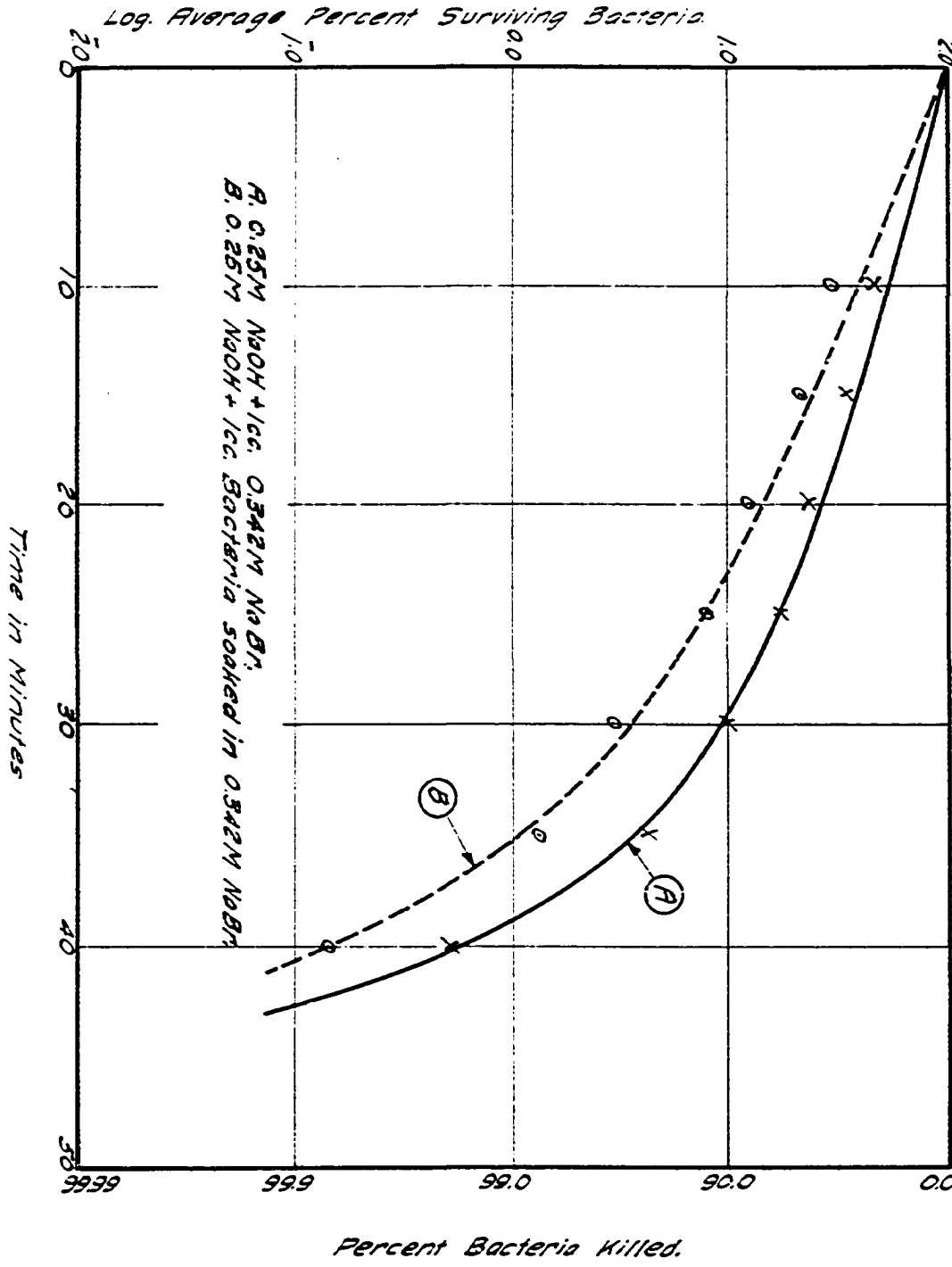

Fig. 7. Effect of Preliminary Soaking in NaBr Solution on Bacteria (No 25) in NaOH at 60°C.

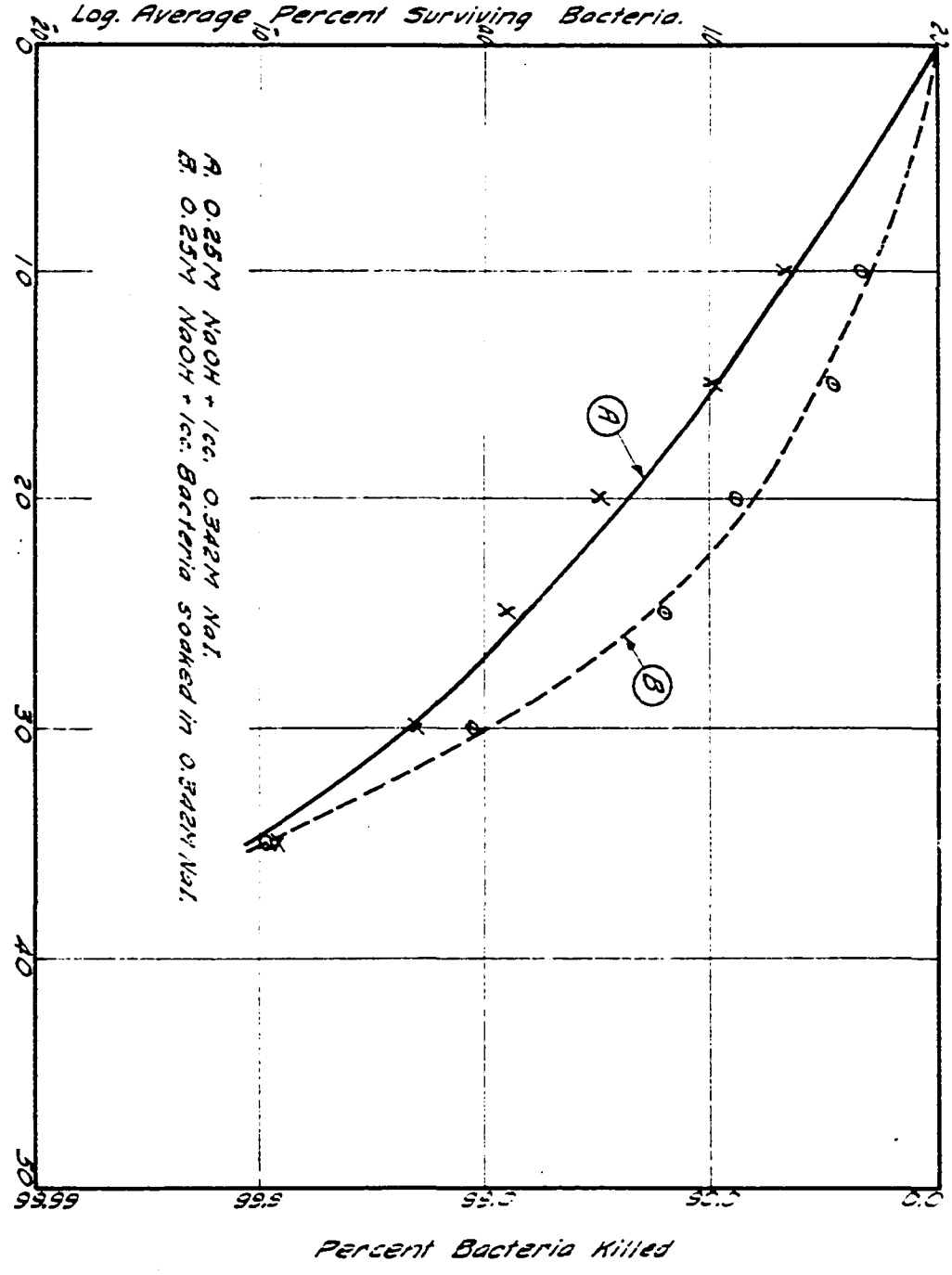

Fig. 8 Effect of Preliminary Soaking in Nal Solution on Bacteria (No. 25) in NaOH at 0°C.

Discussion of Data

The effect of the preliminary soaking of the bacterial spores in the different halide solutions before inoculation into the disinfecting alkali solutions, is shown in Curves B, Figs. 6, 7, and 8, respectively. No effect is noted in the cases of sodium chloride and bromide since they show disinfection times of 42.75 and 41.5 minutes, respectively, which agree closely with the times secured when the alkali test solutions were used alone. When 1 cc. of these halides were added to the alkali test solutions, respectively, (Curves A, Figs. 6 and 7) the killing times secured were 44.5 and 42.5 minutes. These times agree well with the other times and hence no importance is attached to the preliminary soaking in case of sodium chloride and sodium bromide.

However, the preliminary soaking in sodium iodide solution reduced the disinfecting time to 35.25 minutes (Curve B, Fig. 8). Also, the addition of 1 cc. of sodium iodide solution to alkali test solution reduced the time to 55.75 minutes (Curve A, Fig. 8). These reductions of more than 5 minutes are considered significant, and, as has been mentioned earlier, this seems to be characteristic where sodium iodide is involved. Later, an explanation will be suggested for this behavior.

It might appear that the preliminary treatment retarded the disinfecting action somewhat during the first 20 or 25 minutes in the cases of sodium chloride and sodium iodide (Curves B, Figs. 6 and 8) and then afterward became more rapid but the

same is not true in the instance of sodium bromide (Curve B, Fig. 7) and hence no significance is given it.

IV. pH Values of Various Test Solutions

It is of some interest to compare the pH values of the various test solutions before and after sterilization. The pH determinations were made at 25°C., using the usual hydrogen platinum electrode and a Leeds and Northrup student's potentiometer (new type) with a portable d'Arsonval galvanometer. E.M.F. readings were converted into pH values by substituting these readings for E in the formula, $pH = \frac{E-0.283}{0.059}$. The results of these determinations are recorded in Table IV.

Table IV.

pH Values of Test Solutions Before and After Sterilization (25°C.).

Test Solution	pH (before sterilization)	pH (after sterilization)
0.25 M NaOH	12.86	12.86
0.25 M NaOH + 0.342 M NaCl	12.88	12.88
0.25 M NaOH + 0.342 M NaBr	12.85	12.84
0.25 M NaOH + 0.342 M NaI	12.86	12.83
0.25 M NaOH + 0.342 M NaF	12.91	12.92
0.342 M NaF	6.68	6.70

Discussion of Data

These solutions were sterilized in an autoclave at 15 pounds for 15 minutes. In the cases of the bromide and iodide-alkali mixtures, the solutions became slightly more acid while the fluoride-hydroxide mixture became more alkaline. After devoting some thought to the matter, the results are probably what one would anticipate. However, it is doubtful if the slight increase or decrease in alkalinity of the test solutions as determined by pH in this range has any particular significance. It is certain that they in no manner account for the great reduction in killing time secured by the halide-hydroxide mixtures when compared with the alkali test solutions of approximately the same pH value.

In the case of 0.542 M NaF alone, the pH values are not what were expected. Hydrolysis should be expected after the following reactions:

$$2NaF + 2\ HOH \rightleftharpoons 2NaOH + H_2F_2$$

or, $$2NaF + HOH \rightleftharpoons NaOH + NaHF$$

An alkaline reaction should be anticipated. The pH values were checked by an associate by another method (quinhydrone) and found to be slightly lower (6.2) at 25°C. The solutions had practically no effect on either red or blue litmus paper. However, when this concentration was introduced in a 0.25 M sodium hydroxide test solution, the alkalinity was increased as determined both by titration and pH methods.

GENERAL DISCUSSION OF RESULTS

Theoretical Considerations.

In the beginning of this thesis, it was pointed out that there was no general agreement as to the type of action involved in disinfection by alkalies. Previous work done in these laboratories seemed to point that concentration of the undissociated molecule of alkali was a very important factor. The addition of a neutral salt (sodium chloride) to the alkali test solutions gave a large reduction in the killing time of bacterial spores and it was thought that by using other neutral halides more information might be secured concerning the type of action involved in these disinfection tests. It seemed logical that the neutral halides might act as accelerants by decreasing the ionization of the alkali and hence decrease the solubility of the undissociated alkali. If the undissociated molecule were more toxic to the cell than its ions, the increased toxicity should result in lowering the killing times. If this assumption were true, it was hoped that some quantitative evidence might be secured which would show a definite relationship between the increased concentration of the undissociated alkali, or its decrease in solubility, and the reduction in killing times.

Consider a mixture of water, sodium hydroxide, and a bacterial suspension in which the water and bacteria constitute two immiscible phases with the sodium hydroxide as a solute in equilibrium between the phases. The sodium hydroxide is more soluble in the water phase and, also, is ionized to a considerable extent,

$$NaOH \rightleftharpoons Na^{+} + OH^{-}.$$

When a highly ionized, water soluble salt is added to the water phase such as sodium chloride, sodium bromide, or sodium iodide, it may be shown, aside from any chemical activity, that it usually decreases the solubility of the more volatile solute in that phase (in this case, the NaOH). The effect on the solute of the added salt is generally proportional to the concentration up to about one molar, the temperature, the pressure, and the nature of the salt or its tendency to react. The decrease in solubility of the solute varies greatly with the nature of the salt added, but is approximately the same for a definite salt, regardless of the nature of the solute. This phenomenon in a general way is commonly known as "activity", "fugacity", "salting-out effect", or "chemical potential".

This same effect may be expressed numerically in a more quantitative fashion by the mass-action law. Its application presupposes a knowledge of the molecular species present, their dissociation constants, and holds in exact form only for dilute solutions. In this instance, it was thought that it would be interesting to make quantitative application of the mass-action law to the problem in hand. It was hoped that some light might be thrown on the germicidal action of alkalies as all the necessary information seemed to be available.

A simple statement of the mass-action law as applied in this problem may be represented by the equation,

$$\frac{C_{Na} + X\, C_{OH^-}}{C_{NaOH}} = K.$$

In this form the mass-action equation becomes a simple tool for determining roughly the change in concentration of the undissociated alkali when the same salt in varying concentrations with a common ion (Na^+) or when different salts with a common ion (Na^+) are added to the sodium hydroxide test solutions.

In order to determine the constant (K) for sodium hydroxide and at the same time the concentration of the undissociated molecule in the 0.25 M sodium hydroxide, it was necessary to compile a table of the ionization values at different concentrations for sodium hydroxide and the different salts added. Curves were plotted and the desired values were selected from them.

Calculation of the Undissociated Alkali in the Different Test Solutions.

After the constant for sodium hydroxide (K = 1.70266) had been determined, a table was compiled showing the different concentrations of the undissociated alkali when the common-ion effect was applied in the mass-action equation. These values are shown in Column 9, Table V. for 0.25 M sodium hydroxide alone and when varying concentrations of the same salt and the same concentration of different salts were used.

Table V.

Concentration in Mols of Undissociated Sodium Hydroxide in Test Solutions

(Where X = Concentration of Undissociated NaOH in Mols)

(1) Series	(2) Test Solution	(3) % Ionization of Salt or Alkali (18°C.)	(4) C_{Na^+} in Mols from Alkali	(5) C_{Na^+} in Mols from Salt Added	(6) C_{Na^+} in Mols from Both Sources	(7) C_{OH^-} in Mols from Alkali	(8) K	(9) C_{NaOH} in Mols
1.	0.25 M NaOH	88.5	0.22125	-	-	0.22125	1.70266	0.0288
2.	0.25 M NaOH + 0.17 M (1%) NaCl	82.8	0.25-X	0.14076	0.39-X	0.25-X	1.70266	0.04
3.	0.25 M NaOH + 0.34 M (2%) NaCl	79.3	0.25-X	0.2696	0.519-X	0.25-X	1.70266	0.0525
4.	0.25 M NaOH + 0.51 M (3%) NaCl	77.4	0.25-X	0.3947	0.645-X	0.25-X	1.70266	0.064
5.	0.25 M NaOH + 0.34 M NaBr	79.2	0.25-X	0.2692	0.519-X	0.25-X	1.70266	0.0525
6.	0.25 M NaOH + 0.34 M NaI	79.8	0.25-X	0.2713	0.521-X	0.25-X	1.70266	0.0535
x7.	0.25 M NaOH + 0.34 M NaF	69.1	0.29-X	0.2349	0.525-X	0.29-X	1.70266	0.062

xThe calculation in the case of fluoride-hydroxide mixture (Series 7) needs some explanation. It was shown by titration of the mixture with a standard acid that the alkalinity was increased 0.04 N, making the fluoride-hydroxide test solution 0.29 M instead of 0.25 M, that of the original alkali. This increased alkalinity was due to the hydrolysis of the sodium fluoride. On this basis, the increased concentration of the undissociated alkali was secured.

Comparison of Different Concentrations of Sodium Chloride in Sodium Hydroxide.

If the undissociated molecule of alkali is the primary factor in this instance of alkali disinfection, it is necessary to assume that the killing time should be inversely proportional to the concentration of the undissociated molecule of sodium hydroxide. These values were computed for the first four series from the data in Column 9 (Table V) in which different sodium chloride concentrations were added to the same concentration of alkali test solution. The actual killing times were taken from work done in these laboratories which had been previously reported (11). The results are shown in Table VI.

Table VI.

Comparison of Percent Reduction in Killing Times (Theoretical With Actual) With Different Concentrations of Sodium Chloride in Alkali Test Solutions

(1) Series	(2) Test Solution	(3) C_{NaOH} in Mols	(4) Computed Killing Times = $\frac{1}{C_{NaOH}}$	(5) Experimental Killing Times in Minutes (60°C)	(6) Percent Reduced Killing Times (Theoretical)	(7) Percent Reduced Killing Times (Actual)	(8) Percent Difference in Reduced Killing Times (Theoretical from Actual)
1.	0.25 M NaOH + 0 NaCl	0.0288	34.73	42.5	100.0	100.0	0.0
2.	0.25 M NaOH + 0.17 M NaCl	0.04	25.0	30.6	71.9	72.0	+0.1
3.	0.25 M NaOH + 0.34 M NaCl	0.0525	19.1	23.4	55.0	55.1	+0.1
4.	0.25 M NaOH + 0.51 M NaCl	0.064	15.62	19.9	44.9	46.8	+1.9

Comparison of Same Concentration of Different Sodium Halides in Sodium Hydroxide.

More valuable information was gained when the values in Column 9, Series 5, 6 and 7, were compared in a similar manner. In these series, the same concentrations of different salts were added to the same concentration of sodium hydroxide test solution. The results are compiled in Table VII.

Table VII

Comparison of Percent Reduction in Killing Times (Theoretical With Actual) With Same Concentration of Different Sodium Halides in Alkali Test Solutions

(1) Series	(2) Test Solution	(3) C_{NaOH} in Mols	(4) Computed Killing Times = $\frac{1}{C_{NaOH}}$	(5) Experimental Killing Times in Minutes (60°C)	(6) Percent Reduced Killing Times (Theoretical)	(7) Percent Reduced Killing Times (Actual)	(8) Percent Difference in Reduced Killing Times (Theoretical from actual)
1.	0.25 M NaOH + 0 salt	0.0288	34.73	42.85	100.0	100.0	0.0
2.	0.25 M NaOH + 0.34 M NaCl	0.0525	19.0	23.0	54.8	53.7	+1.1
3.	0.25 M NaOH + 0.34 M NaBr	0.0525	19.0	23.75	54.8	55.4	-0.6
4.	0.25 M NaOH + 0.34 M NaI	0.0535	18.7	17.4	53.8	40.6	-13.2
5.	0.25 M NaOH + 0.34 M NaF	0.062	16.1	21.0	46.3	49.0	-2.7

Comparison of Results, Theoretical and Experimental.

Examination of the data in Columns 6 and 7 (Table VI) shows a remarkable agreement between the assumed theoretical percent reductions in killing times and the percent reductions actually secured from experimental data. In Column 4, the assumption was made that the killing times should be inversely proportional to the concentration of the undissociated sodium hydroxide. The percent reductions should have been 71.9, 55.0, and 44.9 percents (Column 6) respectively, when 0.17 M (1%), 0.34 M (2%), and 0.51 M (3%) sodium chloride was added to the 0.25 M sodium hydroxide test solution. The actual killing times show percent reductions of 72, 55.1, and 46.8 percents, respectively, (Column 7). The deviation of the theoretical from the actual reductions is shown in Column 8. The reductions are almost in exact agreement with the predicted results from the assumption, excepting in the last instance, which may easily be considered within the limits of experimental error.

The variations are quite interesting where the same concentrations of the different sodium halides (NaCl, NaBr, NaI and NaF) are added to the same concentration of sodium hydroxide test solution (Column 8, Table VII). The experimental killing time for 0.25 M sodium hydroxide is 42.85 minutes (Series 1, Column 5.). This is an average of all alkali controls run in Series 2, 3, 4, and 5.

In the instances of the chloride-hydroxide and the bromide-hydroxide mixtures (Series 2 and 3), the deviations of the calculated percent reductions from the experimental percent

reductions are +1.1 and -0.5, respectively. These deviations may be considered within the limits of experimental error since there is a difference of only 0.1 percent in the ionization of sodium chloride and sodium bromide.

The increased germicidal efficiency of the iodide-hydroxide test solution is more difficult of explanation. In Column 8, the difference is 13.2 percent lower than the calculated percent reduction in killing time. It should be recalled in this connection that in the first experiments with this mixture when the 5 cc. portions of test solution were withdrawn at different time intervals and made just alkaline (methyl orange) in the 50 cc. of sulfuric acid, that free iodine was liberated immediately (starch test). Free iodine is a powerful germicide. The killing time was reduced to 8 minutes. However, when the 5 cc. portions were made just acid to phenolphthalein but quite alkaline to methyl orange, no free iodine was liberated and the killing time was 17.4 minutes (average). This lowering of more than 5 minutes over the killing times of the chloride- and the bromide-hydroxide mixture can not be explained on the basis of the increased ionization sodium iodide.

Several explanations are suggested for this increased germicidal efficiency. It may be considered that sodium iodide in acting as an accelerant may form a more toxic compound (sodium iodate) with the sodium hydroxide. Sodium iodate is prepared by adding finely divided iodine to hot concentrated

solutions of sodium hydroxide. In this instance, it is possible that the first parts of the 5 cc. portions of the test solutions introduced in the sulfuric acid for neutralization, formed hydriodic acid. The amounts formed decrease as the amounts of test solutions added increase and the mixture approaches neutralization. Hydriodic acid is very easily oxidized even by the oxygen of the air and, undoubtedly, the free iodine produced in the first experiments with this mixture, was formed in this manner. In these experiments, the free iodine remained in the somewhat acid medium (just alkaline to methyl orange) while in the more alkaline solutions (just acid to phenolphthalein) no evidence was secured by the starch test. At the same time, in the latter experiments, the killing times was considerably reduced below that of the chloride- and bromide-hydroxide mixtures. It may be possible that the freed iodine was converted into minute amounts of sodium iodate which may be the effective agency in lowering the killing time. Some recent data (unpublished) points that a compound of this nature is very toxic in an alkali test solution. However, no evidence is available that this substance is formed.

A second explanation may be advanced that the sodium iodide increases the "activity" of the sodium hydroxide by producing a larger amount of the undissociated alkali than anticipated by the method of calculation employed and hence accounts for the reduced killing time. Again, no data are available bearing directly on this suggestion.

Still another suggested explanation is that the minute amounts of free iodine liberated by the reactions suggested in a preceding paragraph may have been adsorbed by the injured walls of the bacterial cells. In this manner, the amount of free iodine may have been lowered to such an extent that none was detected by the starch test which is considered quite delicate. This assumption seems quite tenable. However, again, no data are available to bear it out.

In the case where sodium fluoride is added to the alkali test solution (Series 5), the difference in percent reduction is 2.7 percent lower than the calculated percent reduction. However, if one considers the average time of the alkali controls run in this series (45.25 minutes) instead of the average time for all the controls in all the series (42.85 minutes), the percent reduction in actual killing time becomes 48.6 instead of 49.0 (Column 7, Series 5) and the difference in Column 8 becomes 2.3 percent. Furthermore, if one considered the times of an individual experiment (Fig. 5, Curves C and D) the percent reduction becomes 48 and the variation from the calculated value would be 2.3 instead of 2.7 percent. Even the greater variation is considered in good agreement with the theoretical values. It should be remembered, that the increased alkalinity of the mixture to 0.29 N was considered in the calculations.

On the whole, in Series 2, 3 and 5, the experimental results show a remarkable agreement with the calculated values based on the mass-action law and that the concentration of the

undissociated sodium hydroxide is the main factor in the germicidal action of sodium hydroxide. Only the instance of the iodide-hydroxide mixture (Series 4) demands further explanation. This might well offer a problem for further study.

Of the suggested explanations for this increased efficiency, the sodium iodate theory seems the least plausible since the concentration of the sodium hydroxide was low and the temperature of the mixture was low under the conditions of the experiment for the formation of sodium iodate even though sodium iodate may be found to be quite toxic. The increased "activity" theory is not considered seriously because it was known from the first experiments with this mixture that free iodine was liberated and steps had to be taken to prevent its formation in detected amounts. It seems logical that the adsorption of the free iodine by the bacterial cells should be given more consideration. It is reasonable to assume that walls of the bacterial cells would be greatly softened and injured and, in this manner, the adsorption properties for the iodine would be increased and hence the toxicity greater. It is thought that this theory is worthy of most consideration.

SUMMARY AND CONCLUSIONS

1. Sodium halides at the concentrations and temperatures employed, had little toxic effect on the bacterial organisms used in the disinfection tests. Sodium fluoride was most effective.

2. Sodium halides added to sodium hydroxide increased greatly the germicidal efficiency of the sodium hydroxide test solution. The addition of sodium iodide produced an abnormally toxic effect.

3. A preliminary soaking in the different sodium halide solutions of the same disinfection tests did not affect the resistance of the bacteria to sodium hydroxide disinfection.

4. The pH values for all the alkali test solutions employed when measured by potentiometric methods were in the same range. The fluoride-hydroxide mixture appeared to be slightly more alkaline.

5. The assumption that the undissociated molecule of alkali is a very important factor in alkali disinfection appears to be justified. By application of the mass-action law and the common-ion effect, remarkable agreement was obtained between the anticipated calculated reductions of killing times and those obtained from experimental data. The iodide-hydroxide mixture appears to be the only exception.

6. Of the several theories suggested for the abnormal toxicity of the iodide-hydroxide mixtures, the "adsorption theory" is held to be the most tenable.

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