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Chemical sterilization with alkalies

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CHEMICAL STERILIZATION WITH ALKALIES

BY

James Roger Hall

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject
Food and Sanitary Chemistry

Approved

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CHEMICAL STERILIZATION WITH ALKALIES

INTRODUCTION

Mechanical washing of bottles is widely used in the milk and beverage industries. Glass bottles which have been returned to the plants after being used, must be thoroughly cleaned and sterilized, in order that the beverage to be placed in them, may be issued for sale in a sanitary condition.

The simultaneous cleaning and sterilization of the bottles is accomplished in mechanical bottle washing, by two methods, the soaker and the hydro method. By the first method the dirty bottles, after being placed in a conveyor belt, are slowly drawn through the several compartments, containing the hot washing solution, usually two or three, and finally through a rinse water compartment, after which they may be further rinsed by jets of water. In the hydro type of washer, the bottles in the conveyor belt are subjected to intermittent jets of the hot washing solution, and finally rinsed in the same manner.

The washing solution usually consists of a sodium hydroxide solution to which has been added one or several of the various sodium salts such as sodium carbonate, tri-sodium phosphate, sodium silicate, or sodium tetra-borate. These mild alkalies are added for the express purpose of securing a better rinse and a bright sparkling bottle. The tempera-

ture of the soaker solution in different machines, or compartments of the same machine, varies between 110°-160°F. Temperatures above 140°F., although efficient, are more likely to cause loss by breakage, particularly in the hydro type of washer, due to the hot bottles being subjected to the cold rinse water. This may be avoided by a suitable adjustment of the temperature of the rinse water. Temperatures lower than 120°F. are found to be insufficient to produce a satisfactory bottle, with the alkali content and time of exposure which are generally used in the commercial machines.

The germicidal efficiency of washing solutions may be said to depend upon three factors, viz.,

(1) the temperature

(2) the time of exposure

(3) the composition of the washing solution.

These factors may be varied within certain limits in the different machines and yet produce satisfactory bottles.

The purpose of these experiments is three-fold, first, to arrive at a better understanding of the theoretical principles involved in the use of the salt effect in disinfection, secondly, to determine the relative efficiencies of the strong alkali hydroxides as germicides, and thirdly, to determine the relative germicidal efficiency of several alkali salts when added to the strong alkali hydroxides.

HISTORICAL

In 1896, Paul and Krönig (12), in working with a number of disinfectants, reported that lithium, sodium, and potassium hydroxide solutions were efficient germicidal agents, while ammonium hydroxide solutions were not. These investigators found that the amount of hydroxyl ion present in solution, determined the relative germicidal efficiency. Lithium hydroxide was slightly less efficient than sodium hydroxide, and sodium hydroxide slightly less efficient than potassium hydroxide, but the authors concluded the germicidal powers were nearly the same. This was explained as being due to the relative number of hydroxyl ions present, of which lithium hydroxide had the smallest number and potassium hydroxide the greatest number.

For a number of years, it was generally accepted that the germicidal efficiency of alkalies was in direct ratio to the amount of free hydroxyl ion present. In 1921 it was suggested by Traube and Somogyi (15), that other forces must contribute to germicidal power. These investigators said that these forces included undissociated molecules, surface tension, adsorption, electrical potential, swelling, and osmotic pressure.

Sherman (13) and later, Mudge and Lawler (11) reported that the germicidal efficiency of alkali solutions is directly related to the pH of the solution. Studies were also reported by these investigators upon the effect of temperature,

by which solutions of lower pH could be made equal to solutions of higher pH in germicidal power, by raising the temperature. Weiss (16), in 1921, working on Clostridium botulinium had previously obtained similar results.

Levine, Buchanan, and Lease (2) reported that by increasing the temperature, the same concentration of sodium hydroxide was more effective at the higher temperature. At the same temperature, the higher concentrations of sodium hydroxide were more effective.

In another paper (4), by Levine, Peterson, and Buchanan, studies are reported on sodium hydroxide, sodium carbonate, and tri-sodium phosphate at the same hydrogen-ion concentration. It was found that pH was not the sole factor determining germicidal efficiency in considering the three different salts. However, in considering the same compound, with an increase in pH, an increase in germicidal efficiency was observed.

In another series of experiments (5), these same authors found that sodium hydroxide in comparison to sodium hydroxide-sodium carbonate mixtures is less efficient at the same hydrogen-ion concentration. The effect of added sodium carbonate on a sodium hydroxide solution, while not affecting the pH, is to increase the germicidal power.

Levine, Toulouse, and Buchanan (3,6) reported that equal weights of sodium chloride and sodium carbonate when added to sodium hydroxide solutions, quite markedly decrease the kil-

ling time, while tri-sodium phosphate is less efficient. By increasing the weight of added salts the germicidal efficiency increased, but at a decreasing rate.

It has been suggested (3,5,6,8) that the undissociated sodium hydroxide molecule is responsible for the germicidal action, and that the effect of added salts, in concentrations not germicidal in themselves, is to increase the number of undissociated molecules. Consequently the germicidal power is increased.

Meyers (9) in working with Bacterium coli, observed that with different buffer mixtures of the same pH that different germicidal powers were found. In a later publication (10) Meyers found that pH and buffer index were important in disinfection and that osmotic pressure had some small effect.

Lowman (8) in working with the effect of the addition of the various sodium halides upon the germicidal properties of sodium hydroxide, found that these halides, in equal concentrations, produced the same effect upon the killing time of the sodium hydroxide solutions. Using the mass-action law Lowman reported that the reduction of killing time found was the same, as would be expected, from a consideration of the ionization values of the compound used, if the un-ionized molecules were considered to be the active agents in disinfection.

The present investigation was started to determine if the theory that the undissociated molecule was an important

factor in disinfection was tenable. Also it seemed desirable to investigate the salt effect in disinfection, to determine whether or not it was a phenomenon depending upon the number of molecules added as salts, rather than the weight of the added salts.

TECHNIQUE USED

The apparatus and technique used were the same as described by Levine, Buchanan, and Lease (2), in their original work. Essentially the same technique has been used by later investigators (3,4,5,6,8,10). However, two small changes were introduced into the original technique for the purpose of convenience and refinement in the experiments, namely, the flask was changed, and the indicator used in neutralizing the test alkali solutions before plating, was different.

The flask shown in plate I, was devised to take the place of the three-necked Woulff flask used in previous experiments. It was made from the ordinary form of a 200 cubic centimeter round bottomed pyrex flask, by sealing on an auxiliary neck at a convenient angle. This flask had the following advantages —

(1) it was much easier and convenient to withdraw the five cubic centimeter portions of the alkali test solutions for plating, without touching the sides or neck of the flask. This has been found to be a serious source of error in bacterial counts, by previous investigators, due to the bacteria being retained on the side of the neck, not exposed to the action of the alkali, and being picked up by the pipette in the withdrawal of later portions.

(2) the water bath could be more conveniently located on a laboratory desk or table than when using the

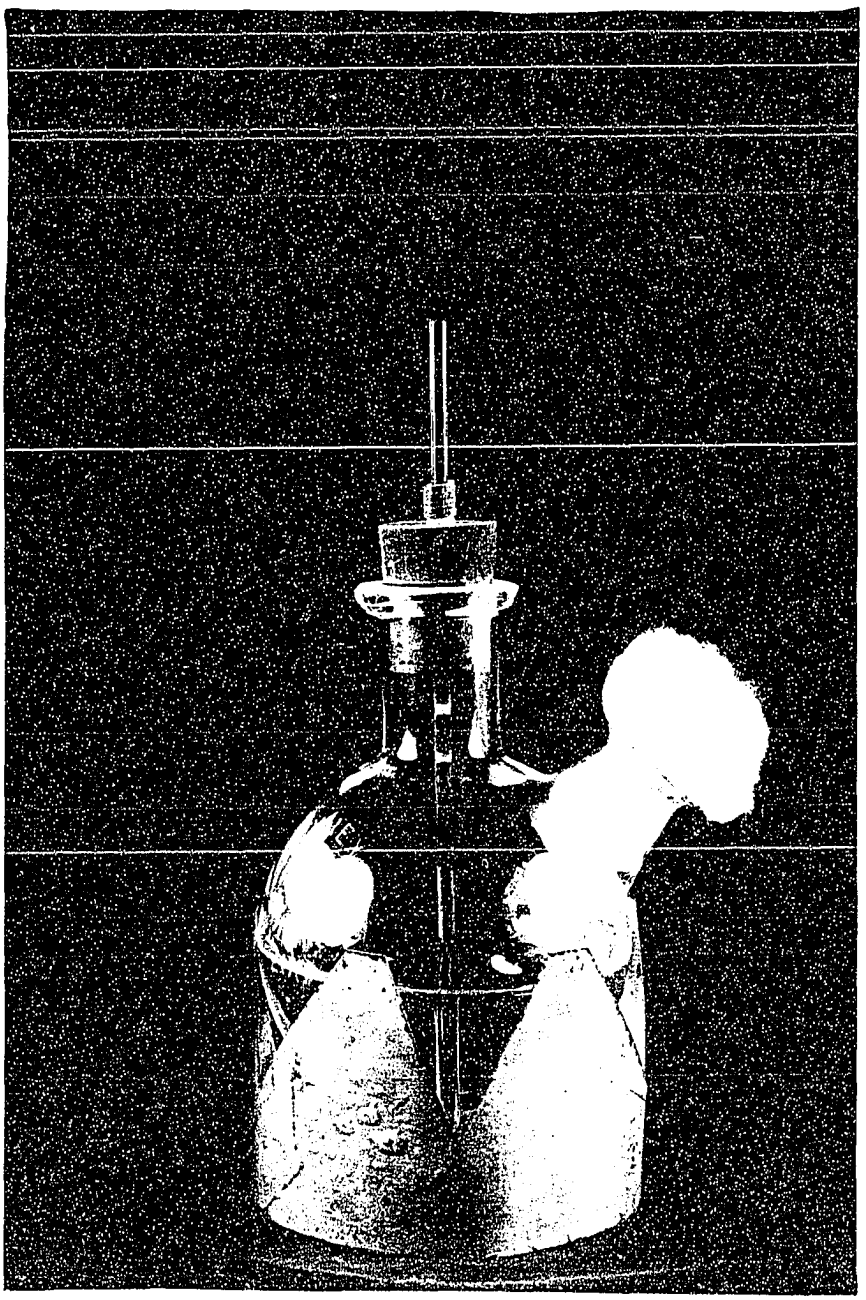


Plate 1. Sideview of flask used in experiments showing stirrer and base for flask.

other flask.

The indicator used when neutralizing the five cubic centimeter portions of the alkali removed from the flask for plating, was the mixed indicator described by Lizius (7). It was decided to use this indicator in order to define more closely the pH of the solutions being plated out. When a solution is alkaline to methyl orange, the pH may easily vary between pH 5 and pH 10. By the use of a three color indicator, with an intermediate color at about pH 7, and neutralizing to this color each time, the pH of the solution should vary but little.

The indicator chosen was made up by dissolving 0.02 gram of methyl red and 0.12 gram of thymol blue in 100 cubic centimeters of 95% ethyl alcohol. Two to three drops (0.1 - 0.15 cc.) were used in each flask containing 45 cubic centimeters of acid solution, which was always just sufficient to neutralize the five cubic centimeters of alkali to be added. Lizius reported that the first change of color from the acid side, was at pH 6, when the color change from red to orange-yellow occurred. Upon the further addition of alkali, this changed through a yellow, to green-yellow and finally at pH 9, a blue-green color appeared. By the use of a hydrogen electrode and the usual potentiometer set-up for pH work, these pH values were determined. As found, these values agreed with the values given by Lizius. The orange-yellow

color was taken as the point to which all solutions were to be adjusted and this was found to be in the range pH 6.5 - 7.0.

ORGANISM EMPLOYED

The organism used was the same as that employed in the original work (2) and it was thought to be ideal for the purpose in view, since it was quite resistant to alkali. This organism is quite fully characterized in the paper by Levine, Buchanan and Lease referred to above (2).

In the first experiments which were made, while new spores were being grown, spores which had been used by another investigator, were employed. These old spores were prepared in April, 1928, and were used by Lowman (8) in his investigations. These spores had been kept in a desiccator over sulfuric acid, and it was found that they had lost considerable of their vitality during their two years of storage.

The loss in vitality is shown by table I and graphically by figure 1 where the spore preparation made in 1928 is compared at 40°C. and 50°C. with the new spores, prepared during this investigation, at 50°C. where the disinfecting solution is in each case 0.5 N sodium hydroxide.

Due to the decrease in vitality of the older spore preparation it was found necessary to carry out the tests with them at 40°C., while the new spores, being more resistant, were tested at 50°C. In the various experiments given in this work the first spores used were the older preparation and will be referred to as "old spores". These tests were carried out at 40°C. The new spores will be referred to

Table I

Showing Surviving Bacteria in 0.5 N Sodium Hydroxide Solution Using Old and New Spore Preparations, at Different Temperatures.

Time in Minutes	Exp. No. 5 3/31/30: 0.5N NaOH at 50°C: Old spores.	Exp. No. 9 4/13/30: 0.5 N NaOH at 40°C: Old spores.	Exp. No. 81 7/2/30: 0.5N NaOH at 50°C: New spores.
	Surviving bacteria in 5 cc.		
0	227,500	755,000	1,580,000
10	58,000	523,000	900,000
20	950	230,000	625,000
25	0	183,000	220,000
30	0	145,000	18,500
35	0	95,200	2,500
40	0	22,000	0
45	0	11,100	0
50	0	2,200	0
55	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	25.49	$\bar{1}$.40645	69.27	1.84055	59.65	1.75558
20	0.42	$\bar{1}$.62074	30.46	1.48379	39.56	1.59722
25			24.24	1.38450	13.92	1.14376
30			19.21	1.28342	1.17	0.06851
35			12.61	1.10069	0.16	$\bar{1}$.19928
40			2.91	0.46447		
45			1.47	$\bar{0}$.16737		
50			0.29	$\bar{1}$.46447		
	K.T.* = 22.5 Min.		K.T. = 55.0 Min.		K.T. = 36.2 Min.	

*K.T. = Killing time for 99.9% of spores.

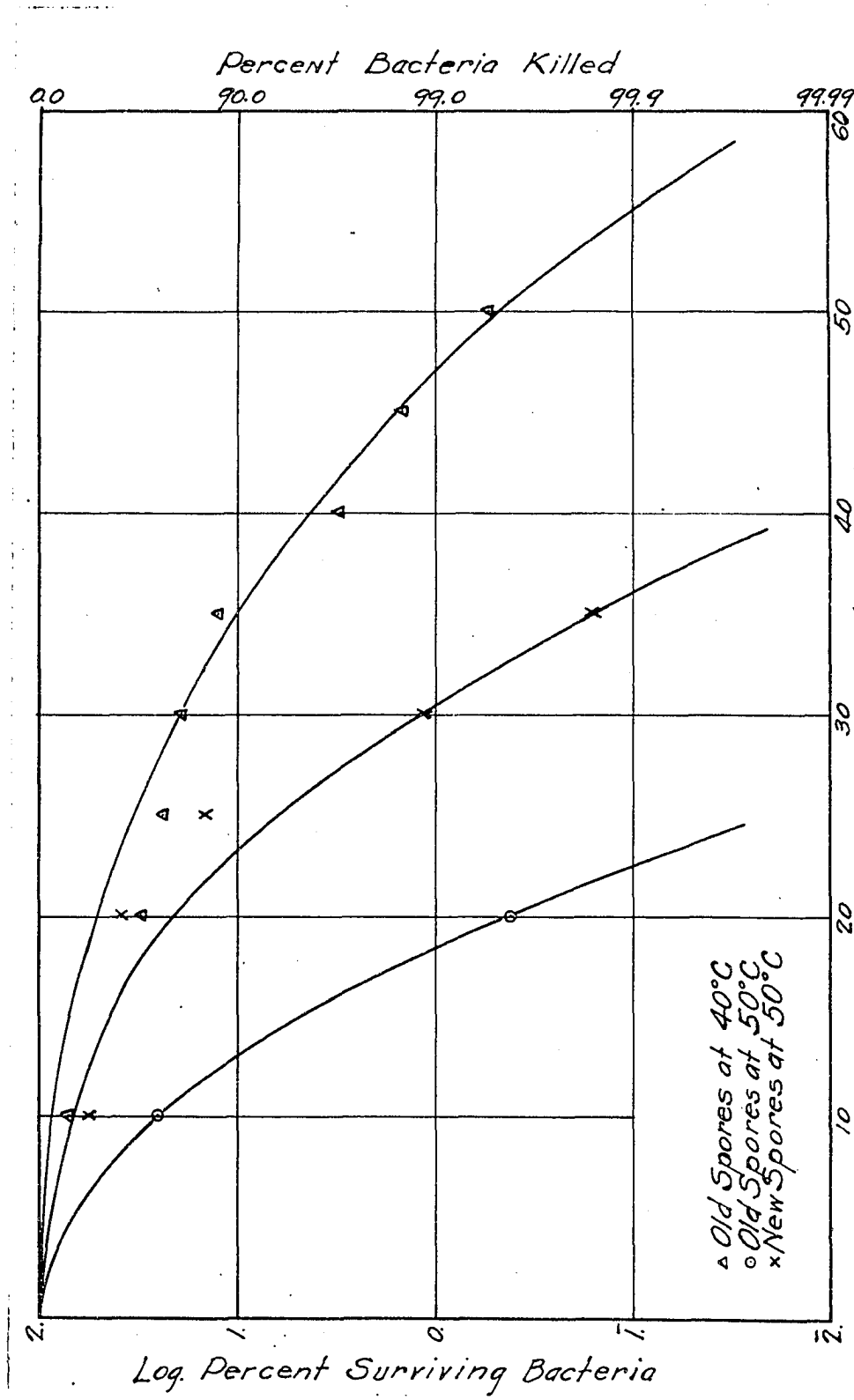


FIG. 1. - Comparative death rates of old and new spores tested against 0.5N NaOH at 40° and 50°C

as such and the tests were all made at 50°C.

The cause of the decrease in vitality of the older spore preparation is not known but it may be due to the desiccation of the spores during the storage over sulfuric acid. It was often observed that losses of weight between weighings which were two or three weeks apart, where none of the dried spore mixture had been removed, amounted to 5 - 10 milligrams.

In making up the spore suspension for the tests where each cubic centimeter should contain about twenty million spores it was found necessary to use 0.4 - 0.5 gram of the dried spore mixture in 10 cc. of water. Lowman in his work of two years previous found that 0.2 gram was sufficient to provide the desired number per cubic centimeter. In the new spore mixture, only 0.1 - 0.2 gram was necessary to provide the same number per cubic centimeter. These data show clearly that not only did the numbers of organisms per milligram of the powdered spores diminish, but that the vitality decreased during the two years of storage.

EXPERIMENTAL

A. Comparison of the Germicidal Power of the Caustic Alkalies.

Previous work has been reported (12) on the different caustic alkalies as germicidal agents. In this investigation the relative killing times of lithium, sodium, potassium, and rubidium hydroxides were determined. These compounds are found in the left hand family of group one of the periodic table and have very similar properties. These alkalies increase in basicity and electrolytic dissociation from lithium hydroxide to rubidium hydroxide. If germicidal power is dependent upon the amount of free hydroxyl ions in solution, then rubidium hydroxide should be a better germicide than potassium hydroxide, and potassium hydroxide better than sodium, etc. Paul and Krönig (12) reported that the germicidal powers of lithium, sodium, and potassium hydroxides were about the same.

Using the technique described above, it was determined to investigate the killing powers of the four alkalies mentioned, to ascertain whether or not significant differences in killing times could be observed.

Lithium hydroxide of Kahlbaum's best reagent grade was obtained in the United States but it was necessary to import rubidium hydroxide from Germany. It would have been very desirable to have included caesium hydroxide in this series of

experiments but it was not possible to obtain this compound. The rubidium hydroxide obtained was of C.P. grade, but due to the cost of this reagent, it was found necessary to reduce the scale on which the experiment was to be run to one-fifth the usual values. This will be described under a later heading.

Since sodium hydroxide is commonly used for disinfection, it was decided to compare the germicidal efficiency of the other alkalies with sodium hydroxide, at the same temperature, and under the same conditions, by including in each experiment, one test solution of sodium hydroxide. Both old and new spore preparations were used. In this manner, a large number of tests upon the killing time of sodium hydroxide was obtained. The killing time was taken to be that time at which 99.9% of the spores introduced were killed. Hereafter, when the term killing time is used, it will be taken to mean 99.9% reduction of numbers of the organisms introduced.

1. Germicidal efficiency of sodium hydroxide.

Sodium hydroxide was used as a standard for comparison in these experiments. In control flasks of twenty-two experiments it was found that the killing times, employing the same test organism, agreed closely with one another, except in one series employing the old spores, which is discussed later. In seven experiments the older spores were used and in fifteen, the new spores were employed. The data for these sodium hydroxide controls are distributed among the different tables in which the results of the various experiments are given.

2. Comparative germicidal efficiency of lithium and sodium hydroxide.

Using the technique referred to above (2), the bacterial spores were inoculated into two flasks containing 0.5 N lithium hydroxide solution and a control flask of 0.5 N sodium hydroxide solution. From these flasks, five cubic centimeter portions were withdrawn at regular intervals and the number of viable organisms determined. The results of four experiments with lithium hydroxide and two control flasks of 0.5 N sodium hydroxide are given in tables II and III. The results are plotted in figures 2 and 3.

Discussion. From a consideration of the data presented in tables II and III it will be seen that the germicidal properties of lithium hydroxide, in the concentration used, is about the same as sodium hydroxide. Four experiments with lithium hydroxide show an average killing time of 47.2 minutes, while the average of the sodium hydroxide controls was 47.4 minutes. This shows excellent agreement with the work of Paul and Krönig (12).

It was observed in the two runs reported above (tables II and III) that the killing time for sodium hydroxide was about 14% less than had previously been obtained with 0.5 N sodium hydroxide. In the earlier runs made during this investigation, using these spores, the resistance to sodium hydroxide was less than had been obtained by another investigator (8) under similar conditions. For this reason, the temperature used in these experiments was reduced to 40°C.

Table II

Showing Relative Germicidal Efficiency of Lithium Hydroxide and Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 46 6/7/30 0.5N LiOH at 40°C		Exp. No. 47 6/7/30 0.5N LiOH at 40°C		Exp. No. 48 6/7/30 0.5N NaOH at 40°C	
	Surviving bacteria in 5 cc.					
0	1,525,000		1,525,000		1,525,000	
10	1,105,000		1,125,000		1,185,000	
20	460,000		530,000		875,000	
30	195,000		223,500		236,500	
35	102,000		110,000		170,000	
40	12,000		15,000		45,000	
45	7,000		2,000		11,000	
50	500		550		200	
55	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	72.46	1.86009	73.77	1.86788	75.94	1.88045
20	30.16	1.47949	34.75	1.54101	57.38	1.75874
30	12.79	1.10676	14.66	1.16601	15.51	1.19056
35	6.69	0.82533	7.21	0.85807	11.15	1.04718
40	0.77	$\bar{1}$.88871	0.98	$\bar{1}$.99282	2.95	0.46994
45	0.46	$\bar{1}$.66183	0.13	$\bar{1}$.11776	0.72	$\bar{1}$.85812
50	0.03	$\bar{2}$.51570	0.03	$\bar{2}$.55709	0.01	$\bar{2}$.11776
	K.T.* = 48 Min.		K.T.* = 47.8 Min.		K.T.* = 47.0 Min.	

[+]Using old spores.

*K.T. = Killing time for 99.9% spores.

Table III

Showing Relative Germicidal Efficiency of Lithium Hydroxide and Sodium Hydroxide.†

Time in Minutes	Exp.No.49 6/7/30 0.5N LiOH at 40°C		Exp.No.50 6/7/30 0.5N LiOH at 40°C		Exp.No.51 6/7/30 0.5N NaOH at 40°C	
	Surviving bacteria in 5 cc.					
0	940,000		940,000		940,000	
10	575,000		645,000		665,000	
20	350,000		345,000		405,000	
30	79,000		92,500		163,500	
35	43,000		37,500		84,000	
40	13,000		12,000		36,000	
45	3,500		1,750		12,000	
50	75		50		150	
55	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	61.17	1.78654	68.62	1.83643	70.74	1.84969
20	37.23	1.57094	36.70	1.56469	43.09	1.63433
30	8.40	0.92450	9.84	0.99301	17.39	1.24039
35	4.57	0.66034	3.99	0.60090	8.94	0.95115
40	1.38	0.14081	1.28	0.10605	3.83	0.58317
45	0.37	$\bar{1}$.57094	0.19	$\bar{1}$.26991	1.28	0.10605
50	0.01	$\bar{3}$.90193	0.01	$\bar{3}$.72584	0.02	$\bar{2}$.20296
	K.T.* = 46 Min.		K.T. = 47 Min.		K.T. = 47.8 Min.	

†Using old spores.

*K.T. = Killing time for 99.9% spores.

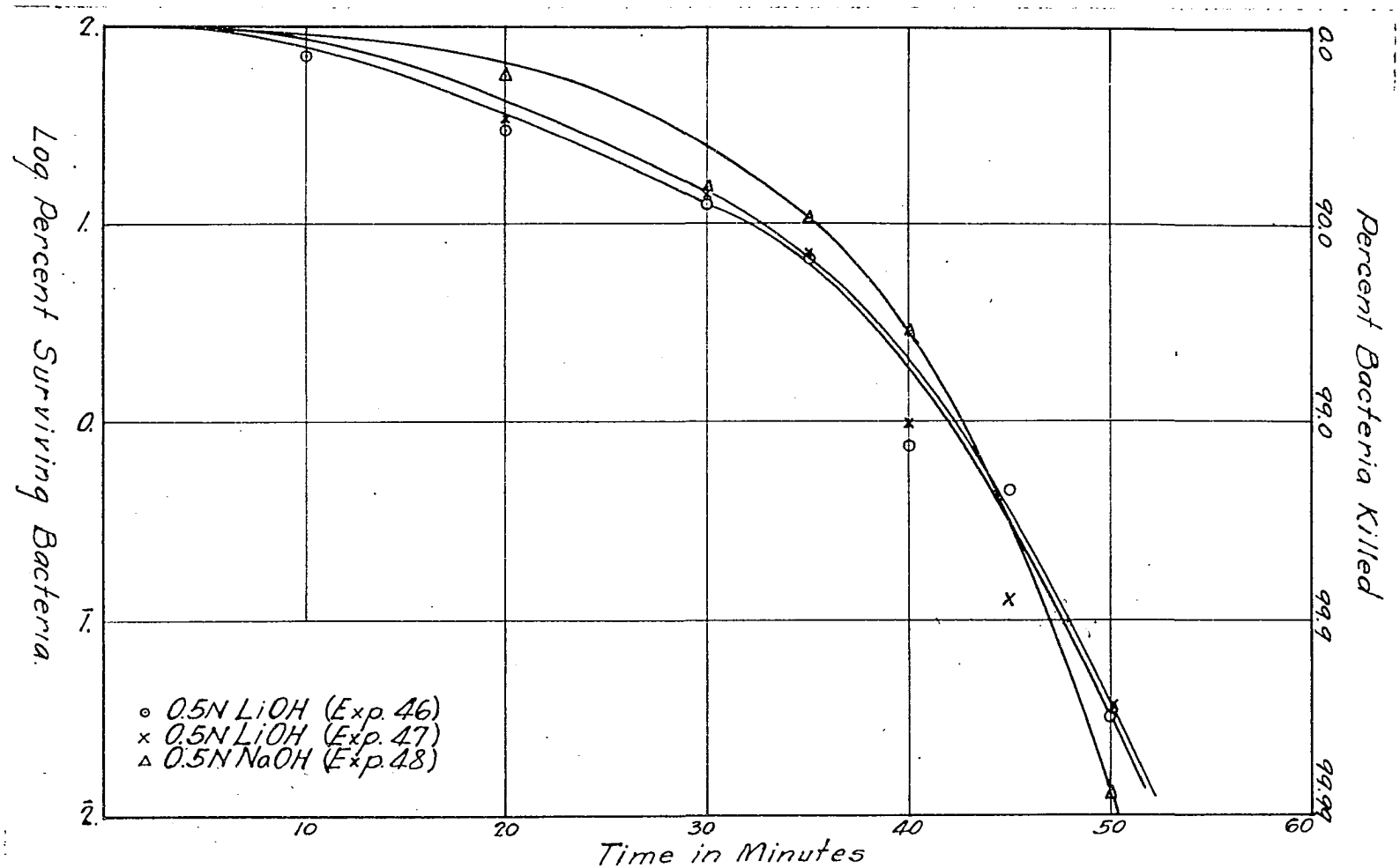


FIG. 2 - Comparison of germicidal efficiency of LiOH and NaOH at 40°C.

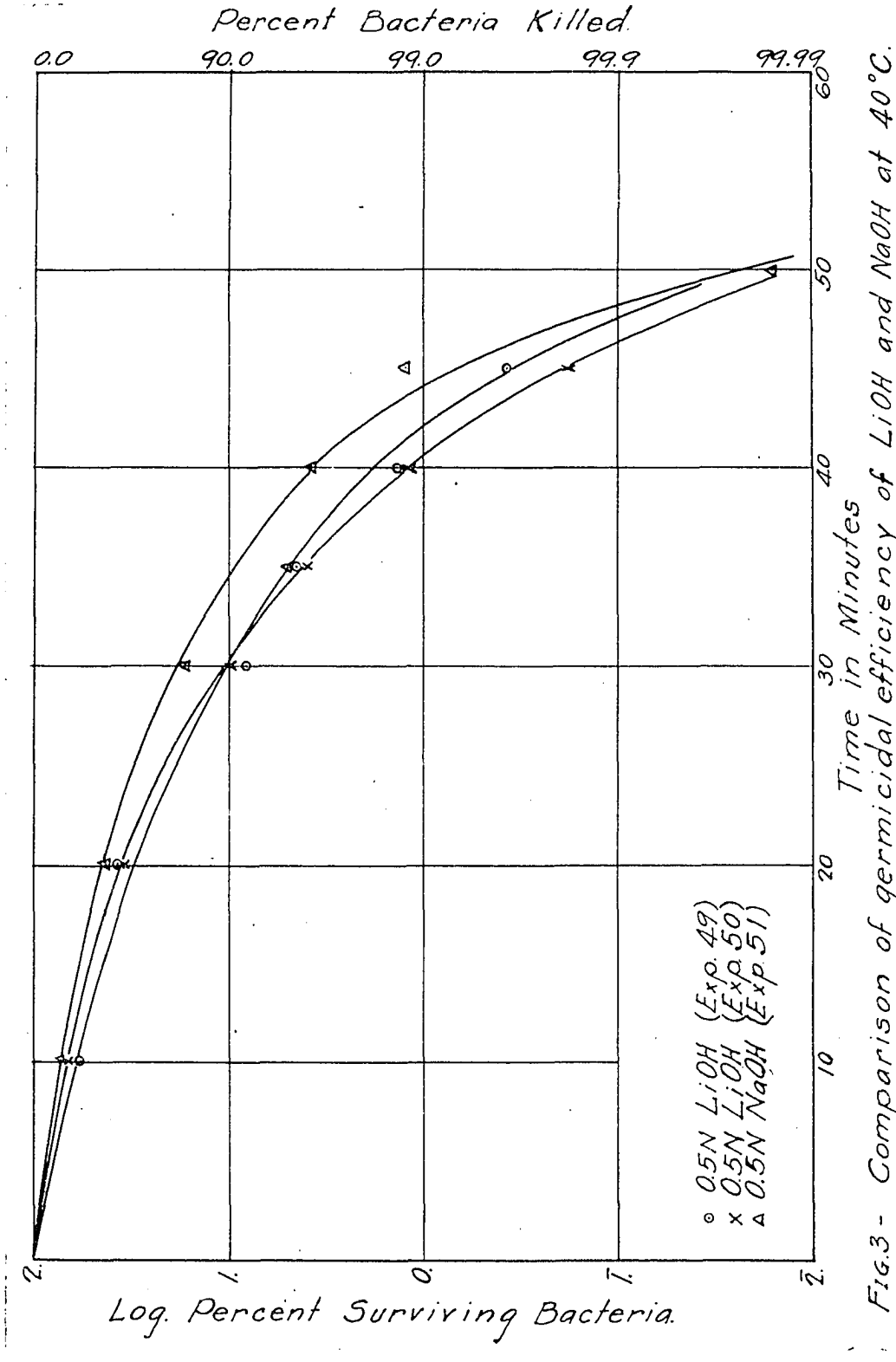


FIG. 3 - Comparison of germicidal efficiency of LiOH and NaOH at 40°C.

The new decrease in resistance in this series, (experiments 46 - 51) is another indication that this spore mixture, for some reason, was losing its vitality. As these spores had been stored for about a month over sulfuric acid, previous to this observation, it is suggested that absorption of fumes from the sulfuric acid might be responsible for the phenomenon of reduced resistance. If this is the case, storage over calcium chloride would be preferable.

3. Comparative germicidal efficiency of potassium hydroxide, sodium hydroxide, and a potassium hydroxide-sodium hydroxide mixture.

A solution of 0.5 N potassium hydroxide was tested against 0.5 N sodium hydroxide in the usual way. The third flask in each experiment contained 100 cubic centimeters of 0.5 N alkali, which was made up by adding 50 cubic centimeters of 0.5 N potassium hydroxide to 50 cubic centimeters of 0.5 N sodium hydroxide. Nine experiments were run in all, triplicate experiments being made on each of the three solutions mentioned.

The data obtained are given in tables IV, V, and VI, and plotted in figures 4, 5, and 6.

Discussion. From a consideration of the data of tables IV, V, and VI it will be seen that sodium hydroxide, potassium hydroxide, and an equal mixture of sodium and potassium hydroxides have equal germicidal power. Average killing times of 54.9 minutes, 54.8 minutes and 55.6 minutes respectively were found. These values show that the germicidal properties of these solutions are nearly identical.

Table IV

Showing Relative Germicidal Efficiency of Sodium Hydroxide, Potassium Hydroxide and a Mixture of Equal Quantities of Sodium and Potassium Hydroxides.+

Time in Minutes	Exp.No.13 4/18/30: 0.5N NaOH at 40°C: Surviving bacteria in 5 cc.		Exp.No.14 4/18/30: 0.5N Alkali (NaOH + KOH) at 40°C. Surviving bacteria in 5 cc.		Exp.No.15 4/18/30: 0.5N KOH at 40°C. Surviving bacteria in 5 cc.	
	Time in Minutes	Log % Survivors	Time in Minutes	Log % Survivors	Time in Minutes	Log % Survivors
0	1,100,000	2.00000	1,100,000	2.00000	1,100,000	2.00000
10	815,000	1.87056	887,500	1.90757	688,000	1.79699
20	387,500	1.54767	885,000	1.90630	505,000	1.66269
30	223,500	1.30868	271,300	1.39285	178,800	1.21177
35	170,500	1.19112	147,800	1.12907	140,300	1.10666
40	117,800	1.03055	109,300	0.99802	96,300	0.94303
45	83,000	0.87848	51,000	0.66697	50,000	0.65837
50	25,800	0.37102	32,800	0.47527	26,500	0.38265
55	1,575	$\bar{1}$.15668	1,550	$\bar{1}$.14973	1,575	$\bar{1}$.15668
60	0		0		0	
	K.T. = 55.5 Min.		K.T. = 55.8 Min.		K.T. = 56.0 Min.	

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	74.23	1.87056	80.82	1.90757	62.66	1.79699
20	35.29	1.54767	80.59	1.90630	45.99	1.66269
30	20.36	1.30868	24.71	1.39285	16.28	1.21177
35	15.53	1.19112	13.46	1.12907	12.78	1.10666
40	10.73	1.03055	9.95	0.99802	8.77	0.94303
45	7.56	0.87848	4.65	0.66697	4.55	0.65837
50	2.35	0.37102	2.99	0.47527	2.41	0.38265
55	0.14	$\bar{1}$.15668	0.14	$\bar{1}$.14973	0.14	$\bar{1}$.15668

+Using old spores.

Table V

Showing Relative Germicidal Efficiency of Sodium Hydroxide, Potassium Hydroxide, and a Mixture of Equal Quantities of Sodium and Potassium Hydroxides.[+]

Time in Minutes	Exp. No. 16 4/27/30: 0.5N NaOH at 40°C: Surviving bacteria in 5 cc.	Exp. No. 17 4/27/30: 0.5N Alkali (NaOH + KOH) at 40°C.	Exp. No. 18 4/27/30: 0.5N KOH at 40°C.
0	885,000	885,000	885,000
10	680,000	435,000	410,000
20	515,000	315,000	295,000
30	390,000	163,000	172,000
35	194,000	101,000	115,000
40	127,500	56,000	48,500
45	67,000	30,000	26,500
50	8,000	4,500	1,450
55	700	1,100	425
60	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	76.84	1.88557	49.15	1.69155	46.33	1.66584
20	58.19	1.76487	35.59	1.55137	33.33	1.52288
30	44.07	1.64412	18.42	1.26525	19.44	1.28859
35	21.92	1.34086	11.41	1.05738	12.99	1.11376
40	14.41	1.15857	6.33	0.80120	5.48	0.73880
45	7.57	0.87913	3.39	0.53018	2.99	0.47631
50	0.904	$\bar{1}$.95615	0.51	$\bar{1}$.70627	0.16	$\bar{1}$.21443
55	0.08	$\bar{2}$.89816	0.12	$\bar{1}$.09445	0.05	$\bar{2}$.68145
	K.T. = 54.5 Min.		K.T. = 55.8 Min.		K.T. = 53.8 Min.	

[+]Using old spores.

Table VI

Showing Relative Germicidal Efficiency of Sodium Hydroxide, Potassium Hydroxide, and a Mixture of Equal Quantities of Sodium and Potassium Hydroxides.[+]

Time in Minutes	Exp. No. 19 4/27/30: 0.5N NaOH at 40°C.		Exp. No. 20 4/27/30: 0.5N Alkali (NaOH + KOH) at 40°C.		Exp. No. 21 4/27/30 0.5N KOH at 40°C.	
	Surviving bacteria in 5 cc.					
0	1,023,000		1,023,000		1,023,000	
10	590,000		585,000		590,000	
20	443,000		440,000		350,000	
30	280,000		257,000		248,000	
35	193,000		119,000		145,000	
40	72,000		87,500		94,500	
45	44,000		42,500		55,500	
50	19,000		14,000		21,500	
55	800		700		850	
60	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	57.67	1.76097	57.19	1.75728	57.67	1.76097
20	43.30	1.63652	43.01	1.63357	34.21	1.53419
30	27.37	1.43728	25.12	1.40005	24.24	1.38457
35	18.87	1.27568	11.63	1.06567	14.17	1.15149
40	7.04	0.84745	8.55	0.93213	9.28	0.96555
45	4.30	0.63357	4.15	0.61851	5.43	0.73441
50	1.86	0.26887	1.37	0.13625	2.10	0.32256
55	0.08	$\bar{2}$.89321	0.07	$\bar{2}$.83522	0.083	$\bar{2}$.91954
	K.T. = 54.5 Min.		K.T. = 55.0 Min.		K.T. = 55.0 Min.	

[+]Using old spores.

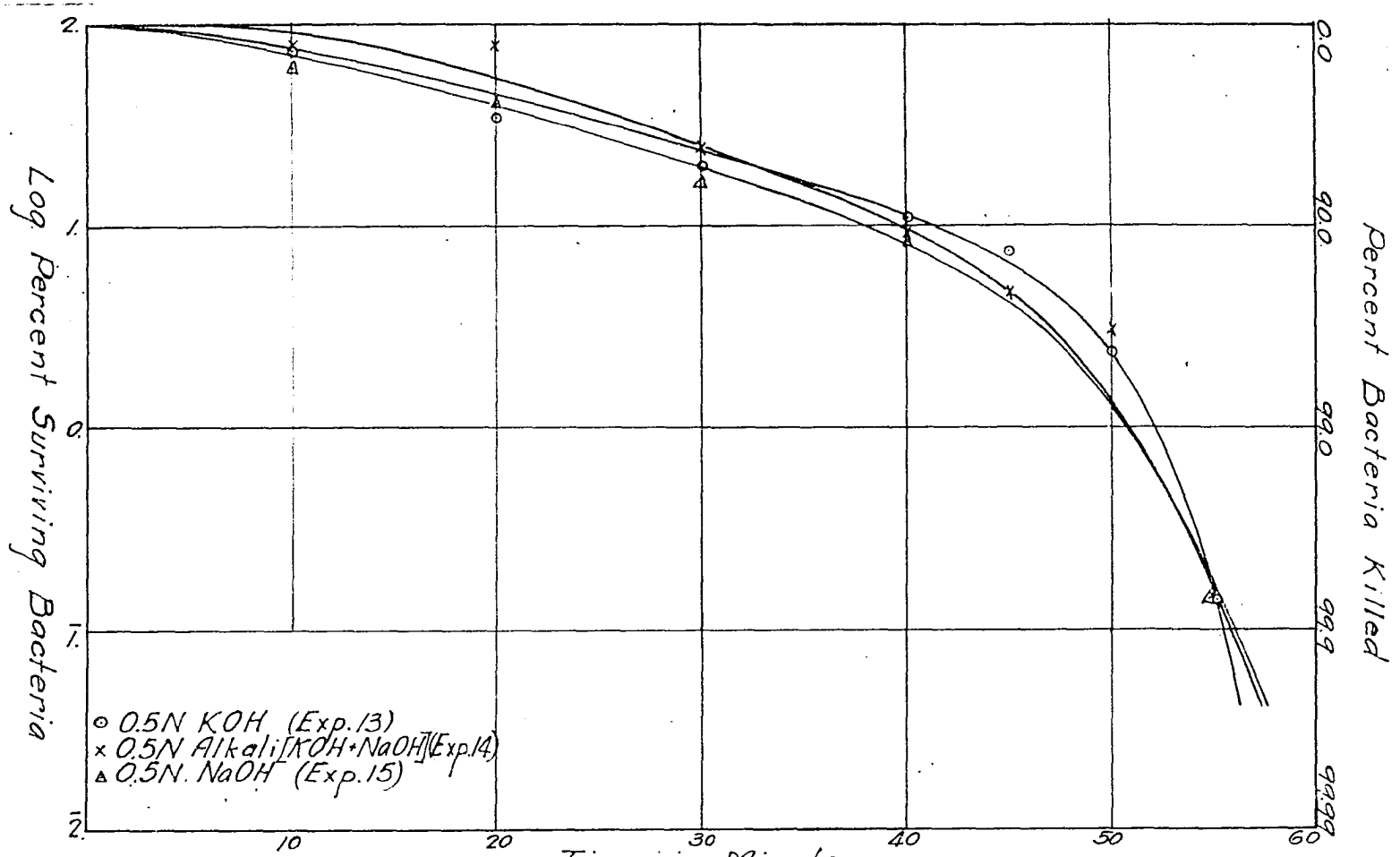


Fig. 4 - Comparison of germicidal efficiency of KOH and NaOH with a mixture of KOH and NaOH at 40°C.

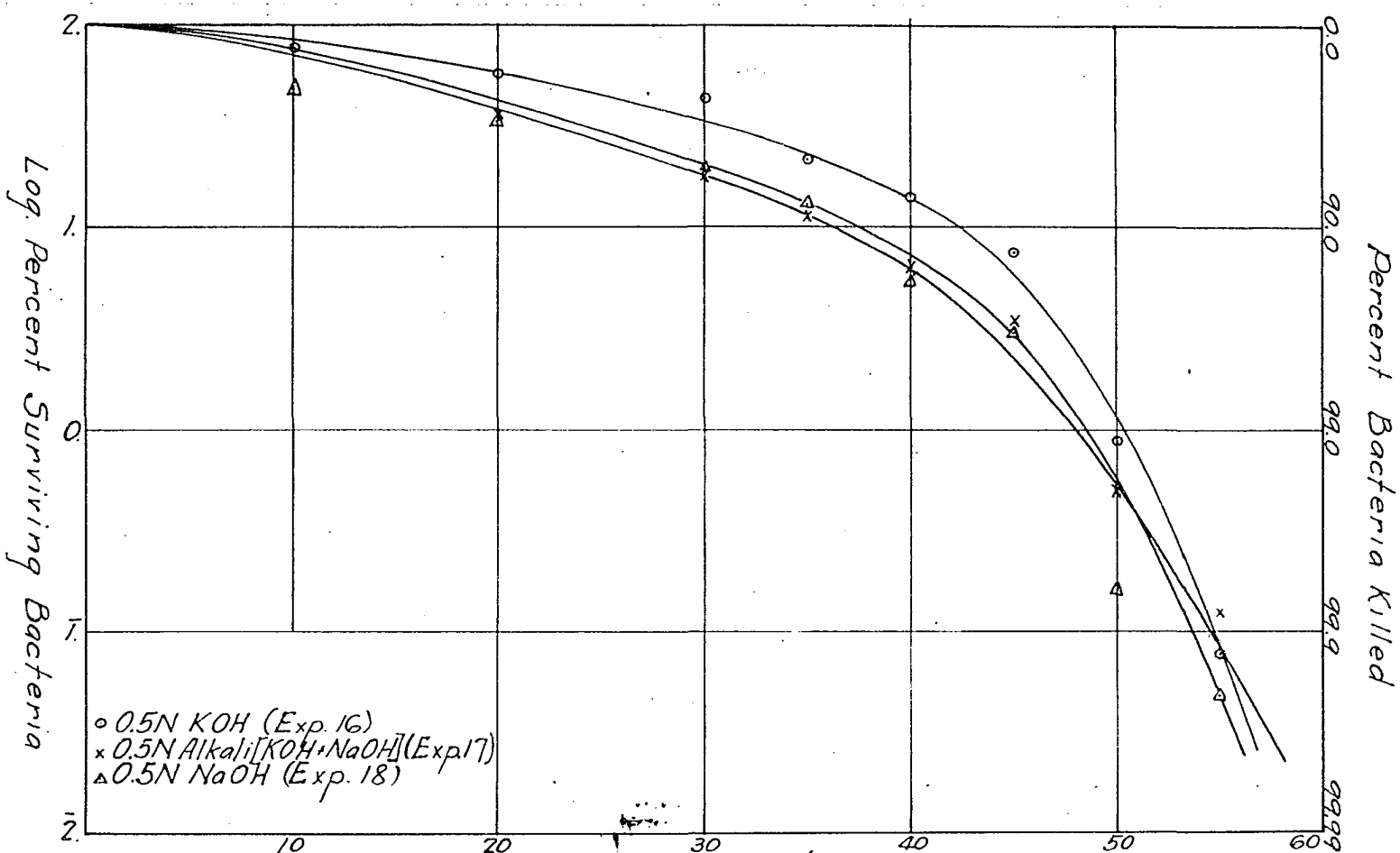


FIG. 5- Comparison of germicidal efficiency of KOH and NaOH with a mixture of KOH and NaOH at 40°C.

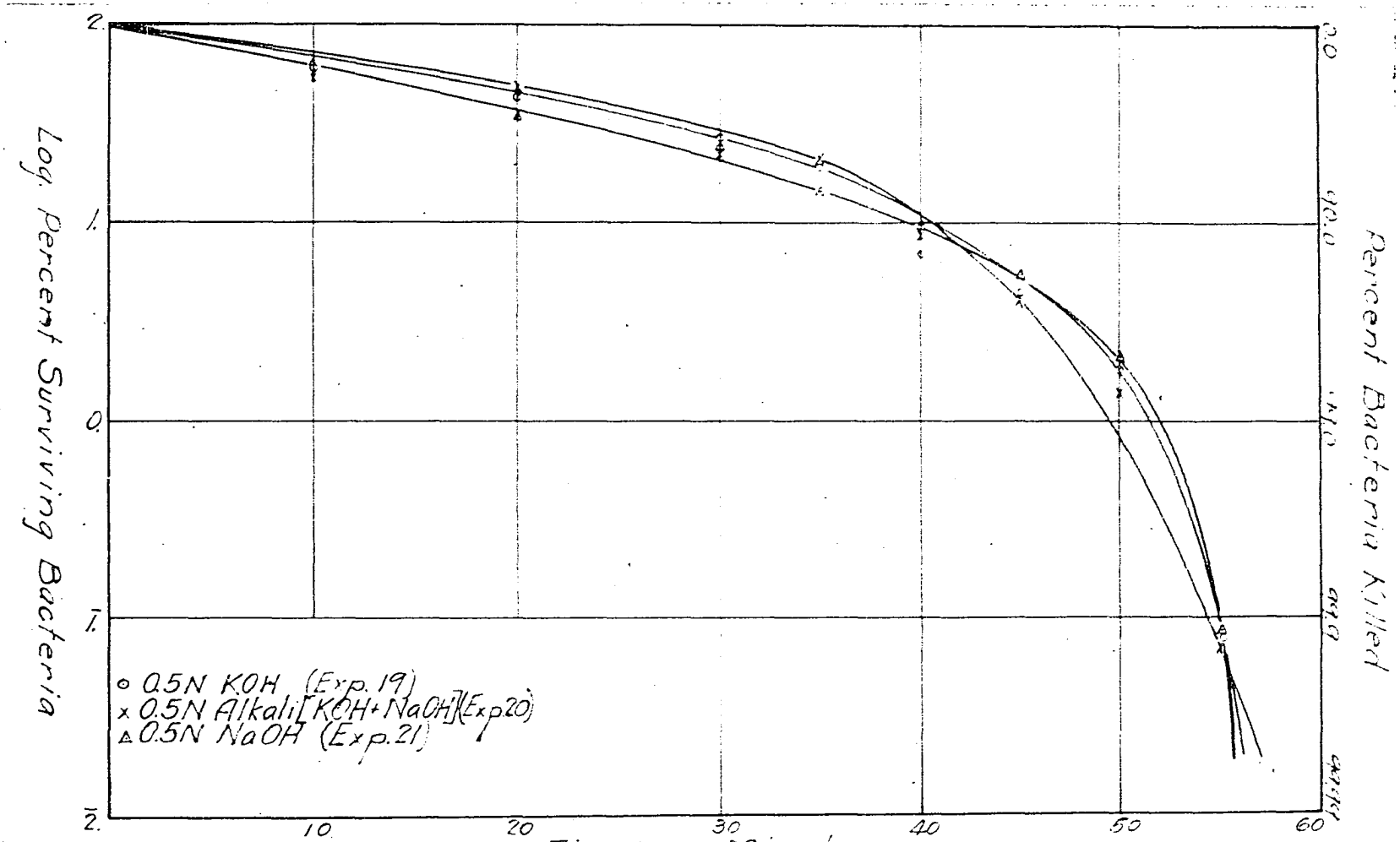


FIG. 6 - Comparison of the germicidal efficiency of KOH and NaOH with a mixture of KOH and NaOH at 40°C.

4. Comparative germicidal efficiency of rubidium and sodium hydroxides.

The technique used in this experiment was slightly different from the usual technique. Since only ten grams of rubidium hydroxide were available, it was necessary to reduce the scale of the experiment to one fifth of the usual values. The number of spores used for inoculation, 20,000,000 per cubic centimeter, was the same. The plate counts obtained were therefore of the same order. Special flasks of the same design as shown in plate 1, were made from 50 cubic centimeter distilling flasks, by cutting off the upper part of the neck including the side arm, and sealing on the auxiliary neck. Nineteen cubic centimeters of the test solutions were used in each flask. This was made up to such a strength, that the introduction of one cubic centimeter of spore suspension brought the concentration of the solution to exactly 0.5 normal.

From these 50 cubic centimeter flasks containing the 0.5 N alkali, were removed one cubic centimeter portions at regular intervals to determine the number of viable organisms. These one cubic centimeter portions were added to nine cubic centimeters of dilute sulfuric acid of sufficient strength to just neutralize the alkali added.

The error likely to be introduced into bacterial counts, by the touching of the pipette, in withdrawing the portions for plating, to the sides of the neck of the flask was found

to be greater in this small scale experiment. This would be expected from a consideration of the relative number of bacteria per unit of volume. One series of experiments with rubidium hydroxide was discarded because the counts obtained were not concordant. In the following experiments the precaution mentioned above, with regard to removing the portions of the test solution for plating from the flask, was carefully observed.

The results obtained in four experiments with rubidium hydroxide and two control flasks of sodium hydroxide are shown in tables VII and VIII. These data are plotted in figures 7 and 8.

Discussion. From a consideration of the data found in tables VII and VIII, it will be seen that 0.5 N rubidium hydroxide is very similar in germicidal properties to 0.5 N sodium hydroxide. Similar reduction in numbers of bacteria are found in the same time intervals, although it must be admitted that the small scale of these experiments is conducive towards errors in bacterial counts and greater variations in counts should be expected. It is noticeable in these small scale experiments that the initial reduction of numbers of bacteria present after ten minutes exposure to the 0.5 N alkali is greater than in experiments on the usual scale.

The average killing time of rubidium hydroxide in four experiments is 33.9 minutes, while the sodium hydroxide controls have an average killing time of 34.0 minutes. No dif-

Table VII

Showing the Relative Germicidal Efficiency of Rubidium Hydroxide and Sodium Hydroxide.+

Time in Minutes	Exp.No.111 7/26/30: 0.5N RbOH at 50°C.		Exp.No.112 7/26/30: 0.5N RbOH at 50°C.		Exp.No.113 7/26/30 0.5N NaOH at 50°C.	
	Surviving bacteria in 1 cc.					
0	1,180,000		1,180,000		1,180,000	
10	339,000		327,000		356,000	
15	163,000		193,000		316,000	
20	94,000		144,000		181,000	
25	75,000		78,000		54,000	
30	10,600		7,900		12,000	
35	1,500		1,500		100	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	28.73	1.45832	27.71	1.44267	30.09	1.47835
15	13.81	1.14031	16.36	1.21368	26.84	1.42881
20	7.97	0.90125	12.20	1.08648	15.34	1.18580
25	6.36	0.80318	6.61	0.82021	4.58	0.66051
30	0.90	$\bar{1}$.95345	0.67	$\bar{1}$.82575	1.02	0.00730
35	0.13	$\bar{1}$.10421	0.13	$\bar{1}$.10421	0.01	$\bar{3}$.92812
	K.T. = 35.8 Min.		K.T. = 35.5 Min.		K.T. = 35.0 Min.	

+Using new spores.

Table VIII

Showing Relative Germicidal Efficiency of Rubidium Hydroxide and Sodium Hydroxide.+

Time in Minutes	Exp. No. 114 7/26/30: 0.5N RbOH at 50°C.		Exp. No. 115 7/26/30: 0.5N RbOH at 50°C.		Exp. No. 116 7/26/30 0.5N NaOH at 50°C.	
	Surviving bacteria in 1 cc.					
0	1,100,000		1,100,000		1,100,000	
10	320,000		327,000		420,000	
15	147,000		146,000		309,000	
20	123,000		102,000		236,000	
25	24,900		32,500		42,000	
30	8,000		2,300		2,250	
35	100		150		100	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	29.09	1.46376	29.73	1.47316	38.18	1.58186
15	13.37	1.12593	13.27	1.12296	28.09	1.44857
20	11.18	1.04852	9.27	0.96721	21.45	1.33152
25	2.26	$\bar{0}$.35481	2.96	$\bar{0}$.47049	3.82	$\bar{0}$.58186
30	0.73	$\bar{1}$.86170	0.21	$\bar{1}$.32034	0.20	$\bar{1}$.29079
35	0.01	$\bar{3}$.95861	0.14	$\bar{2}$.13470	0.01	$\bar{3}$.95861
	K.T. = 32.0 Min.		K.T. = 32.5 Min.		K.T. = 32.8 Min.	

+Using new spores.

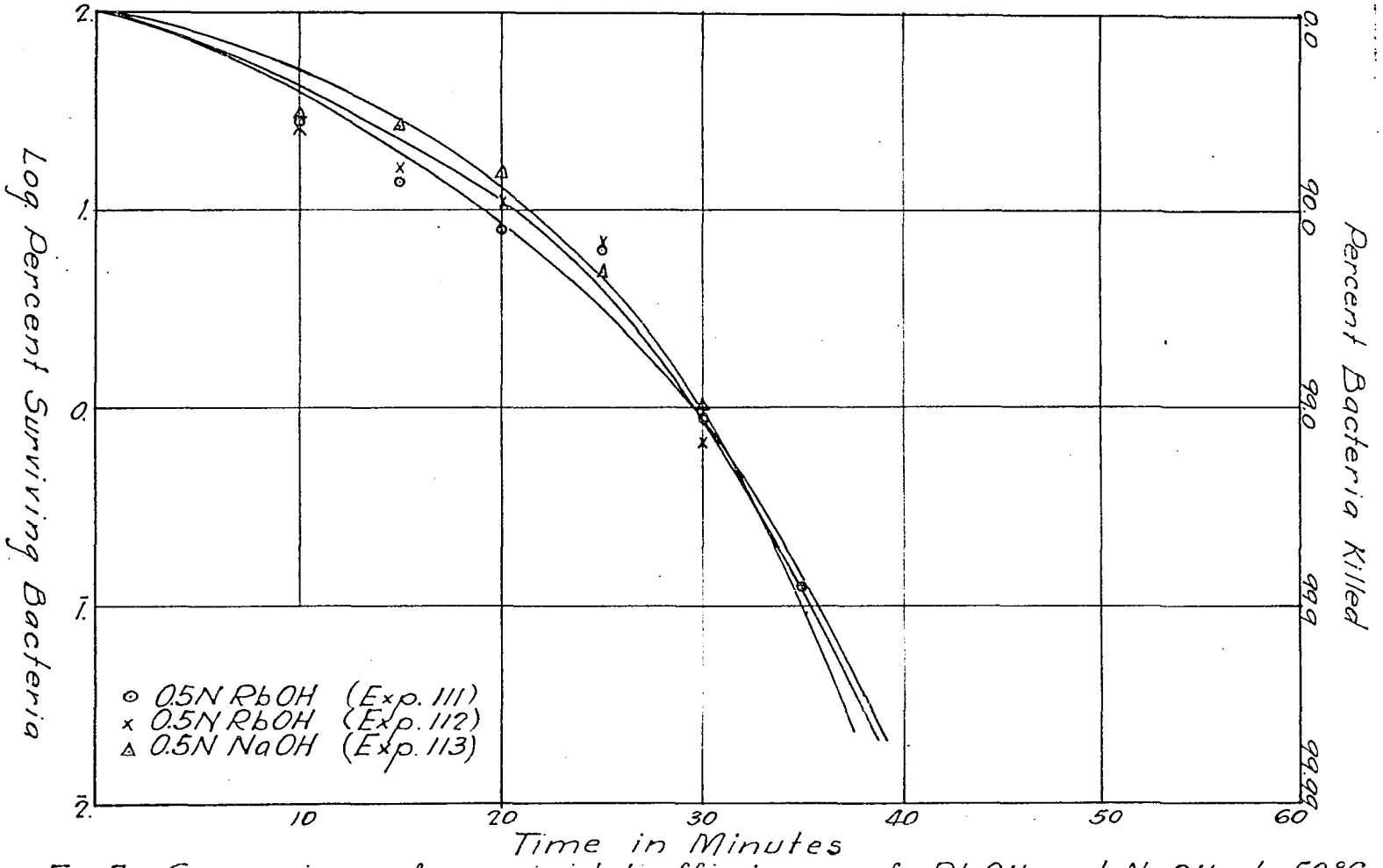


FIG. 7 - Comparison of germicidal efficiency of RbOH and NaOH at 50°C.

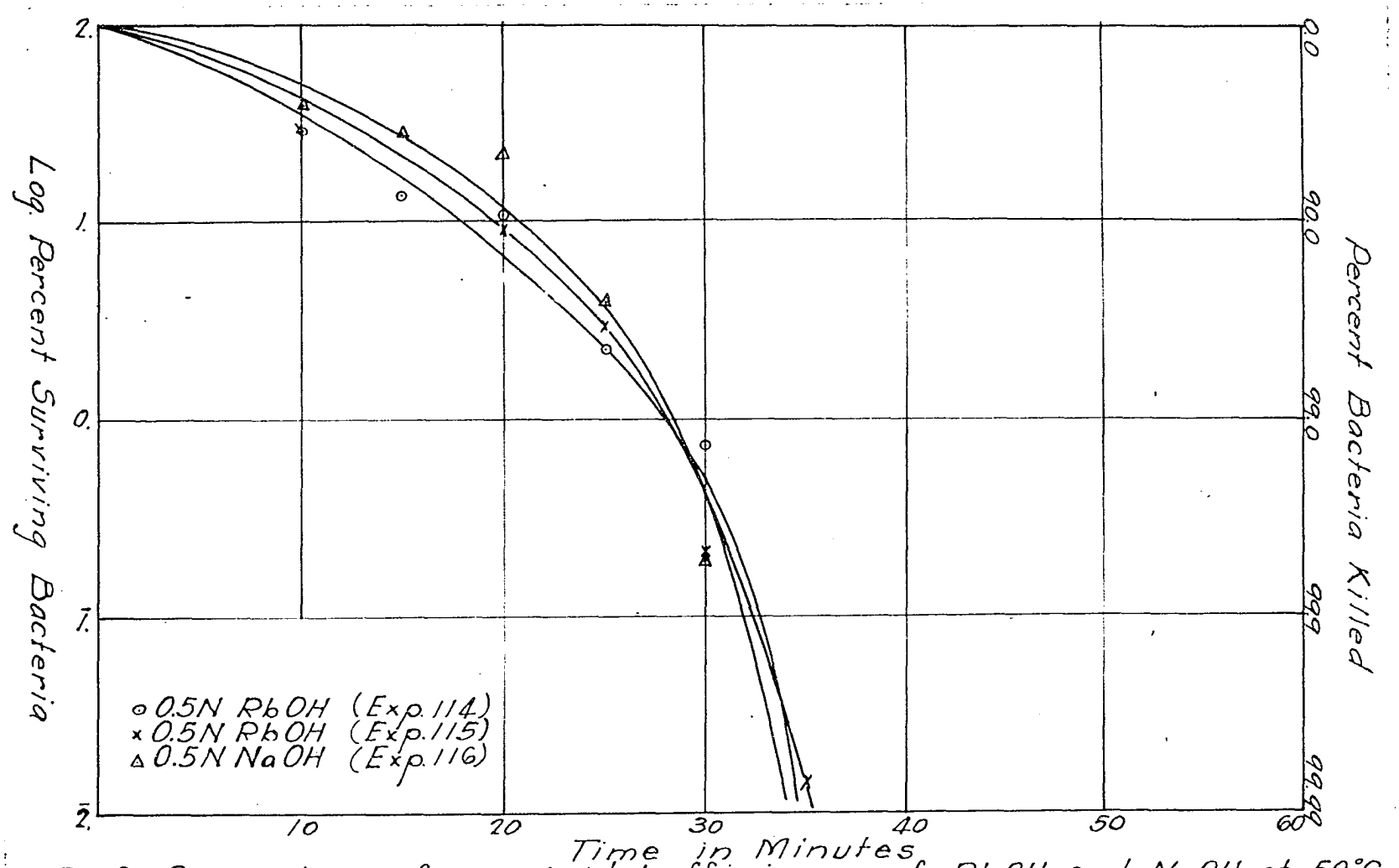


FIG. 8 - Comparison of germicidal efficiency of RbOH and NaOH at 50°C.

ference was found in the comparative germicidal efficiencies of the two alkalies.

General Discussion of Experiments with Caustic Alkalies

From a consideration of the data given in the preceding tables it is evident that solutions of lithium, sodium, potassium, and rubidium hydroxides at the same normality and temperature possess equal germicidal powers towards organism No.25. Differences of two or three minutes in killing times, as were observed are not considered significant as they are well within the limit of experimental error. These results confirm the work of Paul and Krönig (12) in which they reported lithium, sodium and potassium hydroxides in equal molecular concentrations to be equally efficient as germicidal agents.

B. Effect of the Addition of Salts upon the Germicidal Efficiency of Sodium Hydroxide.

The technique used is the same as described above. The salts used in these experiments were of reagent grade. The sodium chloride, sodium nitrate, sodium sulfate, and sodium carbonate were anhydrous salts. The trisodium phosphate and sodium silicate used, contained twelve and five molecules of water of crystallization respectively. With the exception of the sodium silicate, whose behavior on drying was not known, the salts mentioned were dried at 110°C. for six days, to remove all of the water contained. In the case of the sodium phosphate which contained a large amount of water, the efficiency of the drying process was determined by an analysis of the dried salt. A portion of the salt was dissolved in water and titrated with a standard acid, by the method of Smith (14), using phenolphthalein and methyl orange indicators.

This titration was carried on at 55°C. at which temperature the end points, of both indicators used, were at the correct place. From the titration values found, the percent of Na_3PO_4 approached 99.9%, although it is stated in a laboratory handbook (1) that only 11 molecules of water of crystallization are lost at 100°C. It is possible that some decomposition took place.

Since the killing time of sodium hydroxide was taken to be the standard for reference, the three flasks of each run

consisted of two flasks to which different amounts of the dry salt had been added and a control flask of sodium hydroxide. In the tables in which the results are given, the duplicate experiments are reported in the same table although performed in different runs made the same day. For each of the six sodium salts which were to be tested, two amounts of the salt were used. It was the intention to add to the first flask of each run such an amount of the salt so that one-half as much sodium would be added as was contained in the 100 cubic centimeters of 0.5 N sodium hydroxide to be used. In the second flask twice the amount used in the first flask was used. This latter amount provided an equivalent amount of sodium compared to the quantity contained in the 100 cubic centimeters of 0.5 N sodium hydroxide which was to be subsequently added. In this way it might be possible to determine whether or not the salt effect was a molecular effect, i.e., depending upon the number of molecules added, and also to determine the order in which the salts were most effective in reducing the killing time of sodium hydroxide solutions.

1. The effect of the addition of sodium chloride upon the germicidal properties of sodium hydroxide.

Into the first flask of each run there was carefully weighed 1.4613 grams (0.025 mols) of the dried salt. To this, 100 cubic centimeters of 0.5 N sodium hydroxide was added and the flask, with contents, sterilized for 20 mimutes at 20 pounds pressure. When cool, the flask was placed in the water bath, allowed to come to the temperature of the bath and the one cubic centimeter of spore suspension added. Five cubic centimeter portions were withdrawn at regular intervals and the number of viable organisms determined. Into the second flask of the run was placed 2.9225 grams (0.05 mols) of the sodium chloride, the 100 cubic centimeters of 0.5 N sodium hydroxide added and the flask with contents treated in the same fashion as the first flask. Sodium hydroxide controls were also run in each of the duplicate experiments.

The results of these experiments are given in tables IX and X. These values are also shown in figure 9 in which the logarithms for the percent survivors are plotted and an average curve drawn. The killing time from the individual curves (which are not shown) will be found at the end of each table.

For the sake of comparison the germicidal effect of 0.05 mols of NaCl dissolved in water at 60° is shown in figure 9 by the broken line. The data for this curve were taken from the work of Levine, Toulouse, and Buchanan (6).

Discussion. The killing time for 0.5 N sodium hydroxide

which was found to be 35.4 minutes, was reduced to 31.2 minutes by the addition of 0.025 mols of sodium chloride and to 25.3 minutes by the addition of 0.05 mols of sodium chloride. It appears that the addition of the second 0.025 mols of sodium chloride was as effective or a little more so than the first 0.025 mols but the difference is within the experimental error.

Table IX

Showing the Effect of the Addition of Sodium Chloride on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp.No.55 6/19/30: 0.5N NaOH + 0.025 Mols NaCl at 50°C	Exp.No.58 6/19/30: 0.5N NaOH + 0.025 Mols NaCl at 50°C	Exp.No.57 6/19/30 0.5N NaOH at 50°C
	Surviving bacteria in 5 cc.		
0	910,000	1,065,000	910,000
10	635,000	450,000	775,000
15	450,000	330,000	710,000
20	153,000	145,000	485,000
25	18,000	40,000	315,000
30	1,750	3,000	25,000
35	500	500	1,000
40	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	69.78	1.84373	42.25	1.62586	85.16	1.93026
15	49.45	1.69417	30.99	1.49116	78.02	1.89222
20	16.81	1.22560	13.66	1.13551	53.30	1.72670
25	1.98	$\bar{0}$.29623	3.76	$\bar{0}$.57471	34.61	1.53927
30	0.19	$\bar{1}$.28400	0.28	$\bar{1}$.44977	2.75	$\bar{0}$.43890
35	0.05	$\bar{2}$.73993	0.05	$\bar{2}$.73993	0.11	$\bar{1}$.04096
	K.T. = 31.0 Min.		K.T. = 31.3 Min.		K.T. = 35.3 Min.	

[+]Using new spores.

Table X

Showing the Effect of the Addition of Sodium Chloride on the Germicidal Efficiency of Sodium Hydroxide.+

Time in Minutes	Exp. No. 56 6/19/30: 0.5N NaOH + 0.05 Mols NaCl at 50°C		Exp. No. 59 6/19/30: 0.5N NaCl + 0.05 Mols NaCl at 50°C		Exp. No. 60 6/19/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	910,000		1,065,000		1,065,000	
10	495,000		485,000		700,000	
15	195,000		335,000		515,000	
20	33,500		38,500		340,000	
25	500		2,500		245,000	
30	0		0		42,500	
35	0		0		1,250	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	54.40	1.73557	45.54	1.65839	65.73	1.81775
15	21.43	1.33099	31.45	1.49769	48.36	1.68446
20	3.63	0.56600	3.62	0.55811	31.93	1.50413
25	0.05	$\bar{2}$.73993	0.23	$\bar{1}$.37059	23.01	1.36182
30					3.99	0.60104
35					0.12	$\bar{1}$.06956
	K.T. = 24.8 Min.		K.T. = 25.8 Min.		K.T. = 35.5 Min.	

+Using new spores.

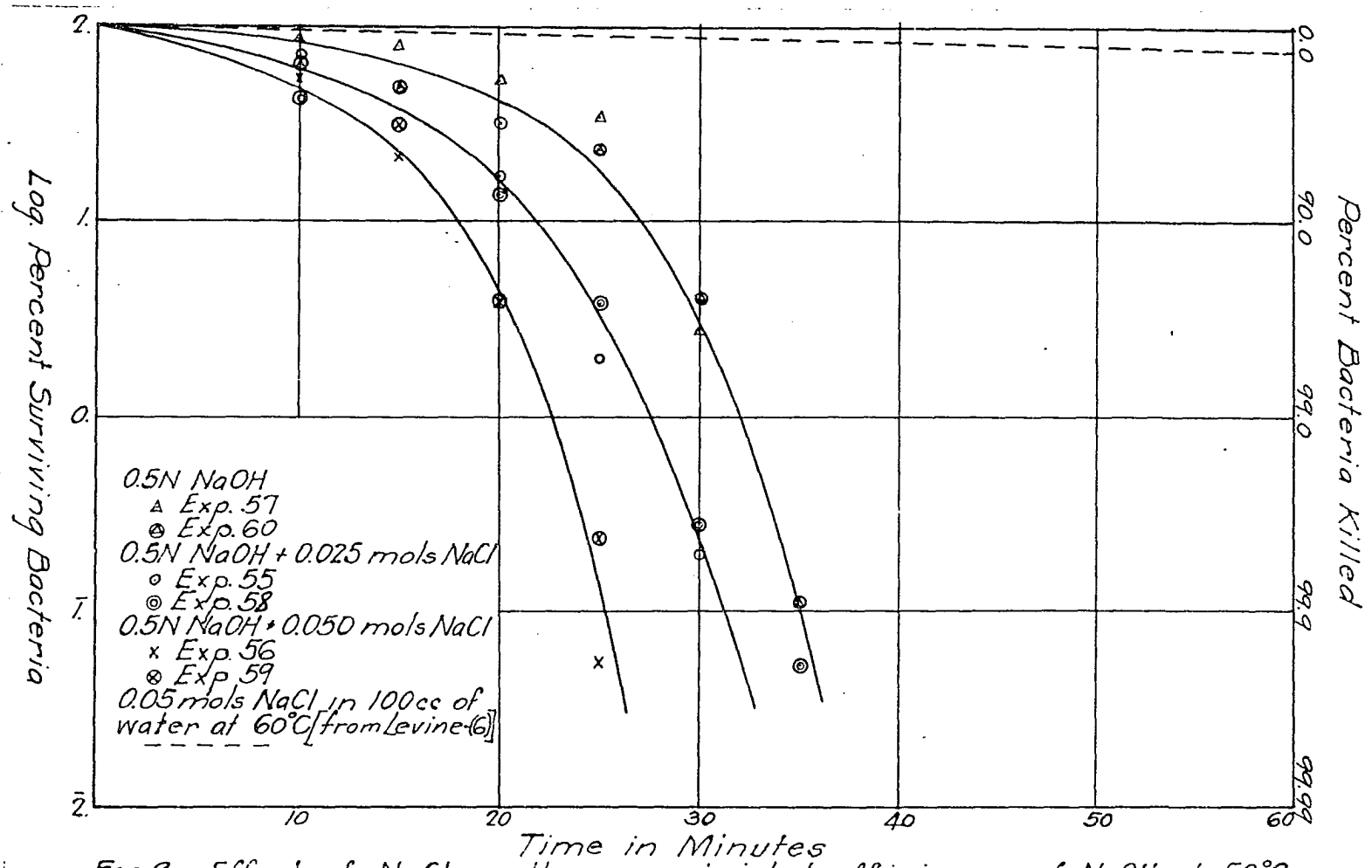


FIG. 9 - Effect of NaCl on the germicidal efficiency of NaOH at 50°C.

2. The effect of the addition of sodium nitrate upon the germicidal properties of sodium hydroxide.

The effect of the addition of sodium nitrate upon the germicidal power of sodium hydroxide was tested in the same manner as described in the tests with sodium chloride.

Of the dry salt, 0.025 mol (2.0253 grams) and 0.05 mol (4.0505 grams) were added to 100 cubic centimeter portions of 0.5 N sodium hydroxide and the number of viable organisms determined at regular intervals.

The data obtained are given in tables XI and XII and plotted in figure 10.

The germicidal power of 0.05 mol of $NaNO_3$ in 100 cubic centimeters of water is shown by the dotted line. A reduction of less than 15% was found after one hour's exposure.

Discussion. The average killing time of 35.7 minutes for 0.5 N sodium hydroxide solutions was reduced to 30.3 minutes by 0.025 mol of sodium nitrate. A further reduction to 24.9 minutes was found by the use of 0.05 mol of sodium nitrate. These results agree closely with the results obtained by similar molecular quantities of sodium chloride.

Table XI

Showing the Effect of the Addition of Sodium Nitrate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 61 6/23/30: 0.5N NaOH + 0.025 Mols $NaNO_3$ at 50°C		Exp. No. 64 6/23/30: 0.5N NaOH + 0.025 Mols $NaNO_3$ at 50°C		Exp. No. 63 6/23/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	995,000		850,000		995,000	
10	625,000		590,000		825,000	
15	390,000		360,000		500,000	
20	136,000		98,500		340,000	
25	9,500		6,500		118,000	
30	2,000		500		11,500	
35	0		0		250	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	62.81	1.79806	69.41	1.84143	82.91	1.91863
15	39.20	1.59324	42.35	1.62688	50.25	1.70115
20	13.67	$\bar{1}$.13572	11.59	$\bar{1}$.06402	34.17	1.53366
25	0.95	$\bar{1}$.97990	0.76	$\bar{1}$.88349	11.86	1.07406
30	0.20	$\bar{1}$.30321	0.06	$\bar{2}$.76955	1.16	0.06288
35					0.03	$\bar{2}$.40012
	K.T. = 30.8 Min.		K.T. = 29.7 Min.		K.T. = 35.3 Min.	

[+]Using new spores.

Table XII

Showing the Effect of the Addition of Sodium Nitrate on the Germicidal Efficiency of Sodium Hydroxide.+

Time in Minutes	Exp. No. 62 6/23/30: 0.5N NaOH + 0.05 Mols $NaNO_3$ at 50°C	Exp. No. 65 6/23/30: 0.5N NaOH + 0.05 Mols $NaNO_3$ at 50°C	Exp. No. 66 6/23/30 0.5N NaOH at 50°C
	Surviving bacteria in 5 cc.		
0	995,000	850,000	850,000
10	470,000	525,000	610,000
15	190,000	225,000	355,000
20	40,500	31,000	255,000
25	650	450	121,500
30	0	0	24,000
35	0	0	4,000
40	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	47.24	1.67428	61.76	1.79074	71.77	1.85591
15	19.10	1.28093	26.47	1.42276	41.77	1.62081
20	4.07	0.60964	3.65	0.56194	30.00	1.47712
25	0.07	$\bar{2}$.81509	0.53	$\bar{2}$.72379	14.30	1.15516
30					2.82	0.45079
35					0.47	$\bar{1}$.67264
	K.T. = 25.0 Min.		K.T. = 24.8 Min.		K.T. = 36.0 Min.	

+Using new spores.

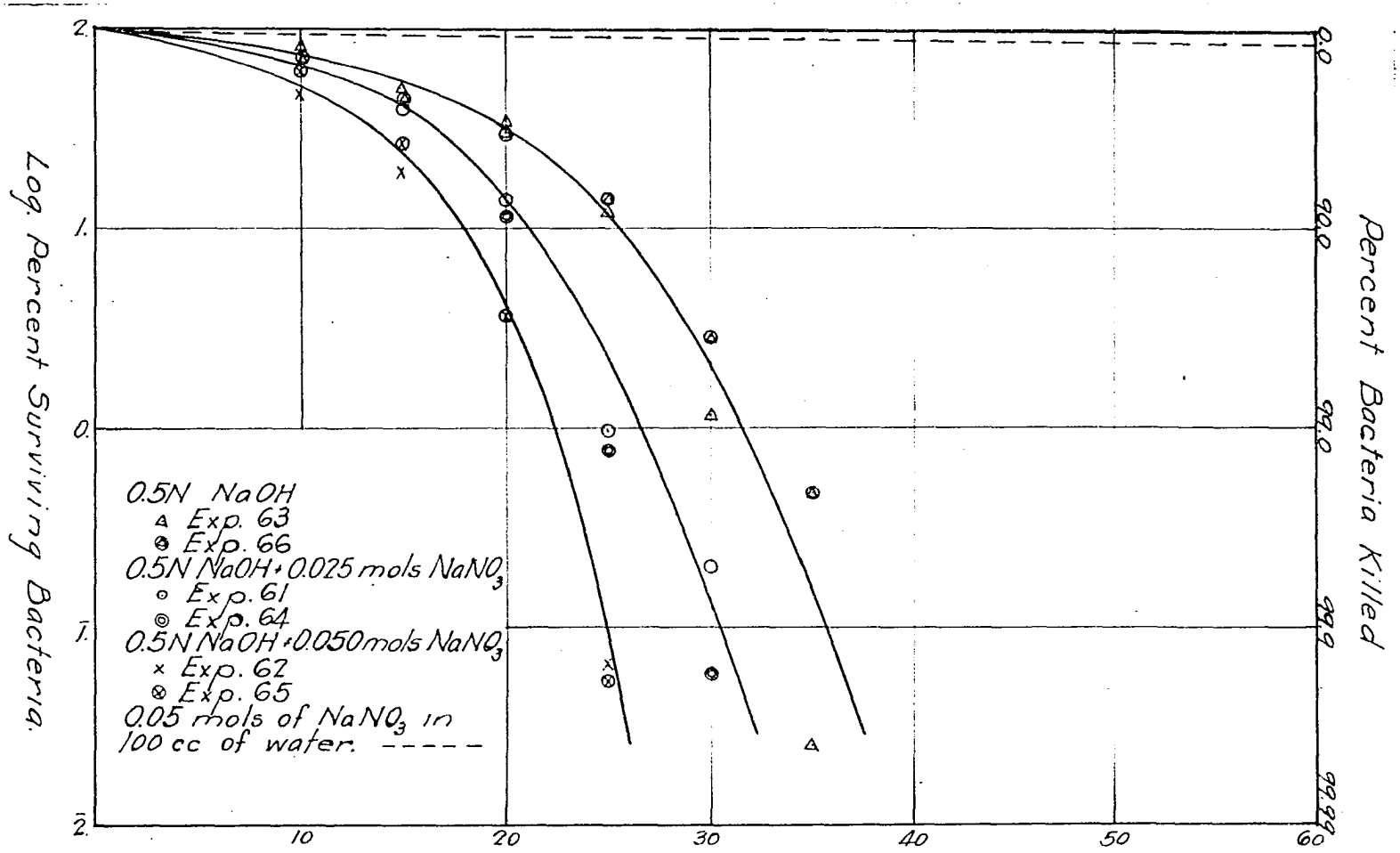


FIG. 10 - Effect of $NaNO_3$ on the germicidal efficiency of NaOH at 50°C.

3. The effect of added sodium carbonate upon the germicidal properties of sodium hydroxide.

Sodium carbonate was tested in the same way that sodium nitrate and sodium chloride were tested.

Of the dry salt, 0.0125 mols (1.3250 grams) and 0.025 mols (2.6500 grams) of sodium carbonate were added to the 100 cubic centimeter portions of 0.5 N sodium hydroxide and the numbers of organisms determined at regular intervals.

The data obtained are given in tables XIII and XIV. These values are plotted in figure 11.

The germicidal effect of 0.05 mols of Na_2CO_3 in 100 cubic centimeters of water at 60°C is shown in figure 11 by the broken line. This curve was taken from the data given by Levine, Toulouse and Buchanan (6).

Discussion. The average killing time of 35.6 minutes for 0.5 N sodium hydroxide solutions was reduced to 29.5 minutes by the introduction of 0.0125 mols of sodium carbonate. A reduction to 25.5 minutes was found by the use of 0.025 mols of sodium carbonate. These reductions in killing time are similar to those found by the use of sodium nitrate and sodium chloride in the preceding two experiments.

Table XIII

Showing the Effect of the Addition of Sodium Carbonate on the Germicidal Efficiency of Sodium Hydroxide.†

Time in Minutes	Exp.No.73 6/29/30: 0.5N NaOH + 0.0125 Mols Na_2CO_3 at 50°C		Exp.No.76 6/29/30: 0.5N NaOH + 0.0125 Mols Na_2CO_3 at 50°C		Exp.No.75 6/29/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	1,475,000		1,385,000		1,475,000	
10	1,073,000		840,000		1,190,000	
15	823,000		690,000		940,000	
20	340,000		209,500		575,000	
25	28,500		21,000		220,000	
30	750		750		19,500	
35	0		0		100	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	72.75	1.86181	60.65	1.78283	80.68	1.90676
15	55.80	1.74661	49.82	1.69740	63.73	1.80434
20	23.05	1.36269	15.13	1.17973	38.98	1.59088
25	1.93	0.28605	1.52	0.18177	14.92	1.17363
30	0.05	$\bar{2}$.70627	0.05	$\bar{2}$.73361	1.32	0.12124
35					0.01	$\bar{3}$.83121
	K.T. = 29.5 Min.		K.T. = 29.5 Min.		K.T. = 35.5 Min.	

†Using new spores.

Table XIV

Showing the Effect of the Addition of Sodium Carbonate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 74 6/29/30: 0.5N NaOH + 0.025 Mols Na_2CO_3 at 50°C	Exp. No. 77 6/29/30: 0.5N NaOH + 0.025 Mols Na_2CO_3 at 50°C	Exp. No. 78 6/29/30 0.5N NaOH at 50°C
	Surviving bacteria in 5 cc.		
0	1,475,000	1,385,000	1,385,000
10	930,000	750,000	800,000
15	785,000	485,000	620,000
20	83,000	56,000	475,000
25	3,000	2,000	199,000
30	0	0	25,000
35	0	0	200
40	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	63.05	1.79969	54.15	1.73361	57.74	1.76164
15	53.22	1.72608	35.02	1.54429	44.77	1.65094
20	5.63	0.75029	4.04	0.60674	34.30	1.53524
25	0.20	$\bar{1}$.30833	0.14	$\bar{1}$.15958	15.05	1.15740
30					1.81	0.25649
35					0.01	$\bar{2}$.15958
	K.T. = 25.5 Min.		K.T. = 25.5 Min.		K.T. = 35.8 Min.	

[+]Using new spores.

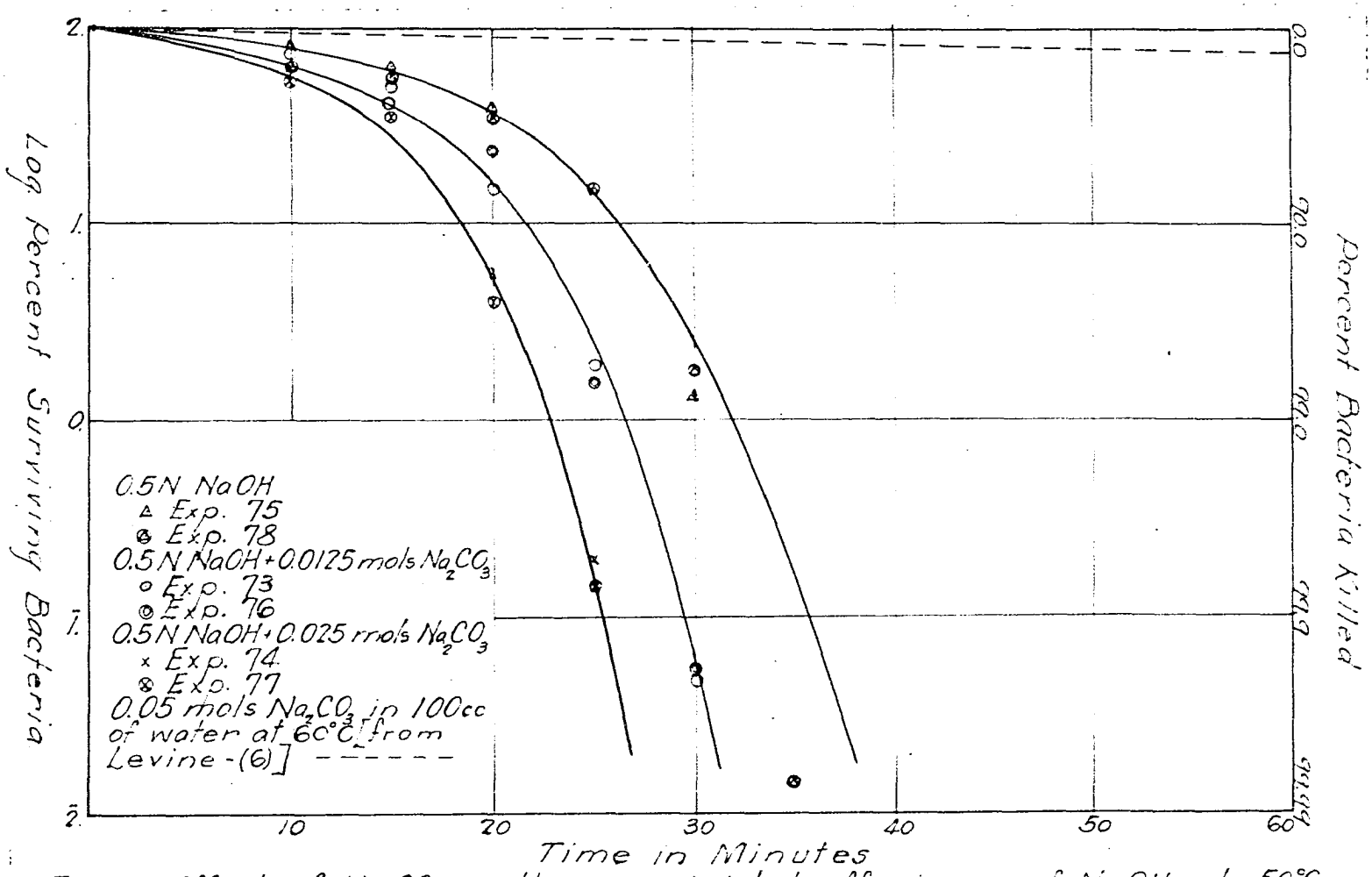


FIG II - Effect of Na_2CO_3 on the germicidal efficiency of NaOH at 50°C

4. The effect of the addition of sodium sulfate upon the germicidal properties of sodium hydroxide.

Sodium sulfate was tested in the same manner as the other salts.

Of the dry salt, 0.0125 mols (1.7758 grams) and 0.025 mols (3.5515 grams) of Na_2SO_4 were added to the 100 cubic centimeter portions of sodium hydroxide. The number of viable organisms was determined at regular intervals and the results are given in tables XV and XVI. These data are plotted in figure 12.

The germicidal power of 0.025 mols of Na_2SO_4 in 100 cubic centimeters of water is shown by the broken line in figure 12. A reduction of less than 15% was observed after one hour's exposure.

Discussion. The average killing time of 0.5 normal sodium hydroxide in these experiments was 35.2 minutes. This was reduced to 29.3 minutes by the addition of 0.0125 mols sodium sulfate. A reduction to 25.6 minutes by 0.025 mols of sodium sulfate was found. These results agree closely with the reduction in killing time found by the use of sodium chloride in previous experiments.

Table XV

Showing the Effect of the Addition of Sodium Sulfate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 91 7/11/30: 0.5N NaOH + 0.0125 Mols Na_2SO_4 at 50°C		Exp. No. 94 7/11/30: 0.5N NaOH + 0.0125 Mols Na_2SO_4 at 50°C		Exp. No. 93 7/11/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	803,500		900,000		803,500	
10	405,000		545,000		575,000	
15	305,000		350,000		300,000	
20	53,000		49,500		155,000	
25	------		2,500		54,000	
30	400		250		5,000	
35	0		0		250	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	50.40	1.70247	60.56	1.78216	71.56	1.85468
15	37.96	1.57931	38.89	1.58983	37.34	1.57213
20	6.60	0.81929	5.50	0.74037	19.29	1.28534
25	----	--------	0.28	$\bar{1}$.44370	6.72	0.82740
30	0.05	$\bar{2}$.69707	0.03	$\bar{2}$.44370	0.62	$\bar{1}$.79398
35					0.03	$\bar{2}$.49295
	K.T. = 29.0 Min.		K.T. = 29.5 Min.		K.T. = 34.7 Min.	

[+]Using new spores.

Table XVI

Showing the Effect of the Addition of Sodium Sulfate on the Germicidal Efficiency of Sodium Hydroxide.

Time in Minutes	Exp. No. 92 7/11/30: 0.5N NaOH + 0.025 Mols Na_2SO_4 at 50°C	Exp. No. 95 7/11/30: 0.5N NaOH + 0.025 Mols Na_2SO_4 at 50°C	Exp. No. 93 7/11/30 0.5N NaOH at 50°C
	Surviving bacteria in 5 cc.		
0	803,500	900,000	900,000
10	460,000	510,000	740,000
15	156,500	184,000	450,000
20	7,000	12,000	295,000
25	1,000	------	70,500
30	0	0	2,500
35	0	0	500
40	0	0	0
Time in Minutes	% Survivors / Log % Survivors	% Survivors / Log % Survivors	% Survivors / Log % Survivors
0	100.00 / 2.00000	100.00 / 2.00000	100.00 / 2.00000
10	57.25 / 1.75777	56.67 / 1.75333	82,22 / 1.91499
15	19.48 / $\bar{1}$.28952	20.44 / 1.31058	50.00 / 1.69897
20	0.87 / $\bar{1}$.94011	1.33 / 0.12494	32.78 / 1.51558
25	0.12 / $\bar{1}$.09501	---- / -------	7.83 / $\bar{0}$.89395
30			0.28 / $\bar{1}$.44370
35			0.06 / $\bar{2}$.74473
	K.T. = 25.3 Min.	K.T. = 25.8 Min.	K.T. = 34.6 Min.

+Using new spores.

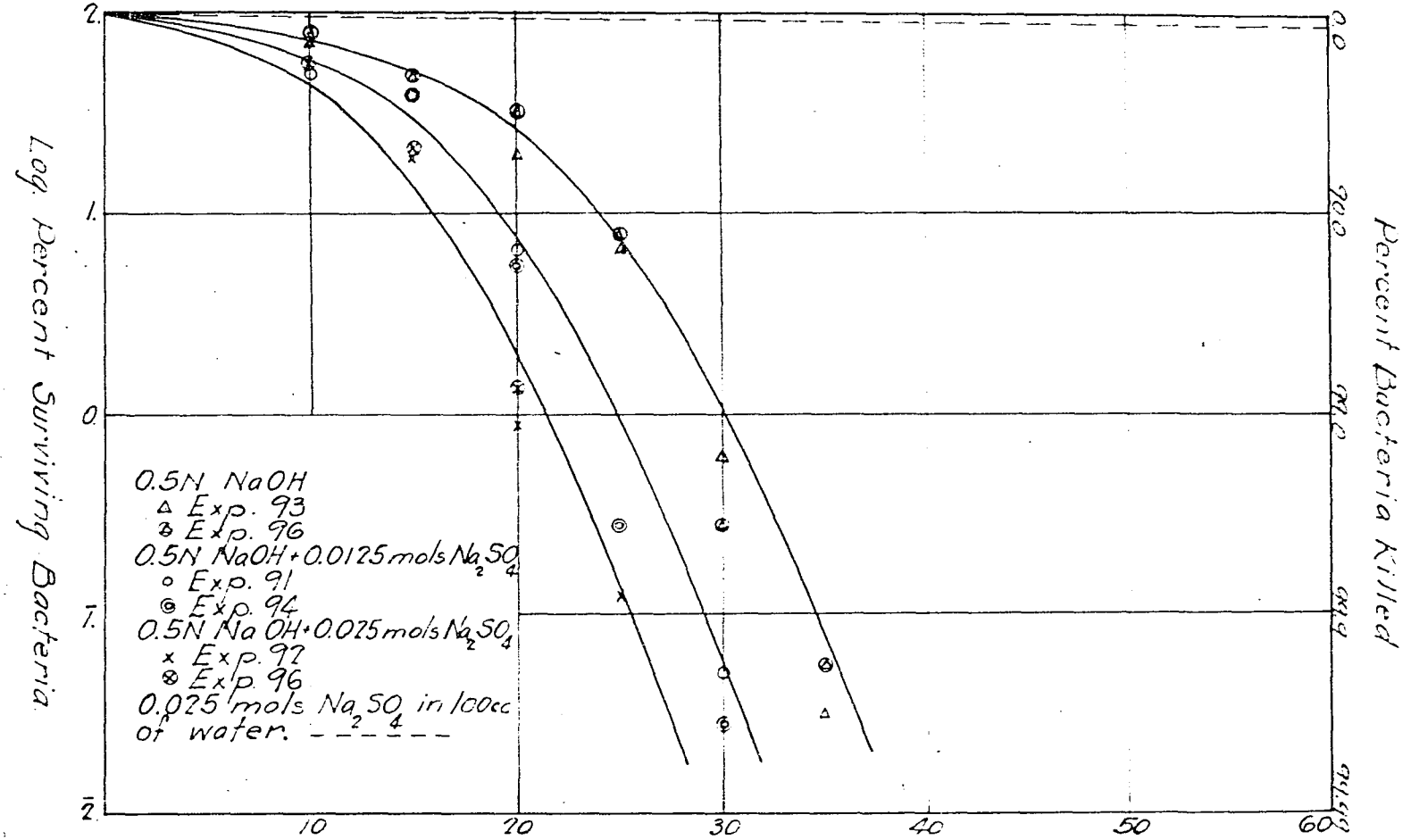


FIG. 12 - Effect of Na_2SO_4 on the germicidal efficiency of NaOH at 50°C.

5. The effect of the addition of sodium silicate upon the germicidal properties of sodium hydroxide.

Sodium meta-silicate*, $Na_2SiO_3 \cdot 5H_2O$ was tested in the usual fashion. It was necessary to analyze the salt in order to determine its compositions. The analysis was made in the usual manner for Na_2O, SiO_2, and water of crystallization (volatile matter at 110°C.). The composition was found to be:

Sodium oxide (Na_2O).....	28.62%
Silica (SiO_2).....	27.10%
Water (matter volatile at 110°C.)..	44.38%
Total.....	100.10%

The $Na_2O:SiO_2$ ratio is 1.056:1. The amount of water found was a trifle high compared to the theoretical amount for $Na_2SiO_3 \cdot 5H_2O$ which is 42.47%.

The behavior of sodium silicate upon dehydration was not known. Therefore, the salt was not heated to make the anhydrous compound but weighed up directly according to the analysis, as $Na_2SiO_3 \cdot 5H_2O$.

For this test, 0.0125 mols (2.7097 grams) and 0.025 mols (5.4194 grams) of sodium silicate were weighed up in duplicate and 100 cubic centimeter portions of 0.5 N sodium hydroxide solution added. The experiments were then made in the usual way.

*Due to the kindness of Mr. C. L. Baker, Chief Chemist of the Philadelphia Quartz Company, Berkeley, California, a pure salt was made available for this experiment.

The data obtained are given in tables XVII and XVIII and have been plotted infigure 13.

The germicidal power of 0.025 mols of $Na_2SiO_3 \cdot 5H_2O$ in 100 cubic centimeters of water is shown by the broken line in figure 13. A reduction in numbers of bacteria after one hour's exposure to this solution, of 70% was observed.

Discussion. The average killing time for 0.5 N sodium hydroxide in these experiments was 35.5 minutes. The addition of 0.0125 mols of sodium silicate reduced the killing time to 25.9 minutes. By the use of 0.025 mols of sodium silicate a killing time of 21.5 minutes was observed. The values are lower in the case of sodium silicate, than the values obtained by the use of sodium chloride.

Table XVII

Showing the Effect of the Addition of Sodium Silicate on the Germicidal Efficiency of Sodium Hydroxide.+

Time in Minutes	Exp. No. 79 7/2/30: 0.5N NaOH + 0.0125 Mols $Na_2SiO_3 5H_2O$ at 50°C.	Exp. No. 82 7/2/30: 0.5N NaOH + 0.0125 Mols $Na_2SiO_3 5H_2O$ at 50°C.	Exp. No. 81 7/2/30: 0.5N NaOH at 50°C.
	Surviving bacteria in 5 cc.		
0	1,580,000	1,500,000	1,580,000
5	1,160,000	1,380,000	----------
10	930,000	1,005,000	900,000
15	420,000	475,000	840,000
20	61,000	82,000	625,000
25	2,500	2,500	220,000
30	0	0	18,500
35	0	0	2,500
40	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
5	73.42	1.86580	92.00	1.96379	-------	-------
10	58.86	1.76982	66.70	1.82413	59.65	1.75558
15	26.52	1.42459	31.67	1.50060	53.16	1.72562
20	3.86	0.58667	5.47	0.73772	39.56	1.59722
25	0.16	$\bar{1}$.19928	0.32	$\bar{1}$.12185	13.92	1.14376
30					1.17	0.06851
35					0.16	$\bar{1}$.19928
	K.T. = 25.8 Min.		K.T. = 26.0 Min.		K.T. = 35.9 Min.	

+Using new spores.

Table XVIII

Showing the Effect of the Addition of Sodium Silicate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 80 7/2/30: 0.5N NaOH+0.025 Mols $Na_2SiO_3 5H_2O$ at 50°C		Exp. No. 83 7/2/30: 0.5N NaOH+0.025 Mols $Na_2SiO_3 5H_2O$ at 50°C		Exp. No. 84 7/2/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	1,580,000		1,500,000		1,500,000	
5	1,200,000		1,150,000		---------	
10	760,000		670,000		1,130,000	
15	250,000		135,000		890,000	
20	9,500		1,500		635,000	
25	0		0		290,000	
30	0		0		61,000	
35	0		0		1,500	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
5	75.95	1.88052	76.67	1.88461	------	-------
10	48.10	1.68215	44.67	1.64998	75.33	1.87699
15	15.82	$\bar{1}$.19928	9.00	0.95424	59.33	1.77330
20	0.60	$\bar{1}$.77906	0.10	$\bar{1}$.00000	42.33	1.62668
25					19.33	1.28631
30					4.07	0.60924
35					0.10	$\bar{1}$.00000
	K.T. = 21.9 Min.		K.T. = 21.1 Min.		K.T. = 35.1 Min.	

[+]Using new spores.

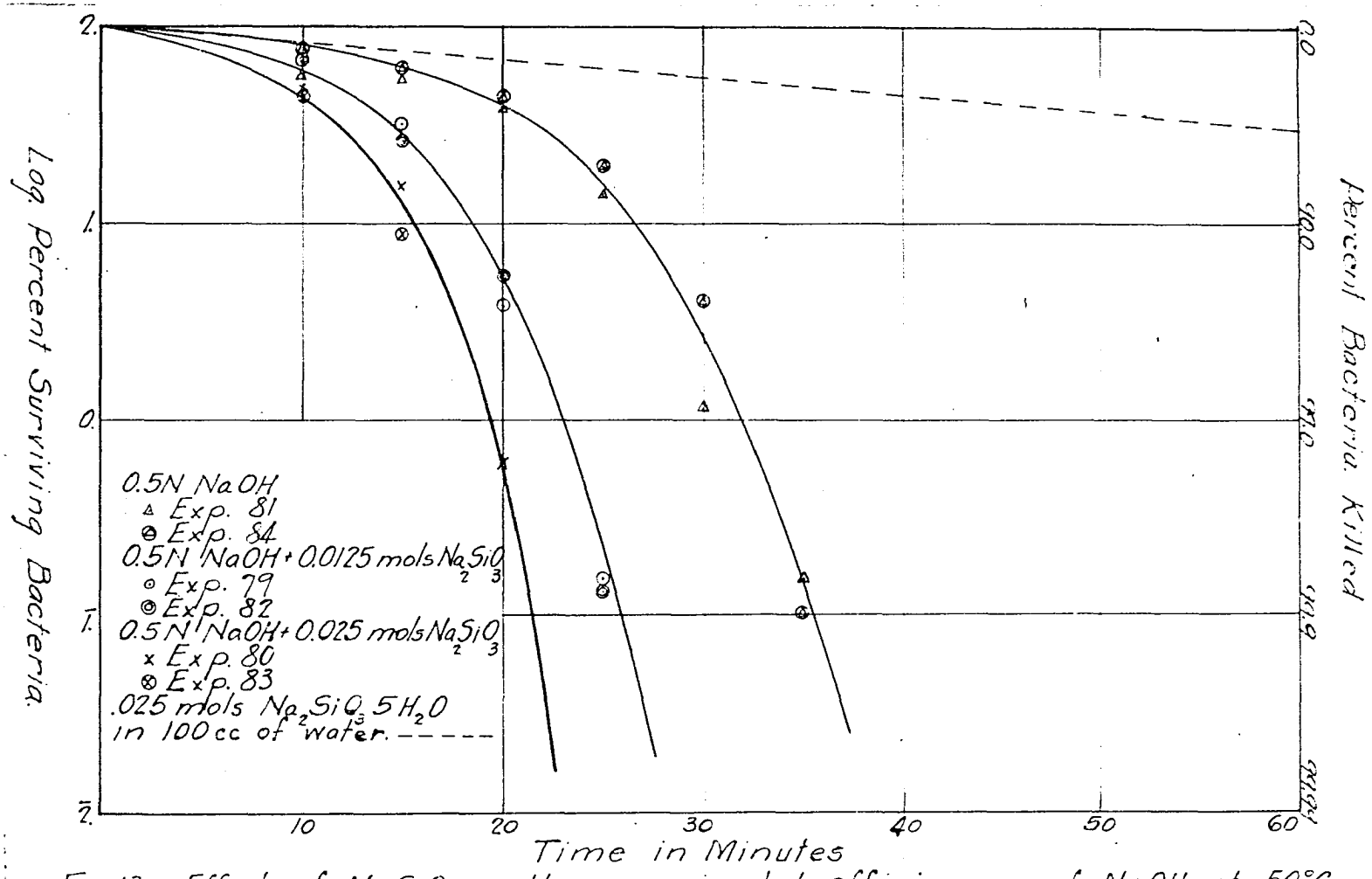


FIG. 13 - Effect of Na_2SiO_3 on the germicidal efficiency of NaOH at 50°C

6. The effect of the addition of sodium phosphate upon the germicidal properties of sodium hydroxide.

Sodium phosphate was tested in the same way as the other salts mentioned.

Of the dry salt, 0.0083 mol (1.3613 grams) and 0.0166 mol (2.7227 grams) were added to the 100 cubic centimeter portions of 0.5 N sodium hydroxide. The number of viable organisms were determined in the usual way at regular intervals. The data obtained are shown in tables XIX and XX. These values are plotted in figure 14.

The germicidal properties of 0.0083 mol Na_3PO_4 in 100 cubic centimeters of water are shown by the broken line in figure 14. After one hour's exposure at 70° the reduction in numbers of bacteria was about 45%. The data for this curve were taken from the data of Levine, Peterson and Buchanan (4).

Discussion. The average killing time, for 0.5 N sodium hydroxide, of 35.3 minutes, was reduced to 29.5 minutes by the addition of 0.0083 mol of Na_3PO_4. A reduction to 25 minutes was observed by the addition of 0.0166 mol of Na_3PO_4. These values check with the values obtained by the use of sodium chloride.

Table XIX

Showing the Effect of the Addition of Sodium Phosphate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 85 7/5/30: 0.5N NaOH + 0.0083 Mols Na_3PO_4 at 50°C.		Exp. No. 88 7/5/30: 0.5N NaOH + 0.0083 Mols Na_3PO_4 at 50°C		Exp. No. 87 7/5/30 0.5N NaOH at 50°C	
	Surviving bacteria in 5 cc.					
0	850,000		1,350,000		850,000	
10	560,000		1,045,000		690,000	
15	400,000		900,000		615,000	
20	112,000		350,000		300,000	
25	11,500		12,000		118,000	
30	150		750		22,500	
35	0		0		------	
40	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	65.88	1.81877	77.41	1.88879	81.18	1.90943
15	47.06	1.67264	66.67	1.82391	72.35	1.85946
20	13.18	1.11980	25.93	$\bar{1}$.41374	35.29	1.54770
25	1.35	0.13128	0.89	$\bar{1}$.94885	13.88	1.14246
30	0.02	$\bar{2}$.24667	0.06	$\bar{2}$.74473	2.65	0.42276
35					----	-------
	K.T. = 29.2 Min.		K.T. = 29.8 Min.		K.T. = 35.2 Min.	

[+]Using new spores.

Table XX

Showing the Effect of the Addition of Sodium Phosphate on the Germicidal Efficiency of Sodium Hydroxide.[+]

Time in Minutes	Exp. No. 86 7/5/30: 0.5N NaOH + 0.0166 Mols Na_3PO_4 at 50°C	Exp. No. 89 7/5/30: 0.5N NaOH + 0.0166 Mols Na_3PO_4 at 50°C	Exp. No. 90 7/5/30 0.5N NaOH at 50°C.
	Surviving bacteria in 5 cc.		
0	850,000	1,350,000	1,350,000
10	505,000	1,010,000	1,220,000
15	365,000	595,000	975,000
20	53,500	61,500	570,000
25	1,000	500	189,500
30	0	0	15,000
35	0	0	2,000
40	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	59.41	1.77387	74.82	1.87399	90.37	1.95603
15	42.94	1.63287	44.08	1.64419	72.22	1.85867
20	6.29	$\bar{0}$.79893	4.56	$\bar{0}$.65855	42.22	1.62554
25	0.12	$\bar{1}$.07058	0.04	$\bar{2}$.56864	14.04	1.14728
30					1.11	$\bar{0}$.04576
35					0.15	$\bar{1}$.17070
	K.T. = 25.3 Min.		K.T. = 24.7 Min.		K.T. = 35.4 Min.	

[+]Using new spores.

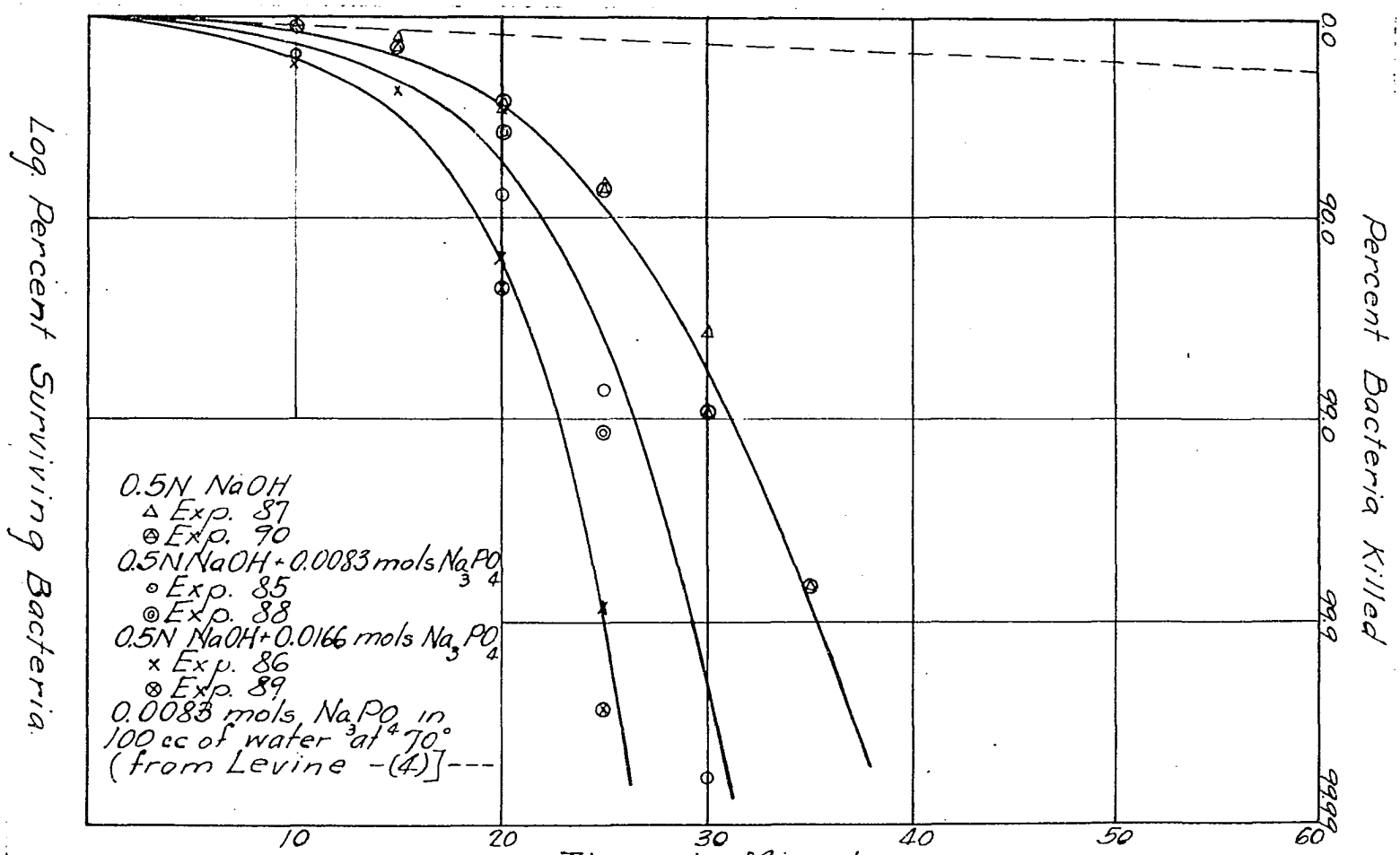


Fig. 14- Effect of Na_3PO_4 on the germicidal efficiency of NaOH at 50°C.

General discussion and conclusions on the effect of the addition of salts upon the germicidal properties of sodium hydroxide.

In the several experiments given above, a number of determinations of the effect of various salts upon the germicidal power of sodium hydroxide have been made. These results are summarized in the following table.

Table XXI

Salt employed	Concentration of added salt in mols of sodium per 100 cc. of 0.5 N NaOH used		
	0.0	0.025	0.050
	Killing time in minutes.		
NaCl	35.3	31.0	24.8
	35.5	31.3	25.8
$NaNO_3$	35.3	30.8	25.0
	36.0	29.7	24.8
Na_2CO_3	35.5	29.5	25.5
	35.8	29.5	25.5
Na_2SO_4	34.7	29.0	25.3
	34.6	29.5	25.8
Na_2SiO_3	35.9	25.8	21.9
	35.1	26.0	21.1
Na_3PO_4	35.2	29.2	25.3
	35.4	29.8	24.7

It has been stated previously that the purpose of these experiments was to determine the relative effect of the same amount of sodium, added as the various salts, upon the germicidal properties of sodium hydroxide. From an inspection of

table XXI, it will be seen that the effect is nearly the same, with the exception of sodium silicate. The effect of 0.025 mols of sodium, added as a salt, upon the germicidal power of 100 cubic centimeters of 0.5 N sodium hydroxide is the same for five of the anions used. This means that sodium chloride, sodium nitrate, sodium sulfate, sodium carbonate or sodium phosphate containing equal weights of sodium, when added to sodium hydroxide, equally increase the germicidal powers of the sodium hydroxide.

The effect of added sodium silicate upon the germicidal power of sodium hydroxide, appears to be greater than any of the other salts used. This is very probably due to the hydrolysis of the sodium silicate, since a high pH is shown by sodium silicate solution in the concentration used, as will be shown later.

In connection with the higher germicidal power that the use of sodium silicate shows, over any of the other salts tested, when added to sodium hydroxide, it might be of interest to consider the experiments that were made with a commercial product, Meta-Sil* which is a commercial form of sodium meta-silicate, $Na_2SiO_3 \cdot 5H_2O$. Meta-Sil contains about 98% $Na_2SiO_3 \cdot 5H_2O$. This compound was used for the more extended experiments on sodium silicate as only a small amount of sod-

*A liberal supply of Meta-Sil was made available for these experiments due to the kindness of Mr. C. L. Baker, of the Philadelphia Quartz Co., of Berkeley, California.

ium silicate of reagent grade was available. The percentage of Na_2O in the two grades of Na_2SiO_3 was identical, 28.6% in each case.

A 0.5 N solution of Meta-Sil was made and the pH determined by the use of a hydrogen electrode and the usual potentiometer set-up. This solution was found to poison the platinum electrode rapidly, and the electrode had to be recoated with platinum black after each determination. The average pH of the solution was found to be 12.89. This pH is very similar to that of 0.25 N sodium hydroxide which was determined to be 13.00. For this reason it was determined to compare the germicidal efficiency of 0.5 N Meta-Sil solution with sodium hydroxide at the same normality and with sodium hydroxide at the same pH. The pH of 0.5 N sodium hydroxide was determined to be 13.22. The data for these experiments are shown in table XXII. These data are plotted in figure 15.

Another series of experiments are shown in table XXIII. In this series a mixture of 50 cubic centimeters of 0.5 N Meta-Sil solution and 50 cubic centimeters of 0.5 N sodium hydroxide solution was tested against 0.5 N sodium hydroxide. Another experiment listed in table XXIII shows the effect on the germicidal efficiency of the addition of one gram of Baker's sodium silicate ($Na_2SiO_2,4H_2O$(C.P.)) to 100 cubic centimeters of 0.5 N sodium hydroxide. This salt contained only 64% as much Na_2O by titration, as Meta-Sil contained. This should be compared to the effect of similar quantities of

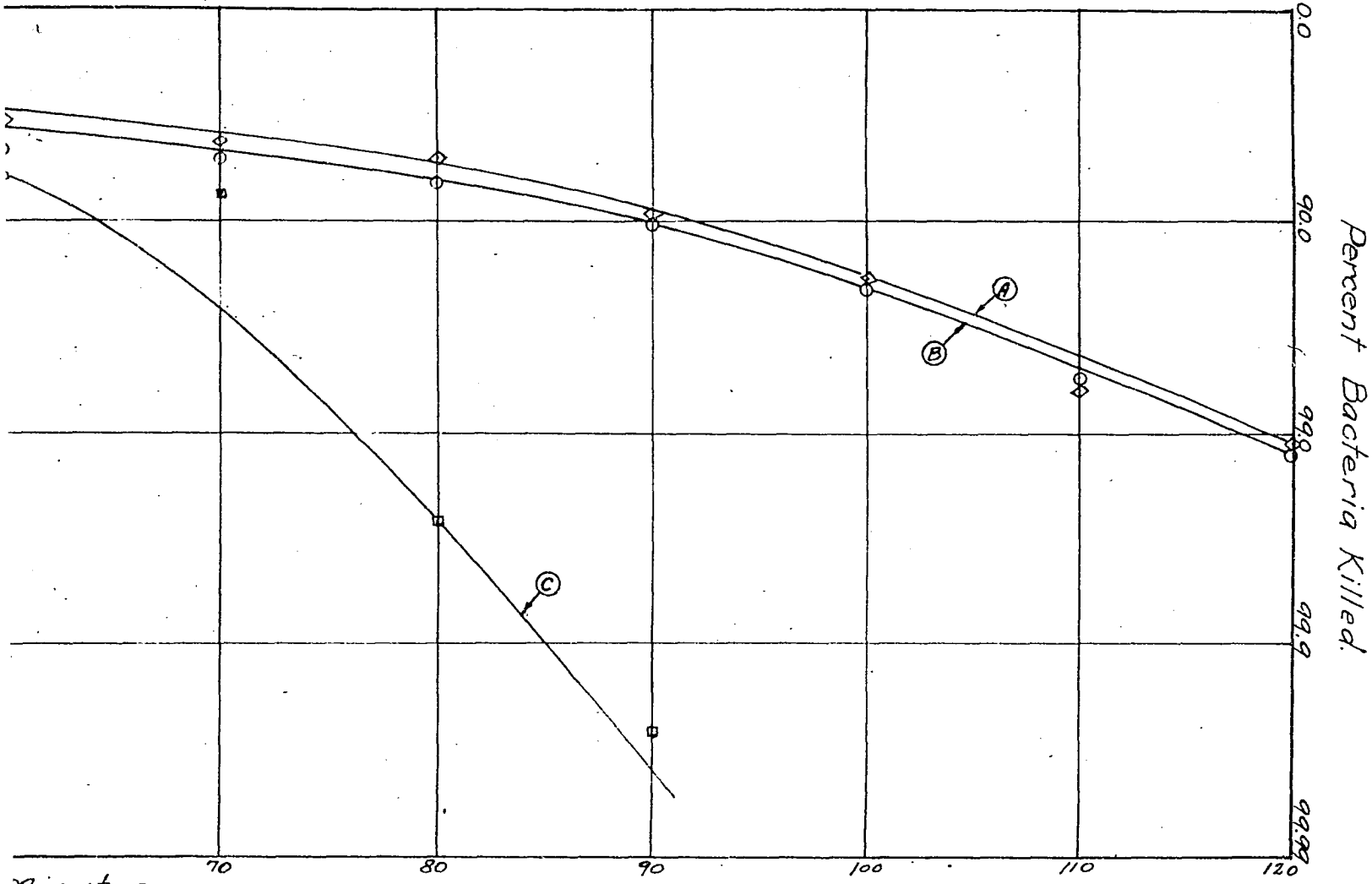
Table XXII

Showing the Germicidal Power of Sodium Silicate Solution as Compared to Sodium Hydroxide at the Same Normality and Sodium Hydroxide at the Same pH.+

Time in Minutes	Exp.No.44 5/26/30: 0.5N Meta-Sil Solution at 40°C. pH 12.89	Exp.No.45 5/26/30: 0.25N NaOH at 40°C: pH 13.00.	Exp.No.42 5/26/30: 0.50N NaOH at 40°C: pH 13.22.
	Surviving bacteria in 5 cc.		
0	1,900,000	1,900,000	850,000
10	1,600,000	1,210,000	465,000
20	1,570,000	1,165,000	185,000
30	1,065,000	965,000	107,000
40	860,000	850,000	42,000
50	825,000	645,000	6,000
60	585,000	420,000	0
70	465,000	380,000	0
80	380,000	290,000	0
90	213,000	188,500	0
100	101,500	94,500	0
110	29,800	34,500	0
120	17,200	15,000	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	84.21	1.92537	63.69	1.80404	54.70	1.73803
20	82.63	1.91715	61.32	1.78758	21.76	1.33775
30	56.05	1.74860	50.79	1.70578	12.59	1.09996
40	45.26	1.65575	44.74	1.65067	4.94	0.69383
50	43.42	1.63770	33.95	1.53081	0.71	$\bar{1}$.84873
60	30.69	1.48837	22.11	1.34450		
70	24.47	1.38870	20.00	1.30103		
80	20.00	1.30103	15.26	1.18365		
90	11.21	1.04963	9.92	0.99656		
100	5.43	0.72772	4.97	0.69668		
110	1.57	0.19547	1.82	0.25907		
120	0.90	$\bar{1}$.95678	0.79	$\bar{1}$.89734		
	K.T. = >120 Min.		K.T. = >120 Min.		K.T. = 54.3 Min.	

+Using old spores.



Comparative germicidal effect of the addition of (Meta-Sil), NaCl, and

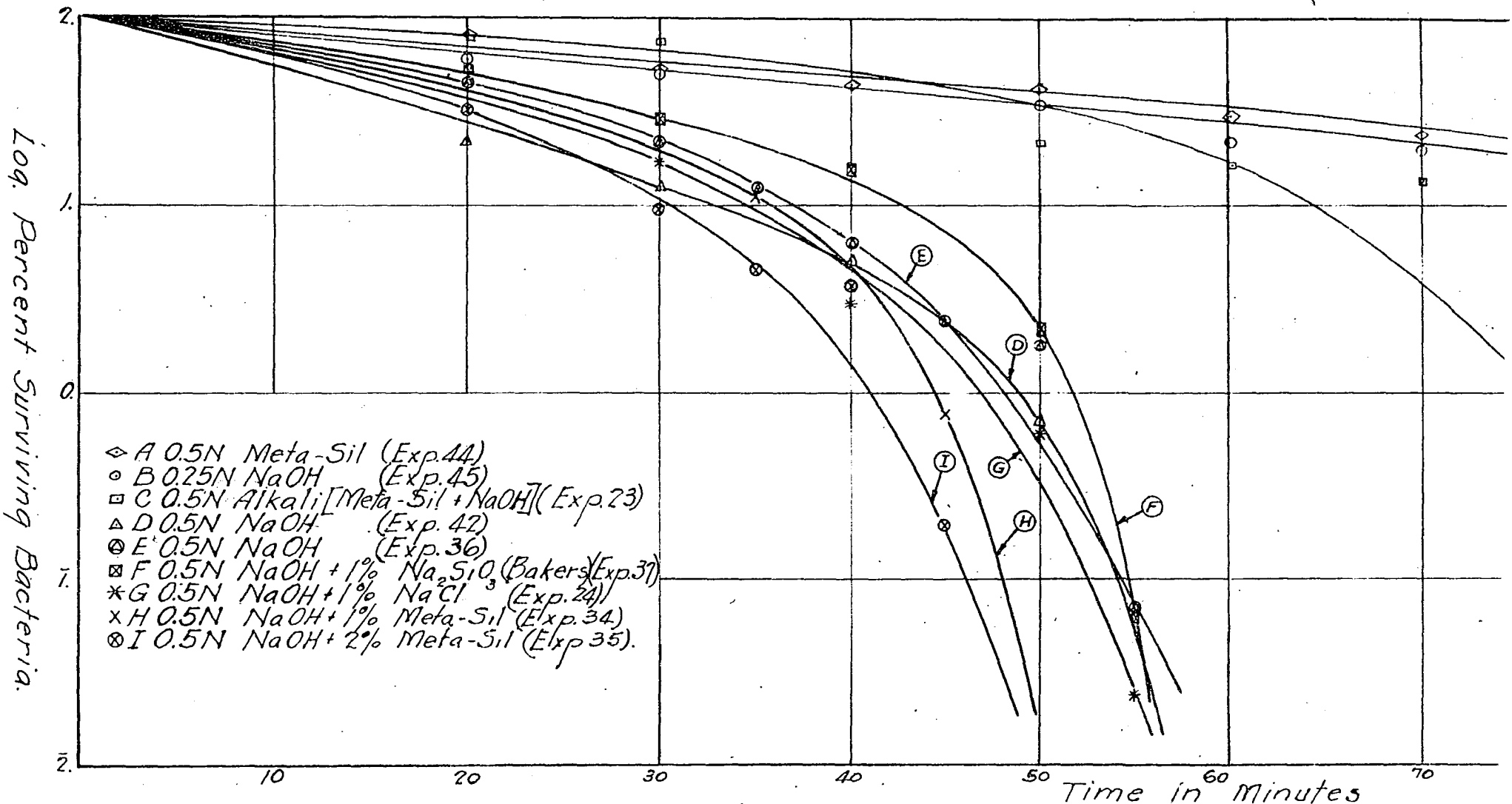


FIG. 15- Germicidal properties of Na_2SiO_3 (Meta-Sil) compared to NaOH. Comparative ge
Na_2SiO_3 (Bakers) to NaOH.

Table XXIII

Germicidal Efficiency of a Mixture of Meta-Sil and Sodium Hydroxide. Comparative Effect of Addition of Sodium Chloride and Sodium Silicate (Bakers C.P.) on the Germicidal Power of Sodium Hydroxide.*

Time in Minutes	Exp.No.23 5/5/30: 0.5N Meta-Sil + 0.5N NaOH at 40°C.	Exp.No.37 5/19/30: 0.5N NaOH + 1 gram $Na_2SiO_3 4H_2O$ (Bakers) at 40°C.	Exp.No.24 5/5/30 0.5N NaOH + 1 gram NaCl at 40°C.
	Surviving bacteria in 5 cc.		
0	1,035,000	940,000	1,035,000
10	930,000	762,500	837,500
20	800,000	510,000	452,500
30	770,000	275,000	175.000
40	510,000	145,000	32,000
50	231,500	20,500	6,500
60	171,000	0	0
70	79,800	0	0
80	3,900	0	0
90	400	0	0
100	0	0	0

Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	89.85	1.95354	81.12	1.90911	80.92	1.90804
20	77.30	1.88815	54.26	1.73444	43.70	1.64068
30	74.40	1.87155	29.26	1.46620	16.91	1.22810
40	49.28	1.69263	15.43	1.18824	3.09	$\bar{0}$.49021
50	22.37	1.34961	2.23	0.33862	0.63	$\bar{1}$.79797
60	16.52	1.21806				
70	7.71	$\bar{0}$.88706				
80	0.38	$\bar{1}$.57522				
90	0.04	$\bar{2}$.58712				
	K.T. = 85.0 Min.		K.T. = 54.8 Min.		K.T. = 52.5 Min.	

*Using old spores.

Meta-Sil, which are shown in table XXIV. For the sake of comparison there is included in table XXIII the data obtained by the addition of one gram of sodium chloride to 100 cubic centimeters of 0.5 N sodium hydroxide. The data of tables XXIII and XXIV are shown graphically in figure 15.

In considering the data above it will be seen that Meta-Sil solutions are not as effective as sodium hydroxide solutions at the same normality but compare very favorably at the same pH with sodium hydroxide. An equal mixture of 0.5 N Meta-Sil solution and 0.5 N sodium hydroxide solution shows a germicidal efficiency about half way between that shown by a 0.5 N sodium hydroxide solution, and that of a 0.5 N Meta-Sil solution. It is especially interesting to note that 0.5 N Meta-Sil solution at a pH of 12.89 possesses equal germicidal power with a 0.25 N sodium hydroxide solution at a slightly higher pH of 13.00.

The addition of one gram and two grams of Meta-Sil respectively to 0.5 N sodium hydroxide is shown to have more effect upon the germicidal power than one gram quantities of either sodium chloride or another sodium silicate (Baker's) of lower Na_2O content. It appears that the greater effect of Meta-Sil upon the germicidal properties of sodium hydroxide depends upon its greater alkalinity since the silicate shown to be least effective has only 64% as much Na_2O as the more effective Meta-Sil. The alkalinity, due to hydrolysis of the salt, appears to be a factor in the reduction of killing

Table XXIV

Effect of the Addition of Meta-Sil on the Germicidal Efficiency of Sodium Hydroxide.+

Time in Minutes	Exp.No.34 5/19/30: 0.5N NaOH + 1 gram: Meta-Sil at 40°C. pH 13.22.		Exp.No.35 5/19/30: 0.5N NaOH + 2 grams: Meta-Sil at 40°C. pH 13.25.		Exp.No.36 5/19/30 0.5N NaOH at 40°C.	
	Surviving bacteria in 5 cc.					
0	504,000		504,000		504,000	
10	450,000		375,000		340,000	
20	300,000		160,000		230,000	
30	85,500		47,300		108,000	
35	58,000		22,500		59,500	
40	32,500		18,500		30,500	
45	4,000		1,000		12,500	
50	50		50		9,000	
55	0		0		350	
60	0		0		0	
Time in Minutes	% Survivors	Log % Survivors	% Survivors	Log % Survivors	% Survivors	Log % Survivors
0	100.00	2.00000	100.00	2.00000	100.00	2.00000
10	89.29	1.95078	74.41	1.87160	67.46	1.82905
20	59.52	1.77469	31.75	1.50169	45.65	1.65940
30	16.96	1.22954	9.39	0.97243	21.43	1.33099
35	11.51	1.06100	4.46	0.64975	11.81	1.07209
40	6.45	$\bar{0}$.80945	3.67	$\bar{0}$.56474	6.05	0.78187
45	0.79	$\bar{1}$.89963	0.20	$\bar{1}$.29757	2.48	0.39448
50	0.01	$\bar{3}$.99654	0.01	$\bar{3}$.99654	1.79	0.25181
55					0.07	$\bar{2}$.84164
	K.T. = 48.3 Min.		K.T. = 46.3 Min.		K.T. = 54.6 Min.	

+Using old spores.

time of sodium hydroxide, when the various salts are added to sodium hydroxide.

From a consideration of the data discussed above, it appears that the anions of salts added to sodium hydroxide have little or no part in the reduction of killing times observed. Equivalent weights of sodium attached to five anions give equal reductions in killing time, therefore, it appears that the amount of sodium ion present in solution is an important factor. The effect of the addition of a salt which provides hydroxyl ions is shown to be greater than similar quantities of other salts. It is suggested that the disinfecting action is due to the hydroxyl ions and to the number of undissociated sodium hydroxide molecules present.

GENERAL SUMMARY

It has been shown that the germicidal powers of the hydroxides of lithium, sodium, potassium, and rubidium are practically the same, when tested against organism No.25.

The effect of the addition of equal quantities of sodium as sodium chloride, sodium nitrate, sodium carbonate, sodium sulfate, or sodium phosphate is to increase equally the germicidal efficiency of sodium hydroxide. Sodium silicate is more effective in increasing the germicidal efficiency of sodium hydroxide. This is explained as being due to the higher alkalinity possessed by the sodium silicate tested, since a sodium silicate of lower Na_2O content is shown to have little effect upon the germicidal powers of sodium hydroxide. Curves are presented to indicate the comparative germicidal powers of the salts listed, upon the test organism. Sodium silicate possesses greatest germicidal powers while the other salts are but weakly germicidal.

It is suggested that the principal agencies in disinfection are the hydroxyl ions and the undissociated sodium hydroxide molecules.

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