A STUDY OF METHODS OF MEASURING GERMICIDAL CHLORINE WITH REFERENCE TO THE OXIDATION-REDUCTION POTENTIAL, STARCH-IODIDE TITRATION AND ORTHO-TOLIDINE TITRATION

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A Study of Methods of Measuring Germicidal Chlorine With Reference to the Oxidation-Reduction Potential, Starch-Iodide Titration and Ortho-Tolidine Titration

Although chlorine has been used for over a century as a means of disinfection, there are still many factors relating to its action and methods for its determination which are as yet not well understood. At the present time there are two accepted methods, the starch-iodide and ortho-tolidine tests, for determining the available chlorine present but there are very few data showing the relative germicidal value of the chlorine thus measured. This is especially true of solutions in which there is organic matter present. A few attempts have been made to correlate the germicidal action of chlorine with the oxidation-reduction potential but this has largely been confined to excessive concentrations of chlorine or to a comparison of the effect of pH on the oxidation-reduction potential and on the germicidal action of the chlorine.

In general an oxidation-reduction reaction may be considered as a process where an oxidizing agent gains electrons and a reducing agent loses electrons. If an unattackable electrode, such as platinum, is immersed in such an oxidation-reduction system, a potential difference is set up at the electrode and can be measured by potentiometric methods. The more oxidized a substance is, the higher will be the electrode potential and the more reduced the substance is, the more negative will be the potential. Regardless of whether the nascent oxygen, nascent chlorine or hypochlorous acid theory is accepted as the explanation for the germicidal action of chlorine, it should be possible to measure it by potentiometric methods. The present work was therefore carried out for the purpose of showing the relationship of the oxidation-reduction potential to varying concentrations of chlorine both with and without the presence of organic matter, and to present further data relative to the accuracy of the starch-iodide and ortho-tolidine titrations in the presence of organic matter as means of measuring the germicidal activity of the chlorine present.

Historical

For a great many years it was thought that the germicidal activity of chlorine was entirely due to oxidation, the chlorine combining with the hydrogen of water to form nascent oxygen according to the reaction:

$$Cl_2 + H_2O \qquad 2HCl + O$$

Nisson (14) in 1890 observed an evolution of chlorine upon the acidification of chloride-of-lime and found that there was an increased germicidal action which he attributed to nascent chlorine. Kronig and Paul (7) in 1897 noted that solutions of chlorine in water were much more germicidal than other strong oxidizing agents and concluded from this that there must be some other bactericidal property present besides that of oxidation. Andrewes and Orton (1) in 1904 were the first to suggest that hypochlorites owe their germicidal activity to hypochlorous acid (HOCl). They pointed out that on the acidification of hypochlorite solutions, hypochlorous acid was liberated and the germicidal activity greatly increased. Holwerda (5) and Charlton and Levine (4) have presented further data indicating that hypochlorite solutions owe much of their germicidal value to undissociated hypochlorous acid.

Rideal and Evans (15) in 1921 were the first to present an oxidation-reduction potential concept of chlorination. Schmelkes (17) found that the oxidation-reduction potential of chlorine solutions, having different pH values, ran parallel to the germicidal activity. Charlton and Levine (4), in referring to some unpublished work by Hellwig, state that the oxidation potentials of available chlorine concentrations of chloramine-T equal to 1,000-, 2,000- and 4,000 parts per million (p.p.m.) are almost identical at a given pH. They therefore conclude, "that even for a specific chlorine compound the oxidation potential is not a dependable measure of germicidal efficiency."

The importance of pH changes in relation to the germicidal activity of chlorine has been pointed out by Johns (6), Mallmann (9), Mallmann and Schalm (10) and Charlton and Levine (3). Charlton and Levine (4) showed that a solution with 100 p.p.m. available chlorine at pH 10.40 gave a killing time of 70 minutes whereas a solution containing 20 p.p.m. available chlorine at pH 8.2 had a killing time of only 4.8 minutes, thus showing that the influence of pH is very great.

Since chlorine tends to be taken up very readily by organic matter many workers, Tilley (19), Beard and Kendall (2) and others have used solutions containing organic matter when testing the germicidal action of chlorine. Wright (21) has shown that certain types of organic matter are more readily attacked by chlorine than are others.

Mallmann (11) has shown that inert substances such as charcoal adsorb chlorine whereas other types of organic matter react with the chlorine and chemically combine with it. He also showed that in the presence of suspended materials the starch-iodide titration and the ortho-tolidine titration tend to measure the available chlorine differently.

McCulloch (12) has shown that in the presence of chicken manure a definite lag precedes the maximum germicidal effect and attributes it to the formation of a new compound, "probably a simple chloramine formed by the action of the hypochlorite upon the ammonia in the chicken manure."

It is generally believed that the chlorine demand must be satisfied and a small amount of residual chlorine remain before disinfection will take place. Tilley and Chapin (20), however, showed that tannery effluents which had been sufficiently chlorinated to become anthrax free in less than two hours showed no available chlorine and that more chlorine could be added without appearing as free or available chlorine. Nachtigall and Ali (13) have shown that some waters can be disinfected with chlorine in amounts less than the chlorine demand of the water. Rudolfs and Ziemba (16) found that in the chlorination of sewage, no matter to what extent of the chlorine demand, there were certain chloro-products formed which would bring about bacterial reductions. On the other hand, they found that neither chloro-peptones nor chloro-proteins, obtained by chlorinating peptone water and nutrient broth, had any inhibiting effect on the growth of bacteria nor were they germicidal.

Experimental and Discussion

There are a number of references in the literature dealing with the relationship of the oxidation-reduction potential of chlorine solutions to their germicidal action. All of these, however, deal with solutions containing such high concentrations of chlorine that they are impracticable both from the standpoint of disinfection and accurate measurement, or they deal with only one concentration of chlorine at varying pH concentrations. In the work presented here an attempt was made to show the relationship of the oxidation-reduction potential to increasing concentrations of chlorine (NaOCl) at a constant pH value. Titration values not to exceed 1.50 p.p.m. were used since this is the range which finds the greatest use in practice. It was hoped that the curves derived from this type of determination might act as the basis for a more accurate method for determining available or germicidal chlorine.

Increasing concentrations of chlorine (NaOCl) were added to equal quantities of distilled water and allowed to stand for 15 minutes to permit the chlorine to come to equilibrium. At the end of this period voltage determinations were made of each of the solutions using a bright platinum electrode and saturated calomel reference electrode. The pH of the solutions was adjusted by the addition of .001 N. HCl and .001 N. NaOH. All pH determinations were made with a glass electrode. For convenience the term oxidation-reduction potential in all of the studies which follow will be represented by the direct voltage reading. Table I and Figure I show these relationships for pH values of 5.00, 7.00 and 9.00.

These data show that at a constant pH, with the addition of increasing concentrations of chlorine (NaOCl), there is first a steep rise in the voltage and then a leveling off. The voltage continues to rise slowly until about 1.10 p.p.m. of chlorine have been added when again there is a sharp rise with each increasing addition of chlorine. This same relationship was found to exist for all pH concentrations between 5.00 and 9.00, the limits within which tests were made. The voltages at pH 5.00 represent the average of several determinations as in this acid range the chlorine is so unstable that identical results could not be obtained from day to day. Chlorine solutions with pH concentrations between 7.00 and 9.00 are quite stable and closely-checking voltages were obtained from day to day. However, it seems doubtful whether this voltage determination would be very practiçal as a means of measuring available chlorine in water and pool supplies, especially in the range between 0.1 p. p.m. and 1.00 p.p.m. of chlorine as there is too little variation in the voltage readings to give an accurate evaluation.

In order to demonstrate the relationship between these oxidation-reductions potentials and the germicidal activity of chlorine solutions, increasing concentrations of sodium hypochlorite, not to exceed 1.50 p.p.m., were added to flasks containing 1,000 mls. of distilled water. These were then

Table I

The Effect of pH on the Oxidation-Reduction

Potential of Chlorine Solutions

pH 5.00		pH 7.00		pH 9.00	
P.P.M. Cl_2	Voltage	P.P.M. Cl_2	Voltage	P.P.M. Cl_2	Voltage
0.	0.380	0.	0.260	0.	0.165
0.16	0.630	0.12	0.440	0.18	0.315
0.34	0.645	0.26	0.450	0.35	0.330
0.52	0.655	0.45	0.460	0.53	0.345
0.71	0.665	0.67	0.475	0.71	0.355
0.91	0.675	0.92	0.490	0.89	0.365
1.08	0.685	1.12	0.535	1.06	0.376
1.27	0.720	1.27	0.590	1.24	0.465

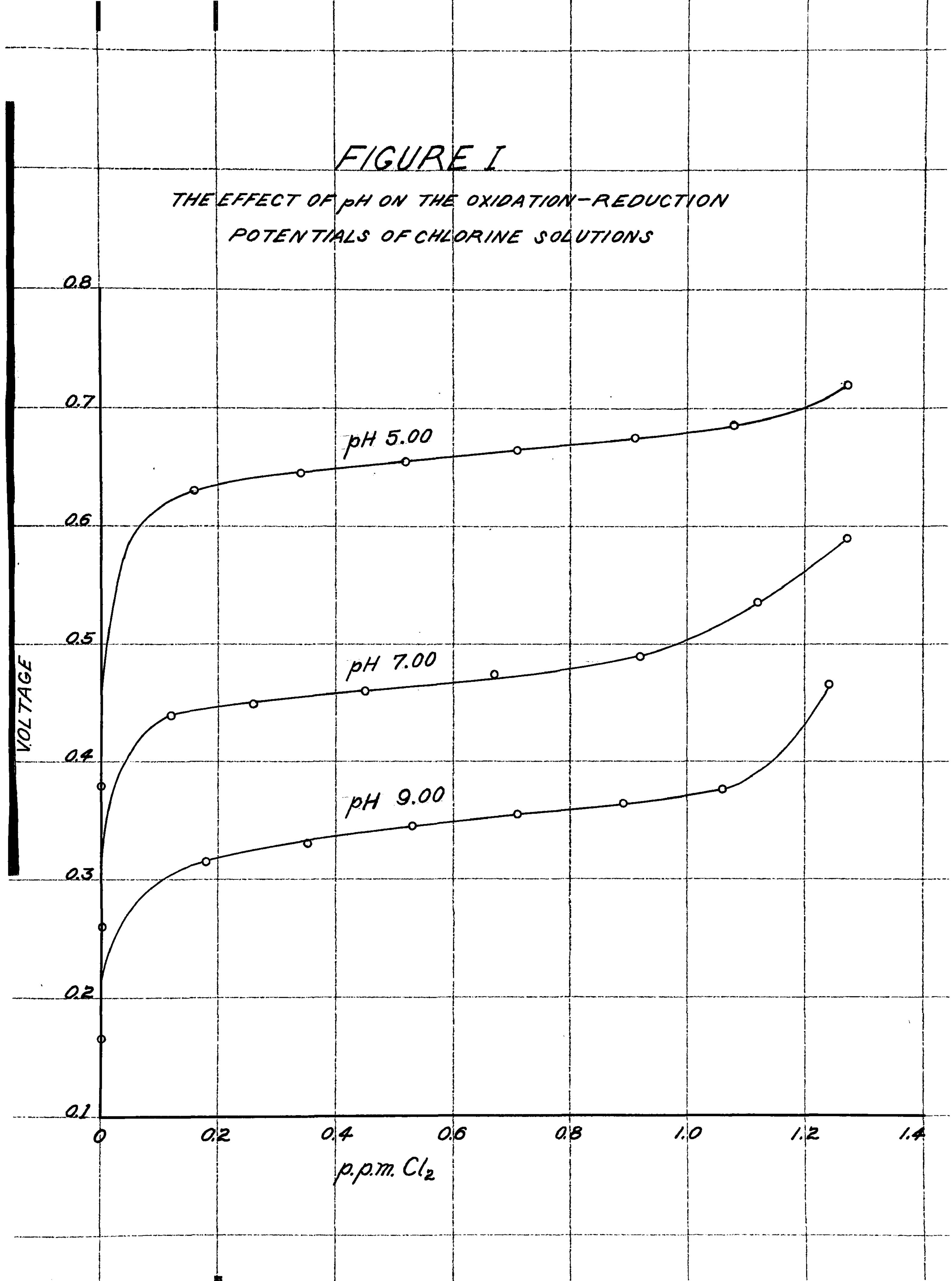


FIGURE I
THE EFFECT OF pH ON THE OXIDATION-REDUCTION POTENTIALS OF CHLORINE SOLUTIONS
pH 5.00
pH 7.00
pH 9.00
VOLTAGE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
p.p.m. Cl_2

allowed to stand for 30 minutes in order that the chlorine might come to equilibrium. At the end of this period the voltage was determined as previously described and the available chlorine checked by the starch-iodide method. Five hundred mls. of each of the solutions were carefully measured and placed in 1,000 ml. flasks and tests to determine the speed of disinfection were made on each of the solutions according to the method of Mallmann and Schalm (10). This consists in adding one ml. of a standard suspension of organisms (*Escherichia coli*) to the 500 mls. of chlorine solution. At the end of 15, 30, 45, 60, 90, 120 and 180 seconds one ml. samples are removed and each is placed in nine mls. of peptone water in order to remove any excess chlorine. These are then plated, incubated at 37° C., and counts made of the surviving bacteria. In the present study, however, instead of adding a constant number of organisms to each of the solutions tested a corresponding increase was made in the number of organisms added with each increase in chlorine concentration. Tests were run at pH concentrations 5.00, 7.00 and 9.00. The results are presented in Tables II, III and IV, and in Figs. II, III and IV.

It is quite evident from these data that there is a very direct relationship between the oxidation-reduction potential of a chlorine solution and its germicidal action. This is especially apparent at pH 7.00 and pH 9.00 where there is a leveling off and then a definite rise in the potential tending towards a step formation. In each case there is this same leveling off and then sudden increase in the percent

Table II

The Relationship of Oxidation-Reduction Potentials

to the Germicidal Activity of Chlorine at pH 5.00

Voltage	P.P.M. Cl_2	Bacteria added per ml.	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.380	0.	30,000	0.						
0.695	0.18	30,000	11.7	21.4	31.0	60.0	82.7	94.7	99.7
0.710	0.35	60,000	19.9	33.0	51.0	75.0	92.2	98.9	100
0.720	0.53	90,000	35.0	46.6	61.5	87.9	99.4	100	100
0.730	0.71	120,000	40.0	57.7	79.4	95.3	99.9	100	100
0.740	0.89	150,000	50.0	69.4	89.4	99.4	100	100	100
0.745	1.06	180,000	62.0	77.8	93.4	100	100	100	100
0.780	1.24	210,000	87.0	96.3	100	100	100	100	100

FIGURE II

THE RELATIONSHIP OF OXIDATION–REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 5.00

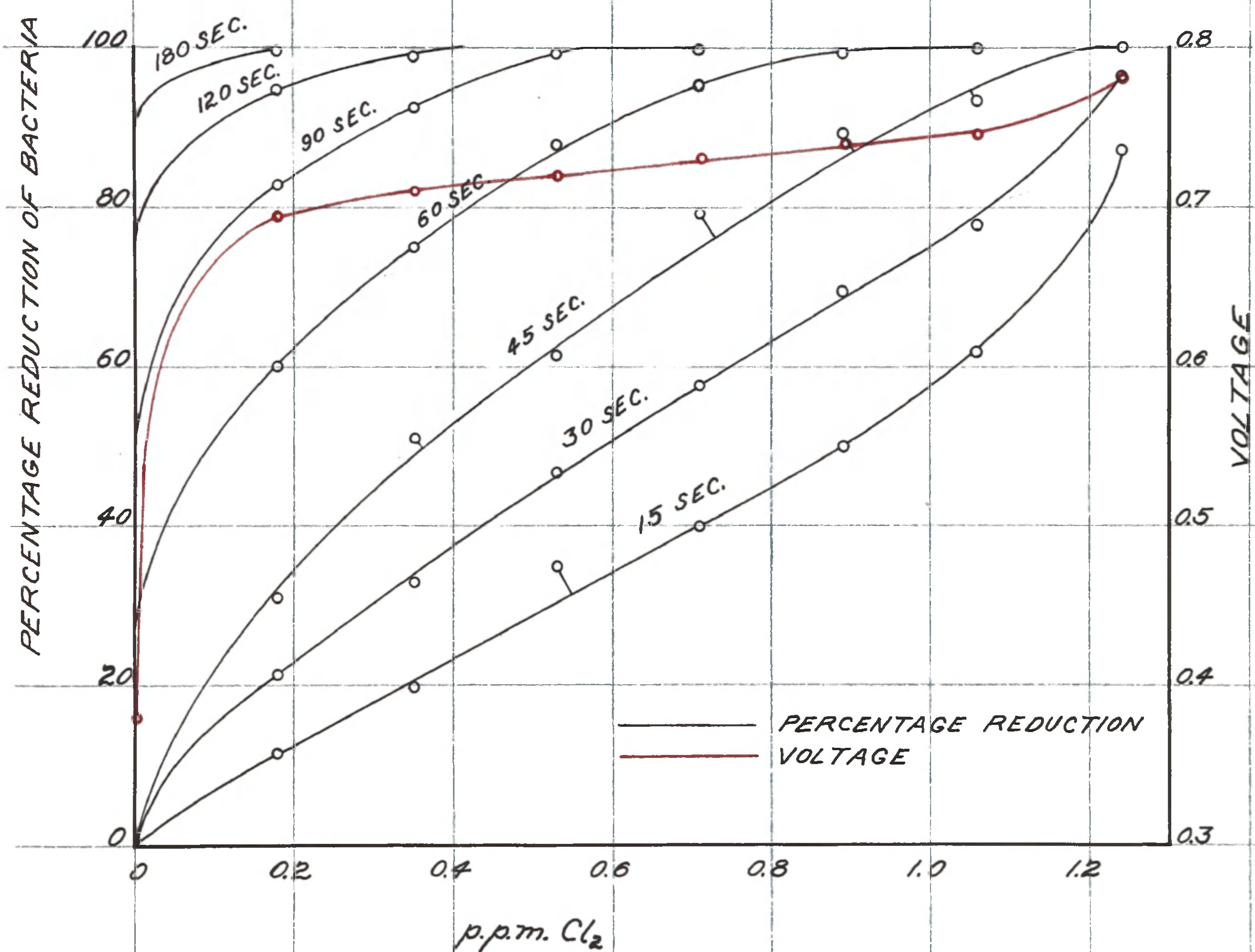


Table III

The Relationship of Oxidation-Reduction Potentials

to the Germicidal Activity of Chlorine at pH 7.00

Voltage	P.P.M. Cl_2	Bacteria added per ml.	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.280	0.	9,800	0.						
0.425	0.18	9,800	5.2	26.6	45.2	56.1	69.4	84.7	99.2
0.455	0.35	19,600	6.4	32.2	59.5	72.2	89.0	97.4	100
0.480	0.53	29,400	16.7	41.9	67.7	81.7	98.3	100	100
0.505	0.71	39,200	23.8	51.6	77.1	89.2	99.9	100	100
0.590	0.89	49,000	74.3	98.0	98.8	99.9	100	100	100
0.660	1.06	58,800	90.0	99.7	100	100	100	100	100

FIGURE III

THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.00

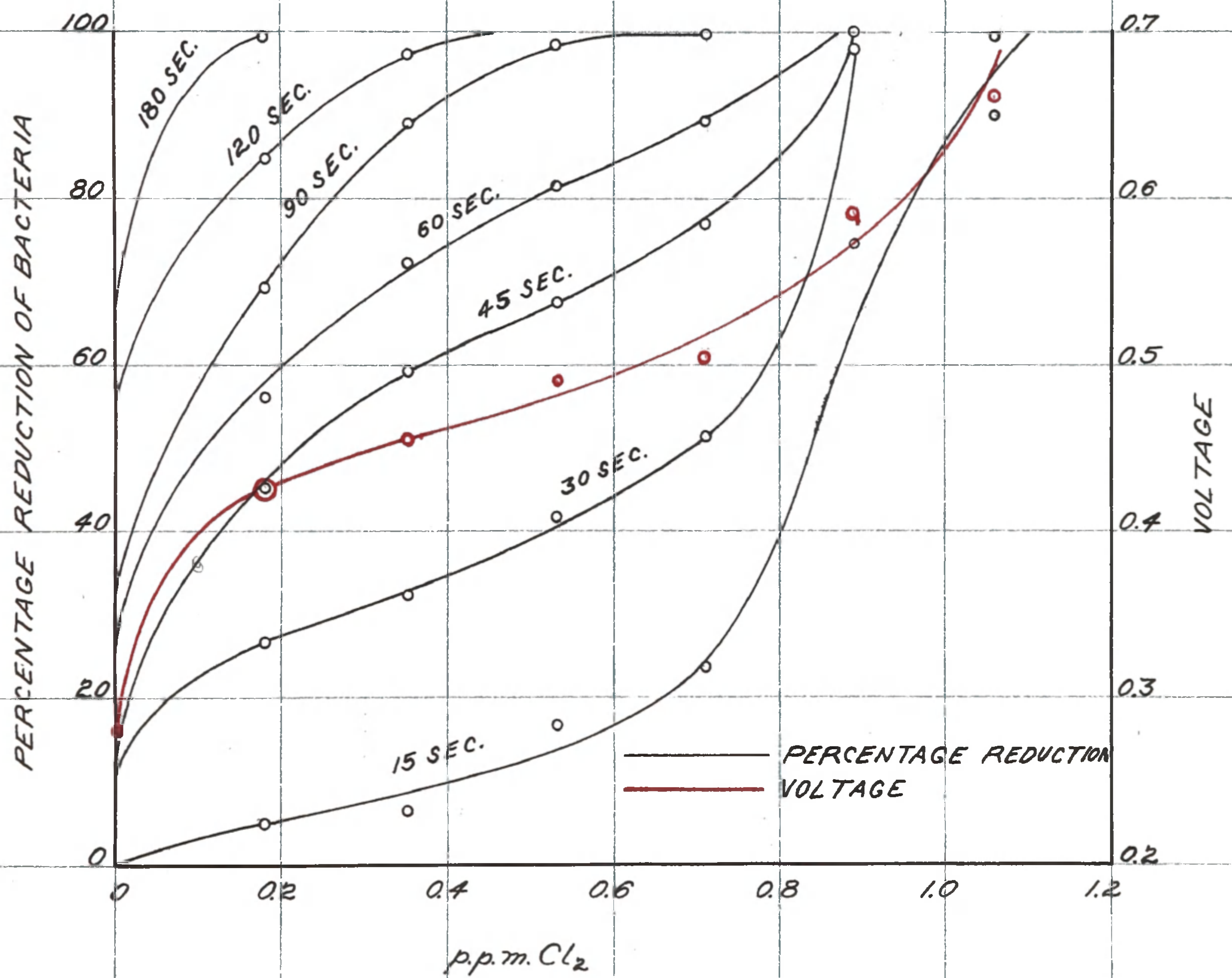


Table IV

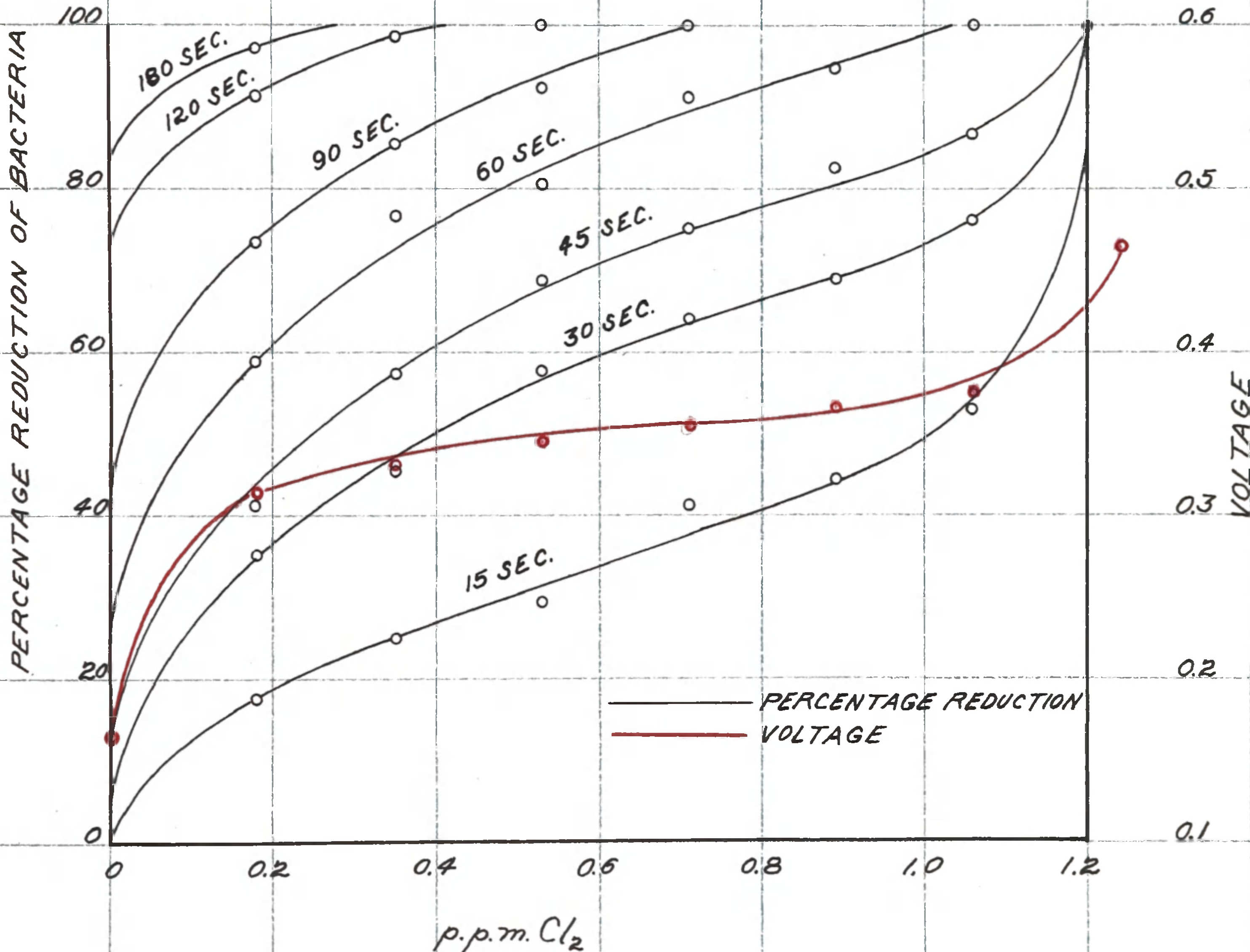
The Relationship of Oxidation-Reduction Potentials

to the Germicidal Activity of Chlorine at pH 9.00

Voltage	P.P.M. Cl_2	Bacteria added per ml.	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.165	0.	3,400	0.						
0.315	0.18	3,400	17.7	35.3	41.2	58.9	73.6	91.2	97.1
0.330	0.35	6,800	25.0	45.6	57.4	76.5	85.4	98.6	100
0.345	0.53	10,200	99.5	57.9	68.7	80.4	92.2	100	100
0.355	0.71	13,600	41.2	64.0	75.0	91.2	100	100	100
0.365	0.89	17,000	44.2	68.9	82.4	94.7	100	100	100
0.376	1.06	20,400	53.0	76.0	86.6	100	100	100	100
0.465	1.24	23,800	99.6	100	100	100	100	100	100

FIGURE IV

THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 9.00



reduction of bacteria, especially at the 15 and 30 second intervals. Since, at the end of 30 seconds, all or most of the bacteria have been killed in the higher chlorine concentrations, the curves tend to approach a straight line during the following time intervals thus following the slowly rising portion of the voltage curve.

There are two probable reasons why this step formation in the killing action of chlorine and its subsequent relationship to the oxidation-reduction potential have never been demonstrated before. In all of the previous work, demonstrating the speed of disinfection of various chlorine compounds, a constant number of organisms was used for each chlorine concentration so that there was merely an addition effect in germicidal action with each increase in the amount of chlorine. However, in the work presented above, with each increase in chlorine concentration a corresponding increase was made in the number of test organisms added, so that the ratio between the amount of chlorine present and the number of organisms was constant at all times. In this way it was possible to demonstrate whether one chlorine concentration had greater germicidal activity than another chlorine concentration and likewise to demonstrate more clearly the relationship between germicidal activity and oxidation-reduction potentials at various chlorine concentrations. In most of the previous work in which an attempt was made to show a correlation between the germicidal activity of chlorine solutions and their oxidation-reduction potentials the mistake was made of using solutions containing too high concentrations of chlorine, where large variations in potential could not possibly be expected to occur. Thus Charlton and Levine (4), in referring to some work by Hellwig in which he used concentrations of chlorine varying from 1,000 p.p.m. to 4,000 p.p.m., found the oxidation-reduction potentials of these solutions to be almost identical at a given pH concentration and concluded that the oxidation-reduction potential is not a dependable measure of its germicidal activity. They also concluded from the work of Schmelkes and Horning (18) "that the oxidation potentials of solutions of different compounds are not proportional to germicidal action", in that the work showed Azochloramid to have a greater germicidal power than chloramine-T, although the latter had a higher oxidation-reduction potential. However, they failed to take into consideration that this was in the presence of organic matter whereas Schmelkes and Horning showed very clearly that in the absence of organic matter chloramine-T had greater germicidal activity than did Azochloramid.

Neither the starch-iodide titration nor the ortho-tolidine titration gave any indication of this difference in germicidal activity between solutions containing different concentrations of chlorine, as did the oxidation-reduction potential. It has been shown by many authors that, in the case of solutions containing the same p.p.m. of chlorine but with different pH concentrations, there was a greatly increased germicidal activity with those solutions which were more acid. Here again the starch-iodide and ortho-tolidine titrations would give misleading results as they would tend to give the same value for each of the solutions. The oxidation-reduction potential, on the other hand, would become more positive with the increasing amounts of acidity and would therefore indicate the increased germicidal activity.

Mallmann (11) has shown that in the presence of certain types of organic matter neither the starch-iodide titration nor the ortho-tolidine titration tend to measure, with any degree of accuracy, the amount of germicidal chlorine present. The following tests were therefore set up to determine further the limitations of these two tests in the presence of organic matter and to demonstrate the role of the oxidation-reduction potential under such conditions. One-tenth percent agar was used as a representative type of insoluble organic matter. The agar was added to flasks containing 1,000 mls. of distilled water and was then heated in the steamer for twenty minutes. After cooling, sufficient chlorine was added to produce the following residuals as previously determined by the starch-iodide titration in distilled water: 0., 0.70, 1.40, 2.10, 2.80, 3.50, 4.20 and 4.90 p.p.m. respectively. These were then allowed to stand at room temperature for 24 hours so that the chlorine and agar might come to equilibrium. At the end of this period the residual chlorine was determined by the starch-iodide titration and voltage determinations were made on each of the solutions as previously described. Five hundred mls. of each solution were then carefully measured out and tests to determine the speed of disinfection were made as before. In these tests a constant number of organisms was used throughout instead of increasing the number with each increase in the amount of chlorine added. These tests were repeated at pH concentrations of 5.00, 7.00 and 9.00. The results are presented in Tables V, VI and VII and in Figs. V, VI and VII.

The results obtained from these studies are quite interesting even though it is rather improbable that such conditions would be encountered in the great majority of instances where chlorine has a practical application as a germicide. However, they tend to show clearly some of the difficulties which may be encountered in successful chlorine treatment and to demonstrate in a very marked way the relationship between the oxidation-reduction potential and the germicidal activity of chlorine solutions.

It will be noted that with successive amounts of chlorine added there is first an increase in the titratable chlorine until a maximum is reached. On the addition of the next successive amounts of chlorine, there is a marked falling off in the amount of titratable chlorine to a minimum value. In the case of pH 7.00 this value is 0. or in other words there is no chlorine present in a form which will show up on titration. On the next successive additions of chlorine the titratable chlorine again increases and continues to do so indefinitely with each increased addition of chlorine. Both the voltage curve and the curves derived from the

Table V

The Relationship of Oxidation-Reduction Potentials

to the Germicidal Activity of Chlorine at pH 5.00

in the Presence of 0.1 Percent Agar*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.360	0.	0.	0.						
0.560	0.70	0.28	22.9	71.4	90.1	97.6	99.7	100	100
0.650	1.40	0.85	56.8	85.6	95.1	100	100	100	100
0.680	2.10	0.99	66.7	94.1	99.1	100	100	100	100
0.600	2.80	0.25	31.4	74.7	93.6	99.6	100	100	100
0.795	3.50	0.64	100	100	100	100	100	100	100
0.825	4.20	0.99	100	100	100	100	100	100	100
0.850	4.90	1.38	100	100	100	100	100	100	100

* Chlorine and agar allowed to come to equilibrium for 24 hours
20,000 organisms per ml.

FIGURE V

THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 5.00 IN THE PRESENCE OF 0.1 PER CENT AGAR

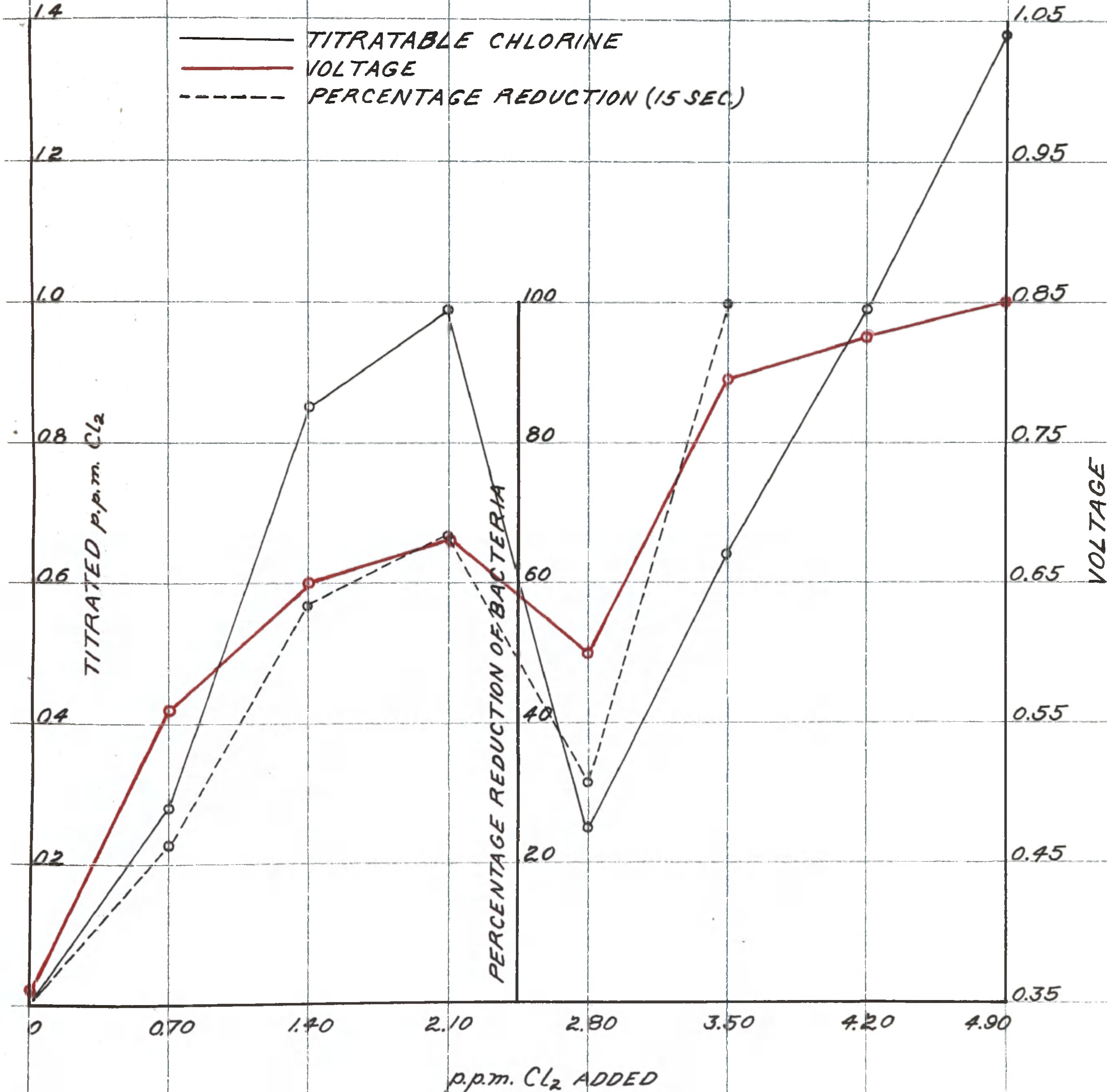


Table VI

The Relationship of Oxidation-Reduction Potentials to the Germicidal Activity of Chlorine at pH 7.00 in the Presence of 0.1 Percent Agar*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.250	0.	0.	0.						
0.365	0.70	0.53	0.4	1.7	3.4	5.0	6.7	8.0	9.7
0.485	1.40	0.71	2.0	4.4	6.7	9.4	12.4	15.4	16.7
0.345	2.10	0.	0	0	0	0	0	0	0
0.310	2.80	0.	0	0	0	0	0	0	0
0.520	3.50	0.21	5.0	7.0	10.0	12.7	19.4	23.6	26.7
0.720	4.20	0.71	94.4	99.9	100	100	100	100	100
0.760	4.90	1.13	100	100	100	100	100	100	100

*Chlorine and agar allowed to come to equilibrium for 24 hrs.
30,000 organisms per ml.

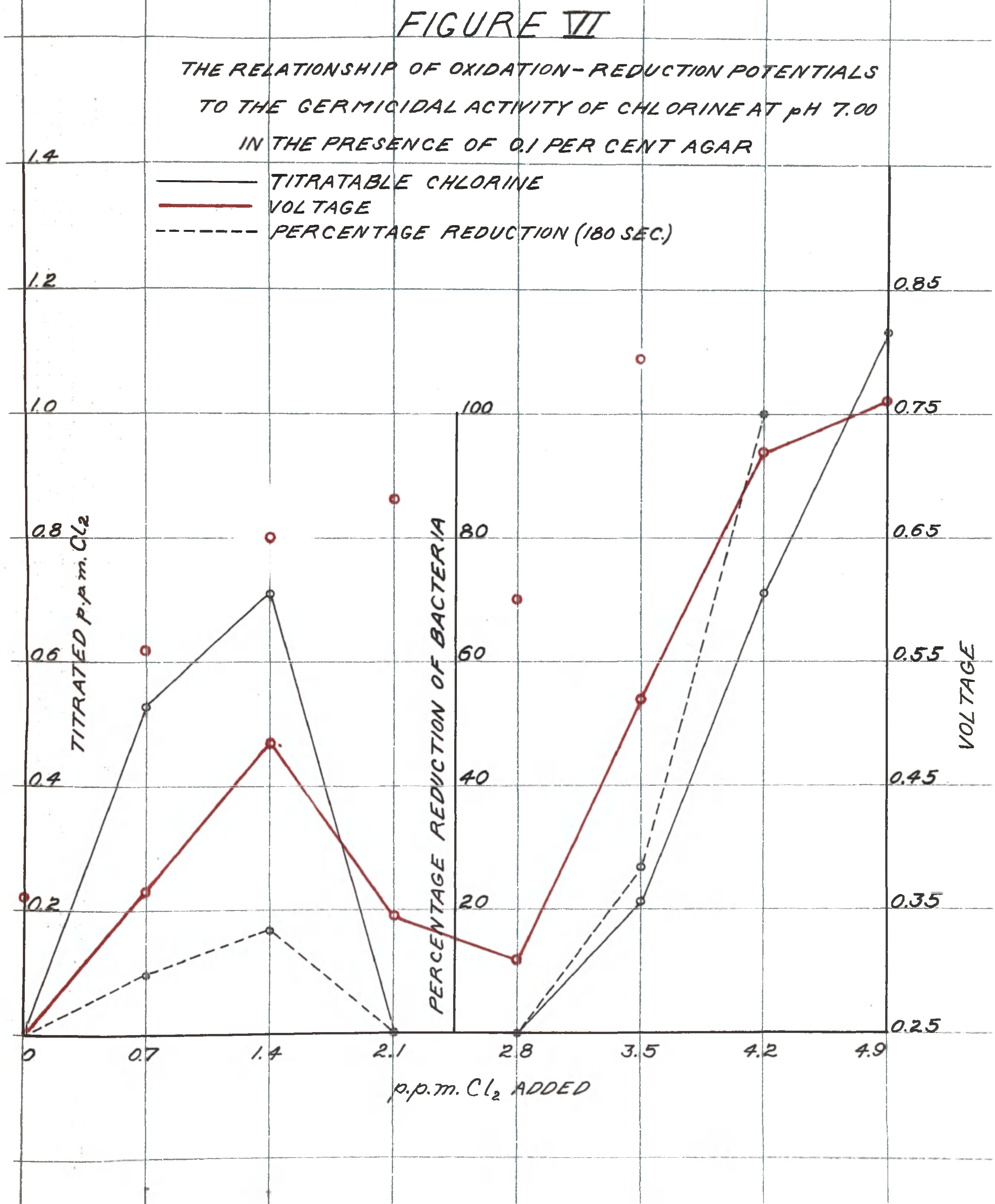


FIGURE VII
THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.00 IN THE PRESENCE OF 0.1 PER CENT AGAR
TITRATABLE CHLORINE
VOLTAGE
PERCENTAGE REDUCTION (180 SEC.)
TITRATED p.p.m. Cl2
PERCENTAGE REDUCTION OF BACTERIA
VOLTAGE
p.p.m. Cl2 ADDED
0
0.7
1.4
2.1
2.8
3.5
4.2
4.9
0.2
0.4
0.6
0.8
1.0
1.2
1.4
20
40
60
80
100
0.25
0.35
0.45
0.55
0.65
0.75
0.85

Table VII

The Relationship of Oxidation-Reduction Potentials

to the Germicidal Activity of Chlorine at pH 9.00

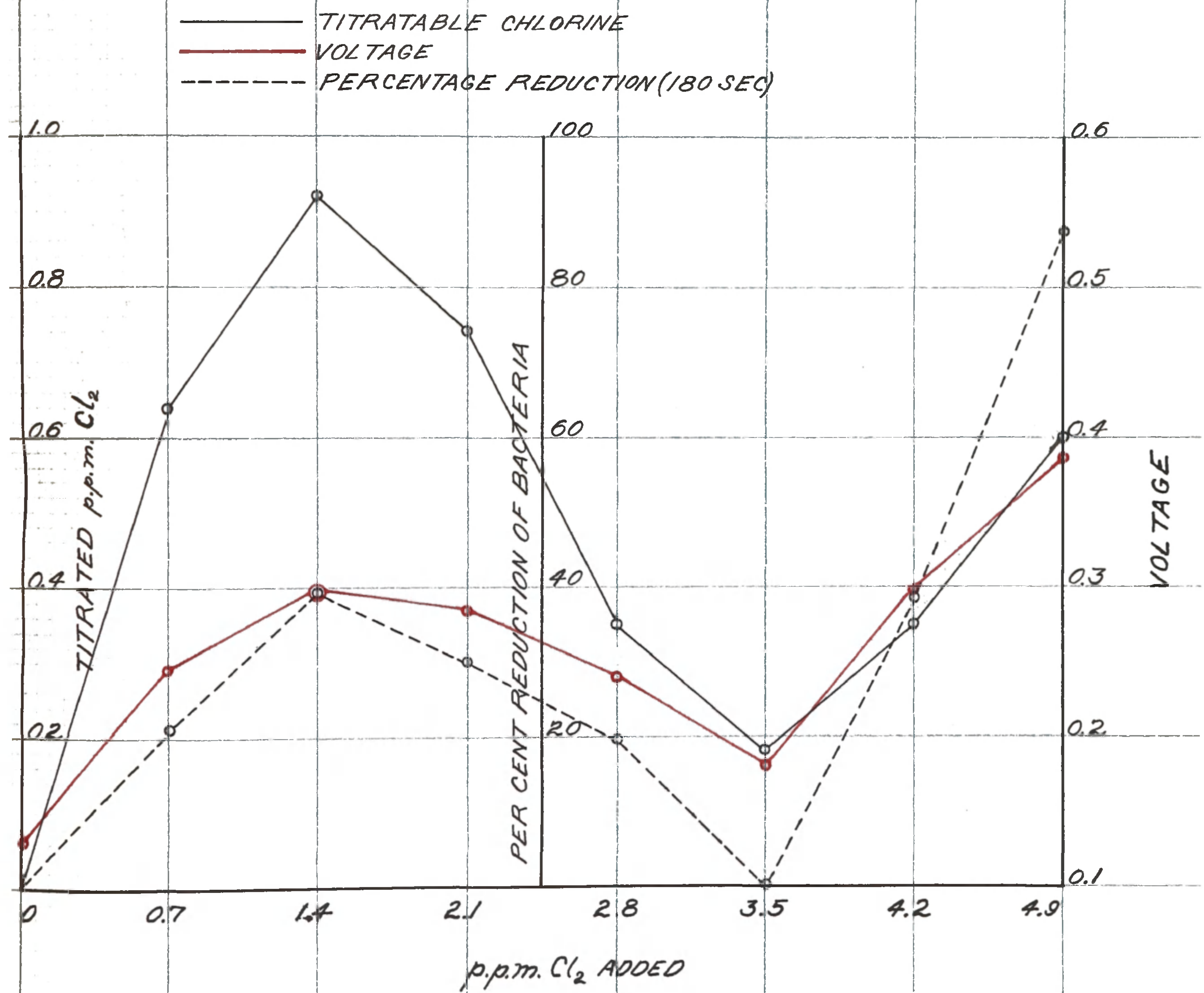
in the Presence of 0.1 Percent Agar*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
				Percentage Reduction of Bacteria					
0.130	0.	0.	0.						
0.245	0.70	0.64	3.1	5.8	11.3	13.4	16.4	18.5	21.3
0.295	1.40	0.92	15.2	19.7	23.7	26.1	28.8	33.4	39.4
0.285	2.10	0.74	9.1	14.3	18.2	21.6	24.3	27.4	30.0
0.240	2.80	0.35	2.2	4.0	11.0	14.0	16.4	18.5	19.7
0.180	3.50	0.18	0	0	0	0	0	0	0
0.298	4.20	0.35	14.3	21.3	23.4	27.0	30.4	36.7	38.5
0.386	4.90	0.60	34.9	45.8	54.0	55.5	67.3	71.9	87.3

*Chlorine and agar allowed to come to equilibrium for 24 hrs.
33,000 organisms per ml.

FIGURE VII

THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 9.00 IN THE PRESENCE OF 0.1 PER CENT AGAR



percentage reduction of bacteria in these different chlorine solutions tend to follow in a general way this same type of curve produced by the titratable chlorine. It will be noted, however, that the voltage curves in each case give a true indication of the degree of germicidal action which may be expected with the various chlorine concentrations whereas the titration curves give values which would lead to conclusions quite different from those actually observed from the curves giving percent reduction of bacteria. For example, in Table V and Fig. V, on the addition of 2.10 p.p.m. of chlorine, a titration value of 0.99 p.p.m. of chlorine is obtained whereas on the addition of 3.50 p.p.m. of chlorine, a titration value of 0.64 p.p.m. is obtained. This would indicate that the solution to which 3.50 p.p.m. of chlorine was added should be much less germicidal than the solution to which 2.10 p.p.m. of chlorine was added. On the other hand, looking at the voltage curve it is seen that on the addition of 2.10 p.p.m. of chlorine a voltage of 0.680 is obtained and on the addition of 3.50 p.p.m. of chlorine a voltage reading of 0.795 is obtained. This would indicate that a much better germicidal action should be obtained on the addition of 3.50 p.p.m. of chlorine than would be obtained on the addition of 2.10 p.p.m. of chlorine. The percentage reduction curve shows that after an exposure of 15 seconds with the addition of 2.10 p.p.m. of chlorine there was 66.70 percent reduction of bacteria whereas on the addition of 3.50 p.p.m. of chlorine there was 100 percent reduction of bacteria.

From this it is quite evident that the oxidation-reduction potential represents a truer picture of the degree of germicidal activity which may be expected than does the starch-iodide titration method. Table VI and VII and Figs. VI and VII show that this same relationship holds good at pH concentrations of 7.00 and 9.00.

In order to demonstrate whether these solutions were more or less germicidal than chlorine solutions in the absence of organic matter (agar), tests were run as before comparing the germicidal action of chlorine in the presence of 0.1 percent agar with solutions made up in distilled water. Solutions of chlorine in the presence of agar were used in concentrations which represented both those before and after the minimal titration value had been reached. These determinations were run at pH concentrations of 5.00, 7.00 and 9.00. The results are shown in Tables VIII, IX and X and in Figs. VIII, IX and X.

Table VIII and Fig. VIII show this relationship at pH 5.00. It may be seen that 2.20 p.p.m. of chlorine in the presence of agar before the minimal titration value is reached has less germicidal action than has 0.53 p.p.m. of chlorine in distilled water and that 0.78 p.p.m. of chlorine in the presence of agar after the minimal titration value has been reached has greater germicidal action than has 0.96 p.p.m. of chlorine in distilled water. Likewise at pH 7.00, Table IX Fig. IX, 0.81 p.p.m. of chlorine in the presence of agar before the minimal titration value has been reached has

Table VIII

The Relative Germicidal Activity of Chlorine in the

Presence and Absence of 0.1 Percent Agar at pH 5.00

Percent agar	Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
				15	30	45	60	90	120	180
				Percentage of Bacteria Surviving						
0.1	0.437	0.	0.	100						
	0.595	0.70	0.35	90.0	84.6	76.0	67.0	44.3	18.9	5.2
	0.665	1.40	1.27	73.0	55.4	40.5	27.9	9.7	3.2	0.9
	0.680	2.10	2.20	65.0	48.6	32.9	23.7	4.8	1.6	.3
	0.690	2.80	0.67	19.4	3.4	1.4	0.3	0.	0.	0.
	0.820	3.50	0.78	0.	0.	0.	0.	0.	0.	0.
0	0.430	0.	0.	100						
	0.730	0.53	0.53	52.2	35.0	20.5	7.9	0.9	0.3	0.
	0.745	0.96	0.96	0.3	0.	0.	0.	0.	0.	0.

55,000 organisms per ml.

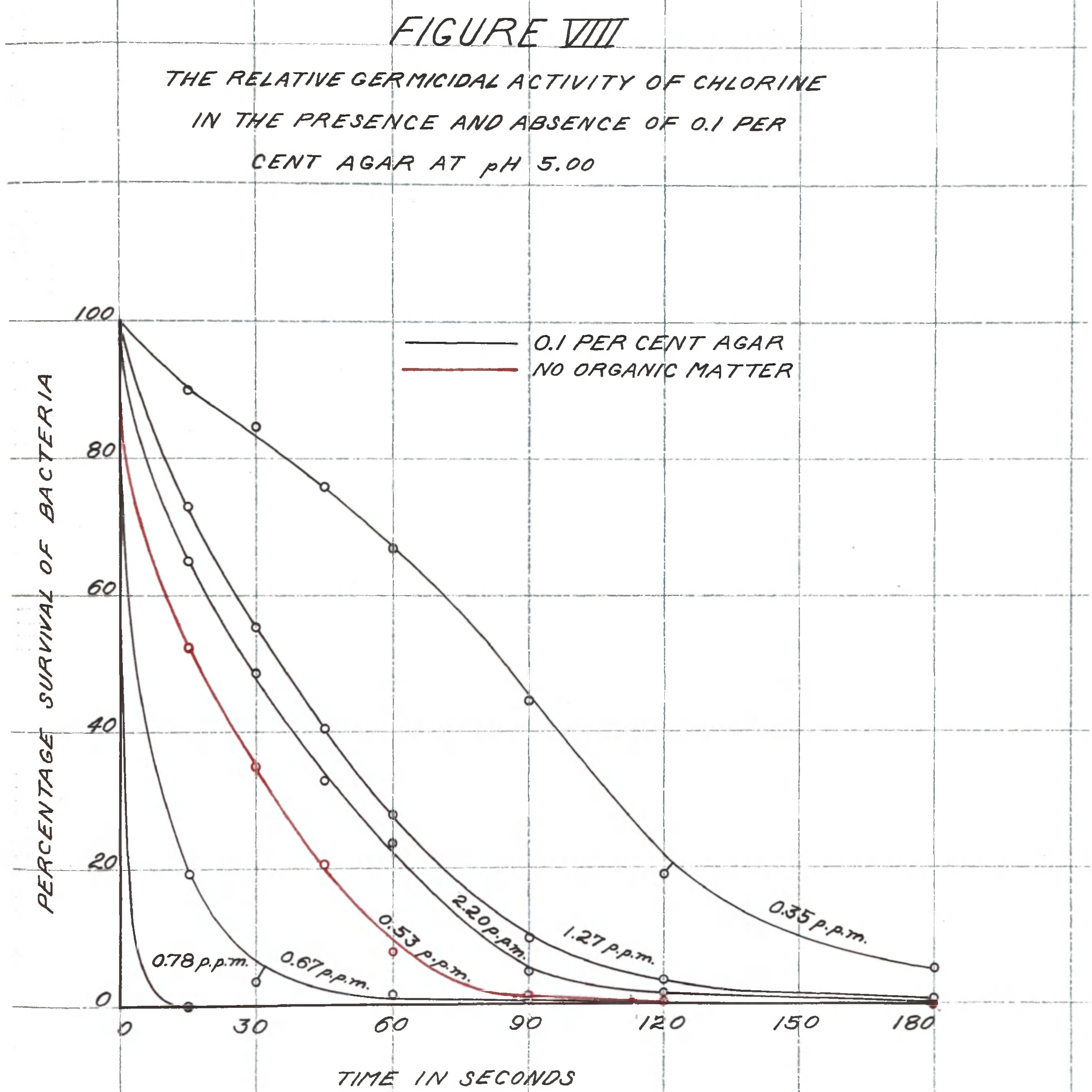


FIGURE VIII
THE RELATIVE GERMICIDAL ACTIVITY OF CHLORINE
IN THE PRESENCE AND ABSENCE OF 0.1 PER
CENT AGAR AT pH 5.00
0.1 PER CENT AGAR
NO ORGANIC MATTER
PERCENTAGE SURVIVAL OF BACTERIA
100
80
60
40
20
0
0
30
60
90
120
150
180
TIME IN SECONDS
0.78 p.p.m.
0.67 p.p.m.
0.53 p.p.m.
2.20 p.p.m.
1.27 p.p.m.
0.35 p.p.m.

Table IX

The Relative Germicidal Activity of Chlorine in the

Presence and Absence of 0.1 Percent Agar at pH 7.00

Percent agar	Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
				15	30	45	60	90	120	180
				Percentage of Bacteria Surviving						
0.1	0.280	0.	0.	100						
	0.380	0.70	0.53	91.6	84.4	76.4	64.8	59.0	51.8	50.0
	0.460	1.40	0.81	90.5	79.0	70.0	62.6	51.4	38.4	27.1
	0.370	2.10	0.28	97.8	94.9	92.0	86.5	79.7	77.8	75.3
	0.710	4.90	0.78	1.8	0.	0.	0.	0.	0.	0.
0.	0.275	0.	0.	100						
	0.445	0.49	0.49	83.6	70.0	62.0	50.0	31.9	20.2	2.9
	0.475	0.88	0.88	72.4	54.7	35.1	18.8	1.4	0.3	0.

27,600 organisms per ml.

FIGURE IX

THE RELATIVE GERMICIDAL ACTIVITY OF CHLORINE IN THE PRESENCE AND ABSENCE OF 0.1 PER CENT AGAR AT pH 7.00

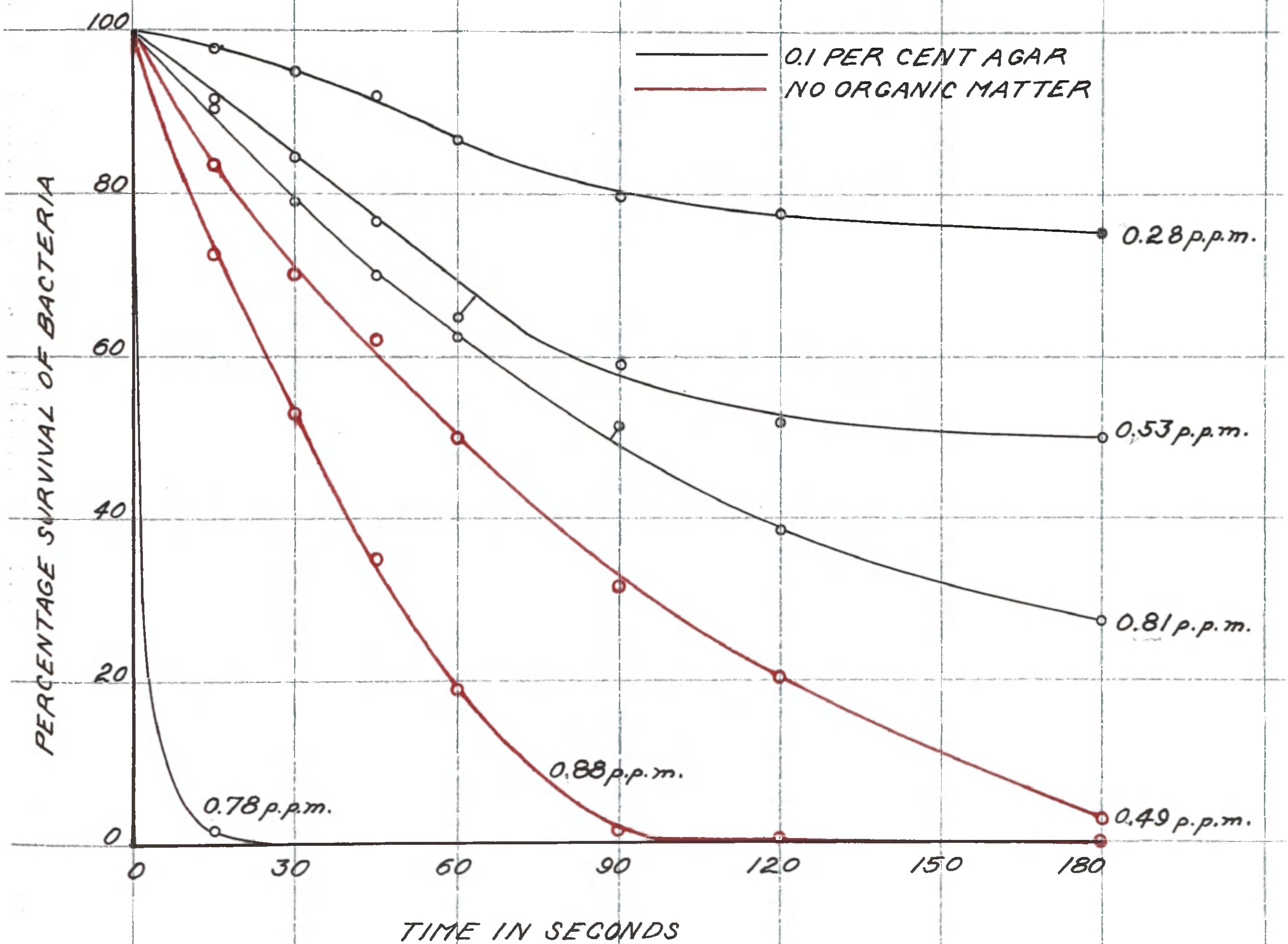


Table X

The Relative Germicidal Activity of Chlorine in the

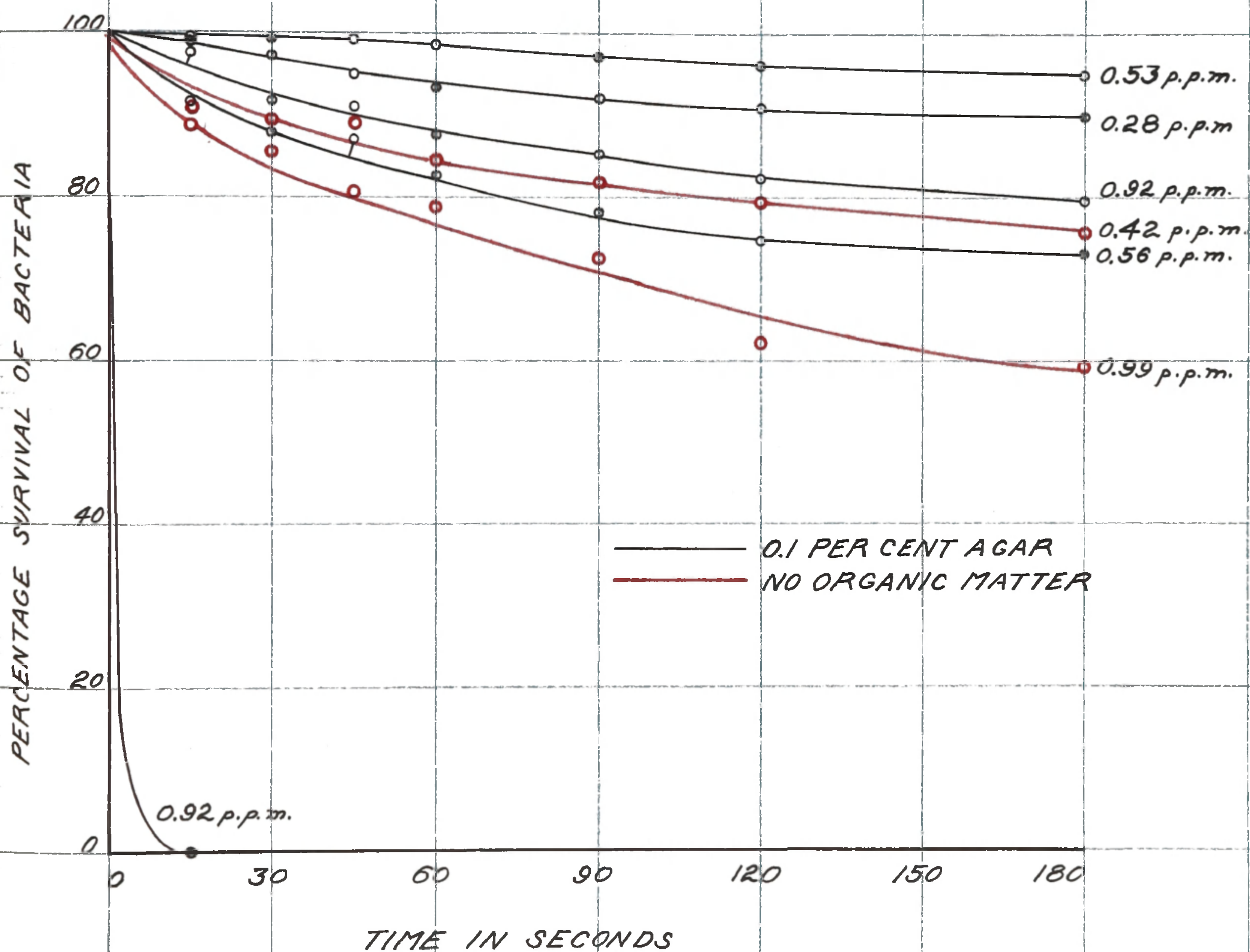
Presence and Absence of 0.1 Percent Agar at pH 9.00

Percent agar	Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
				15	30	45	60	90	120	180
				Percentage of Bacteria Surviving						
0.1	0.165	0.	0.	100						
	0.310	1.40	0.92	97.8	91.7	91.1	87.4	85.0	82.2	79.5
	0.290	2.10	0.53	99.6	99.3	99.3	98.4	97.2	96.0	94.8
	0.305	3.50	0.28	99.0	97.2	94.8	93.5	92.0	90.8	88.9
	0.450	4.20	0.56	91.4	87.7	86.8	82.5	78.0	74.3	73.0
	0.545	4.90	0.92	0.	0.	0.	0.	0.	0.	0.
0.	0.160	0.	0.	100						
	0.330	0.42	0.42	92.9	89.2	88.9	84.4	81.9	79.5	75.8
	0.350	0.99	0.99	88.6	85.6	80.4	78.8	72.4	62.2	59.3

32,700 organisms per ml.

FIGURE X

THE RELATIVE GERMICIDAL ACTIVITY OF CHLORINE IN THE PRESENCE AND ABSENCE OF 0.1 PER CENT AGAR AT pH 9.00



less germicidal value than 0.49 p.p.m. of chlorine in distilled water and 0.78 p.p.m. of chlorine in the presence of agar after the minimal titration has been reached shows much more germicidal action than does 0.88 p.p.m. in distilled water. This same relationship also holds good at pH 9.00, Table X Fig. X, where 0.92 p.p.m. of chlorine in the presence of agar after the minimal titration value has been reached had much more germicidal action than had 0.99 p.p.m. of chlorine in distilled water. As shown in the previous experiment, the oxidation-reduction potential gives the true evaluation of the amount of germicidal action which may be expected to take place whereas the starch-iodide titration method gives very misleading values.

This same relationship, where in one case the chlorine and agar solution is less germicidal than a solution of chlorine alone and in the other case more germicidal than chlorine alone, has been noted previously by Mallmann (8). He found that the addition of 0.3 p.p.m. of chlorine (NaOCl) to a solution containing 3.0 p.p.m. of agar or of a commercial hydrophilic colloid would cause a great increase in the amount of germicidal activity. He also found that if the concentration of agar was greatly increased in proportion to the amount of chlorine, the solution tended to show a greatly decreased germicidal action. This same picture is shown in the present study except that much larger quantities of both agar and chlorine were used.

Table XI and Fig. XI show the effect of 0.01 percent peptone on the germicidal action of chlorine at pH 7.00 and the relationship of the oxidation-reduction potential and starch-iodide titration for residual chlorine to the germicidal action. Sufficient chlorine was added to flasks of sterile peptone solution to give final concentrations of 0.70, 1.40, 2.10 etc. up to 11.90 p.p.m. of chlorine. These were then allowed to stand for 24 hours at room temperature to allow the chlorine and peptone to come to complete equilibrium. At the end of this period the oxidation-reduction potential, residual chlorine by the starch-iodide titration method and germicidal action were determined as previously described.

Here again there was shown to exist a very definite relationship between the oxidation-reduction potential and the germicidal activity of a chlorine solution. The peptone tended to exert what appears to be a poising effect on the voltage, tending to hold it down in a region which is non-germicidal. This poising effect was continued until 9.10 p.p.m. of chlorine had been added; the voltage increasing in a slowly rising line from 0.240 to 0.355. On the next two additions of chlorine up to 10.50 p.p.m. the voltage rose sharply from 0.355 to 0.495 and then leveled off again. The percentage reduction of bacteria tended to follow this voltage curve very closely. Up through the addition of 9.10 p.p.m. of chlorine there was no reduction in the number of bacteria. On the next two additions up to 10.50 p.p.m. of

Table XI

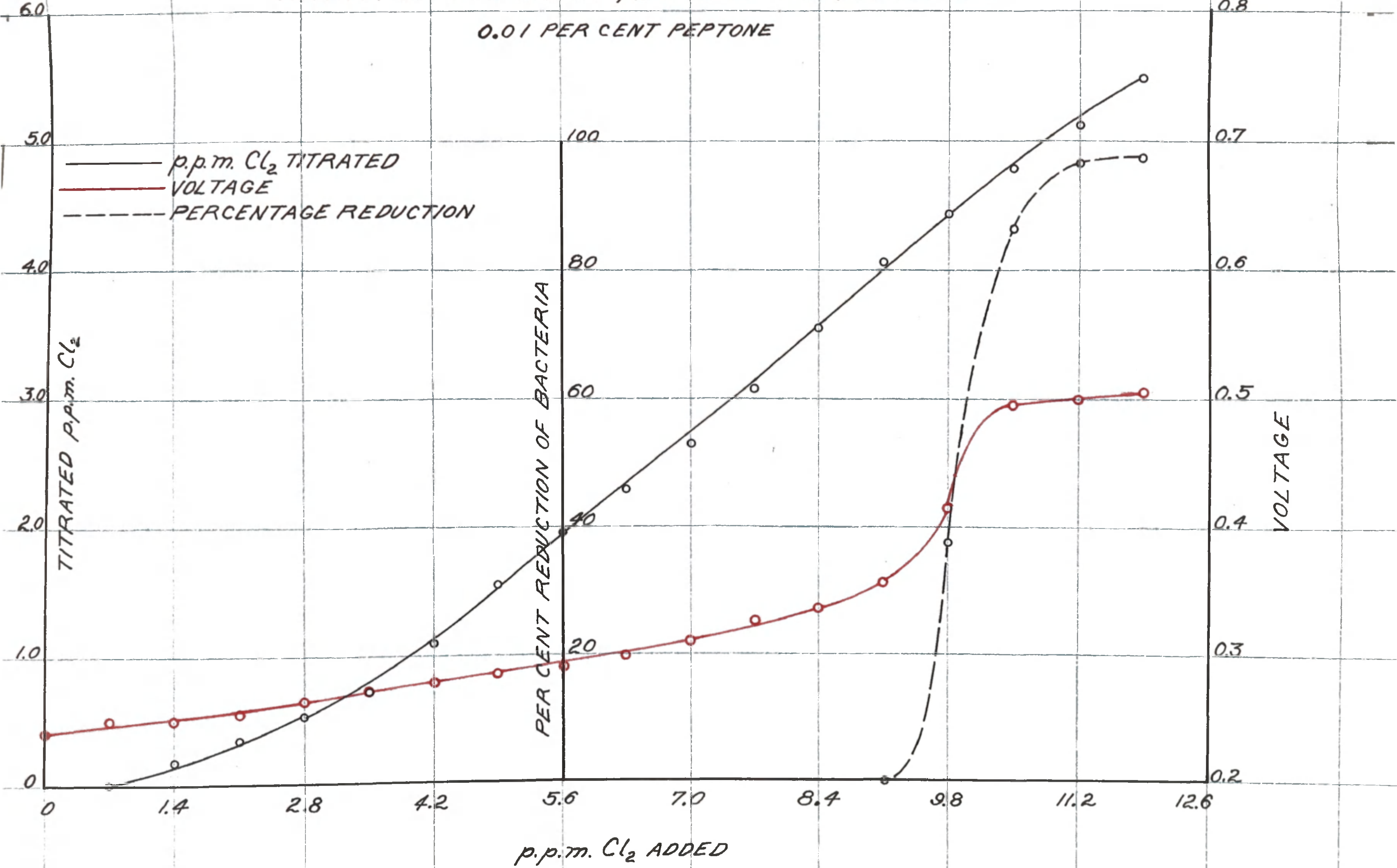
The Relationship of Oxidation-Reduction Potentials to the Germicidal Activity of Chlorine at pH 7.00 in the Presence of 0.01 Percent Peptone*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2	Time of Exposure in Seconds						
			15	30	45	60	90	120	180
			Percentage Reduction of Bacteria						
0.240	0.	0.	0.	0.	0.	0.	0.	0.	0.
0.250	0.70	0.	0.	0.	0.	0.	0.	0.	0.
0.250	1.40	0.18	0.	0.	0.	0.	0.	0.	0.
0.255	2.10	0.35	0.	0.	0.	0.	0.	0.	0.
0.265	2.80	0.53	0.	0.	0.	0.	0.	0.	0.
0.272	3.50	0.71	0.	0.	0.	0.	0.	0.	0.
0.278	4.20	1.10	0.	0.	0.	0.	0.	0.	0.
0.285	4.90	1.55	0.	0.	0.	0.	0.	0.	0.
0.290	5.60	1.95	0.	0.	0.	0.	0.	0.	0.
0.300	6.30	2.30	0.	0.	0.	0.	0.	0.	0.
0.310	7.00	2.65	0.	0.	0.	0.	0.	0.	0.
0.325	7.70	3.08	0.	0.	0.	0.	0.	0.	0.
0.335	8.40	3.55	0.	0.	0.	0.	0.	0.	0.
0.355	9.10	4.07	0.	0.	0.	0.	0.	0.	0.
0.415	9.80	4.43	0.5	7.8	12.1	18.2	24.6	29.2	37.3
0.495	10.50	4.79	8.0	13.8	17.8	22.7	29.2	44.2	86.1
0.500	11.20	5.12	10.5	18.2	22.1	26.7	32.7	61.9	96.5
0.505	11.90	5.49	12.1	16.5	24.0	29.2	41.1	64.4	92.3

*Chlorine and peptone allowed to come to equilibrium for 24 hrs. 30,000 organisms per ml.

FIGURE XI

THE RELATIONSHIP OF OXIDATION-REDUCTION POTENTIALS TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.00 IN THE PRESENCE OF 0.01 PER CENT PEPTONE



chlorine, where the voltage rose sharply into what appears to be the germicidal range, the reduction of bacteria became quite marked, reaching 86.1 percent on the addition of 10.50 p.p.m. of chlorine. On the next two additions of chlorine the germicidal activity only increased slightly, thus tending to follow the portion of the voltage curve as it leveled off. Observation of the curve representing p.p.m. of titratable chlorine shows that there was no correlation between it and the germicidal activity. After the addition of 0.70 p.p.m. of chlorine the titration curve rises steadily to 4.20 p.p.m. of chlorine without showing any germicidal action in the time limits employed. It is quite evident from this that the starch-iodide titration method does not measure germicidal chlorine alone but also measures that chlorine which has been absorbed or is chemically combined and is not free to act in a germicidal capacity.

Since such high titration values were obtained with the peptone solutions up through the addition of 9.10 p.p.m. of chlorine, it was thought that possibly there had been a combination between the chlorine and the peptone to form chloro-peptones, which might act similarly to chloramines. If this were the case they would tend to show little or no germicidal action in the three minute time period employed, whereas if they were allowed to remain in contact with the organisms for a longer period of time they might show germicidal action similar to that shown by chloramines.

Rudolfs and Ziemba (16) showed that peptone solutions chlorinated to different percentages of their one-hour chlorine demand have no germicidal properties. They did show, however, that samples of sewage which had been chlorinated to different percentages of their one-hour chlorine demand tended to form certain "chloro-products" which would bring about bacterial reductions. The work presented here, however, was conducted with peptone solutions which had been chlorinated above their 24-hour chlorine demand and which showed as high as 2.55 p.p.m. of chlorine as measured by the starch-iodide titration.

Chlorine was added to solutions containing 0.01 percent peptone to give final concentrations of 0.84, 1.68, 2.52, 3.36 and 4.20 p.p.m. of chlorine. These were allowed to stand for 24 hours in order for the peptone and chlorine to come to equilibrium. One ml. of a suspension of Esch. coli was then added to each of the flasks and well mixed. At the end of 30 minutes, 1, 2, 3, 4 and 5 hours, samples were withdrawn and plated out as before. Table XII shows the results of these tests.

On the first three additions of chlorine up through 2.52 p.p.m. of chlorine the voltage remained constant at 0.260. On the next two additions it rose to 0.285 and 0.310 respectively. It is interesting to note that as long as the voltage remained constant at 0.260 for the three different amounts of chlorine added, the number of organisms remained relatively constant for each of the time periods at which tests were made. The

Table XII

The Relationship of the Oxidation-Reduction Potential

to the Germicidal Action of "Chloro-Peptones"

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2		Time of Exposure					
		Starch-iodide	Ortho-tolidine	30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
				Number of Bacteria Surviving					
0.260	0.	0.	0.	59,300	34,200	10,700	2,200	1,100	1,100
0.260	0.84	0.35	0.	58,900	51,000	40,900	39,900	35,000	32,400
0.260	1.68	0.88	0.18	58,500	50,100	40,200	40,900	34,700	31,000
0.260	2.52	1.42	0.50	57,100	49,500	41,000	42,100	38,800	31,300
0.285	3.36	1.88	0.85	51,000	32,900	9,900	700	0.	0.
0.310	4.20	2.55	1.20	11,000	300	0.	0.	0.	0.

reductions in bacteria in these three concentrations of chlorine may probably best be explained by the plasmoptysis due to the action of the distilled water over this long period of time and not to any germicidal action due to the presence of "chloro-peptones". The control, made in distilled water, showed a much greater germicidal effect than did these first three solutions containing peptone. The peptone probably in part tended to reduce the difference in osmotic pressure between the organisms and the solution and at the same time offered a substrate suitable for the growth of organisms, such as Esch. coli, so that the killing in these instances was less than that shown by the control. In the two solutions containing the highest concentrations of chlorine where there was an increase in the voltage reading, there was also a marked increase in the amount of germicidal activity. Since the three previous solutions showed titration values as high as 1.42 p.p.m. of chlorine by the starch-iodide method but still showed no germicidal value, it seems probable that this was due to free chlorine which remained in excess of that required to completely chlorinate the peptone or to the formation of small amounts of chloramine. Since the starch-iodide titration gave a value of 1.42 p.p.m. of chlorine and the ortho-tolidine titration gave a value of 0.50 p.p.m. of chlorine, these tests present a very striking example of the complete inadequacy of these two methods as measurements of the germicidal action of chlorine in the presence of organic matter.

Table XIII and Fig. XII give a comparison of the oxidation-reduction potential, starch-iodide titration and ortho-tolidine titration as means of measuring the germicidal activity of chlorine in the presence of 0.1 percent agar at pH 7.00. The chlorine was added to the agar solution and allowed to stand for 12 hours before the determinations were made. It may be seen from Fig. XII that the values for the ortho-tolidine titration and the starch-iodide titration were almost identical up to the addition of 2.10 p.p.m. of chlorine at which concentration all the titratable chlorine disappeared. From this point on the values obtained by these two methods of titration varied widely, the ortho-tolidine titration always giving a much lower value than that obtained by the starch-iodide titration. Since, as was previously shown, the starch iodide-titration, in this particular range, gave values much too low for the actual germicidal value, it is apparent that the ortho-tolidine titration in this particular instance was of much less value since it gave much lower results than the starch-iodide titration.

Table XIV and Fig. XIII give a comparison of these same relationships in the presence of 0.01 percent peptone at pH 7.00. The tests were made as with agar in the above experiment. Here again it is shown that there was no correlation between the three methods for measuring the germicidal activity of a chlorine solution. The voltage which, as

Table XIII

The Oxidation-Reduction Potential, Starch-Iodide and Ortho-Tolidine Titrations as Measures of Available Chlorine in the Presence of 0.1 Percent Agar

P.P.M. Cl_2 added	Voltage	Titrated p.p.m. Cl_2	
		Starch-iodide	Ortho-tolidine
0.	0.260	0.	0.
0.70	0.490	0.53	0.55
1.40	0.380	0.35	0.35
2.10	0.295	0.	0.
2.80	0.410	0.25	0.10
3.50	0.690	0.63	0.35
4.20	0.720	1.06	0.60

FIGURE XII

THE OXIDATION-REDUCTION POTENTIAL, STARCH-IODIDE AND ORTHO-TOLIDINE TITRATIONS AS MEASURES OF AVAILABLE CHLORINE IN THE PRESENCE OF 0.1 PERCENT AGAR

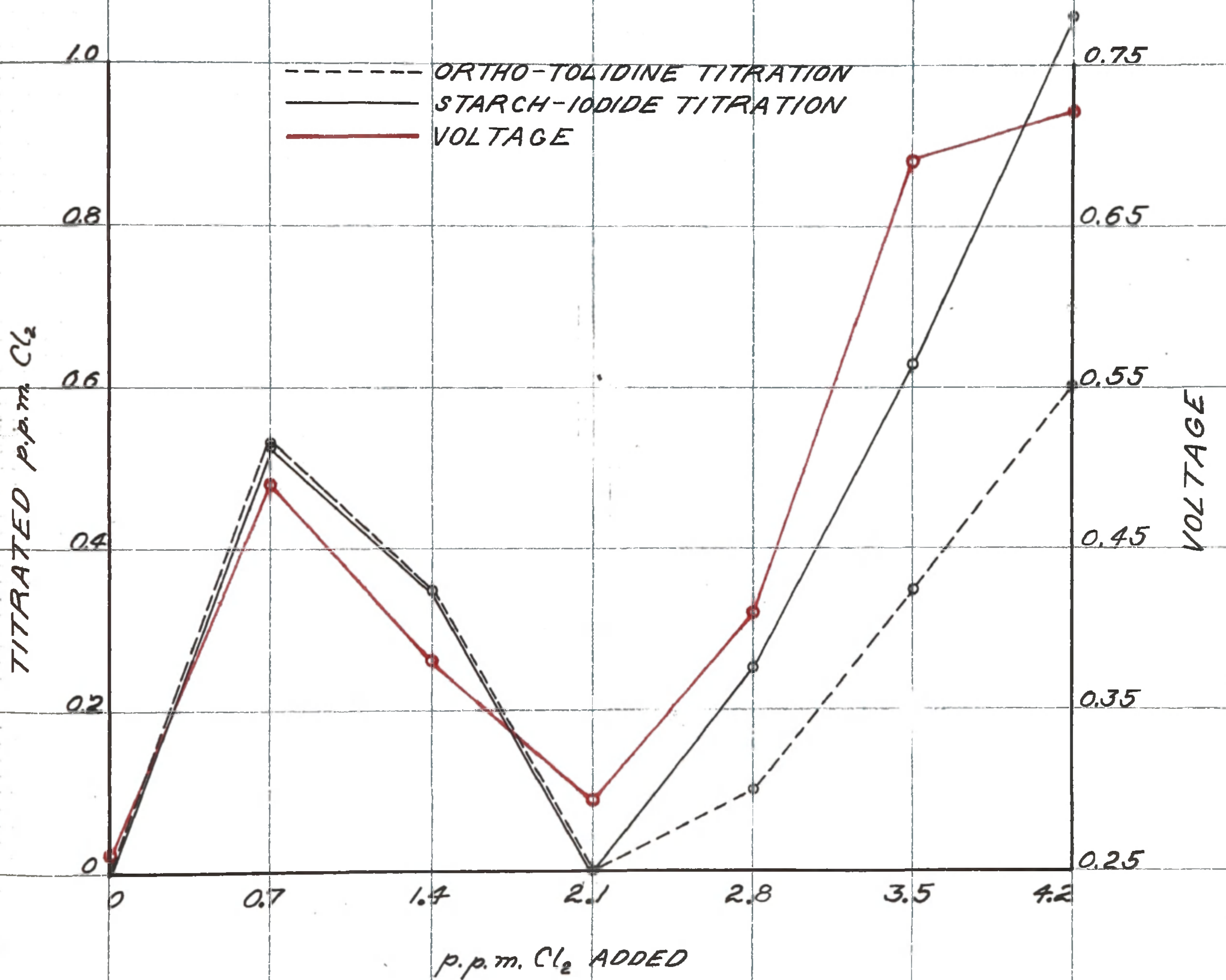


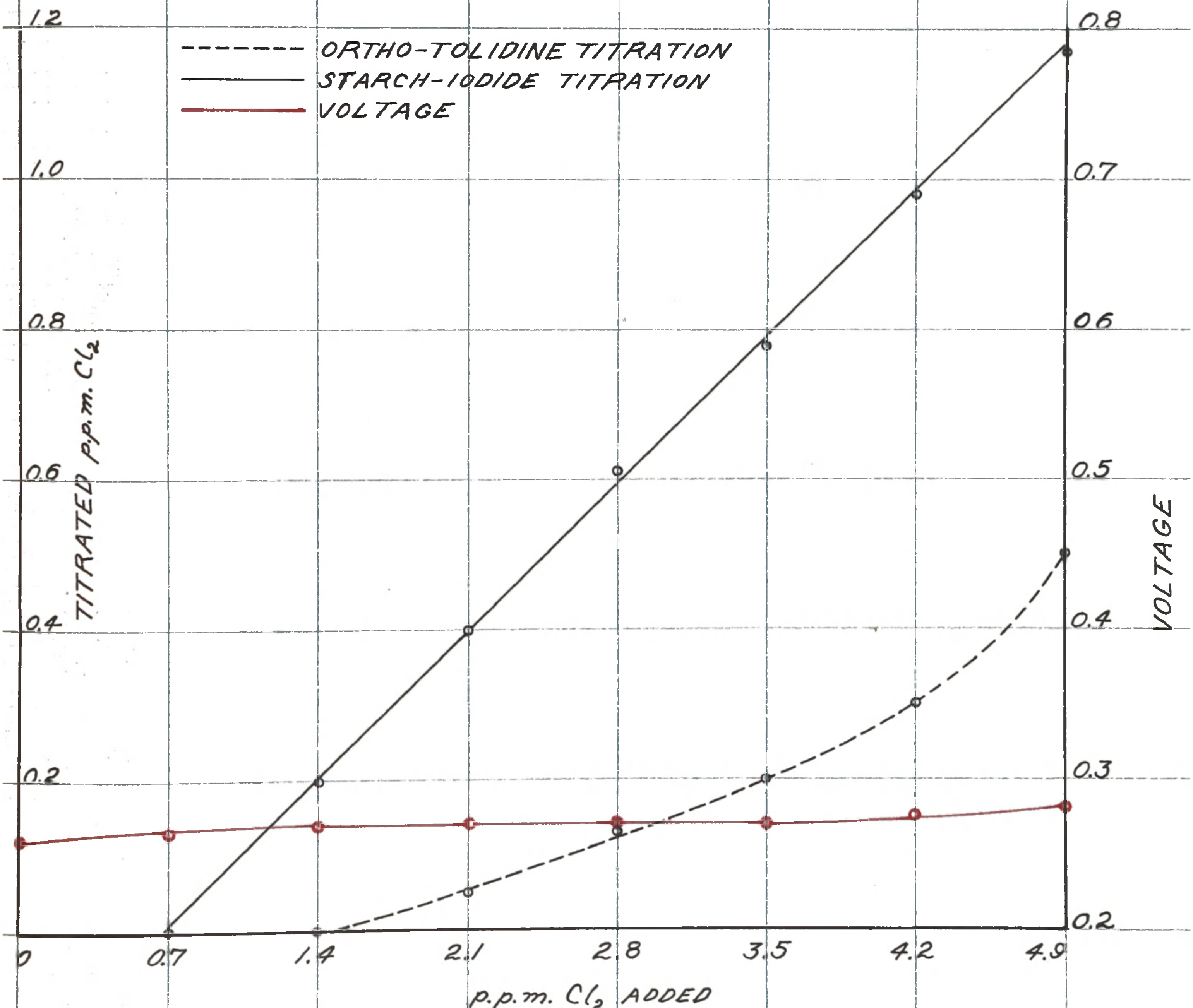
Table XIV

The Oxidation-Reduction Potential, Starch-Iodide and Ortho-Tolidine Titrations as Measures of Available Chlorine in the Presence of 0.01 Percent Peptone

P.P.M. Cl_2 added	Voltage	Titrated p.p.m. Cl_2	
		Starch-iodide	Ortho-tolidine
0.	0.260	0.	0.
0.70	0.265	0.	0.
1.40	0.270	0.20	0.
2.10	0.270	0.40	0.05
2.80	0.270	0.61	0.13
3.50	0.270	0.78	0.20
4.20	0.275	0.98	0.30
4.90	0.280	1.17	0.50

FIGURE XIII

THE OXIDATION-REDUCTION POTENTIAL, STARCH-IODIDE AND ORTHO-TOLIDINE TITRATIONS AS MEASURES OF AVAILABLE CHLORINE IN THE PRESENCE OF 0.01 PER CENT PEPTONE



previously shown, gave a true indication of the amount of germicidal activity to be expected in a peptone solution, remained almost constant, changing only from 0.260 to 0.280 thus indicating that there was no germicidal activity. In contrast, the p.p.m. of chlorine as measured by the starch-iodide titration increased steadily after the addition of the first 0.70 p.p.m. of chlorine to give a final titration value of 1.17 p.p.m. of chlorine on the addition of 4.90 p.p.m. of chlorine. Although the ortho-tolidine titration gave a much lower value than did the starch-iodide titration it still did not represent anywhere near the true germicidal activity of the chlorine solutions tested. The chlorine demand was 1.40 p.p.m. of chlorine instead of 0.70 p.p.m. as measured by the starch-iodide method but from this point on the p.p.m. of chlorine rose steadily up to 0.50 p.p.m. on the addition of 4.90 p.p.m. of chlorine. Both the starch-iodide and ortho-tolidine, therefore, gave values which would indicate a high degree of germicidal activity whereas actually there was no germicidal activity at least for the time periods used.

In the great majority of instances where chlorine finds application as a disinfectant, such as in water and sewage treatment the chlorine is added directly to solutions already containing organisms so that destruction of organisms starts at once before the chlorine has had opportunity to come to complete equilibrium with any organic matter present. Since the previous work was conducted with solutions in which this equilibrium had been allowed to take place, tests were next made to demonstrate the effect of chlorine on adding it to solutions already containing organisms and to demonstrate the value of the starch-iodide titration, ortho-tolidine titration and oxidation-reduction potential as methods of measuring this germicidal action. These determinations were made by adding one ml. of a suspension of Esch. coli to flasks holding 500 mls. of distilled water containing organic matter. After these had been thoroughly mixed, varying amounts of chlorine were added and one ml. samples were withdrawn at the end of 15, 30, 45, 60, 90, 120 and 180 seconds and placed in nine mls. of two percent peptone water to remove any excess chlorine. These were then plated out as before. The determinations of p.p.m. titratable chlorine by both the starch-iodide and ortho-tolidine titrations and the oxidation-reduction potential were made at the end of the three-minute contact period between the chlorine and organic matter.

Table XV and Fig. XIV show these relationships in the presence of 0.1 percent agar at pH 7.00. The results obtained here are quite different from those obtained where the chlorine and organic matter had first been allowed to come to equilibrium. Here there was no rise and then fall in the titratable chlorine, as in the previous experiments, with the addition of increasing amounts of chlorine but a constant and steady

Table XV

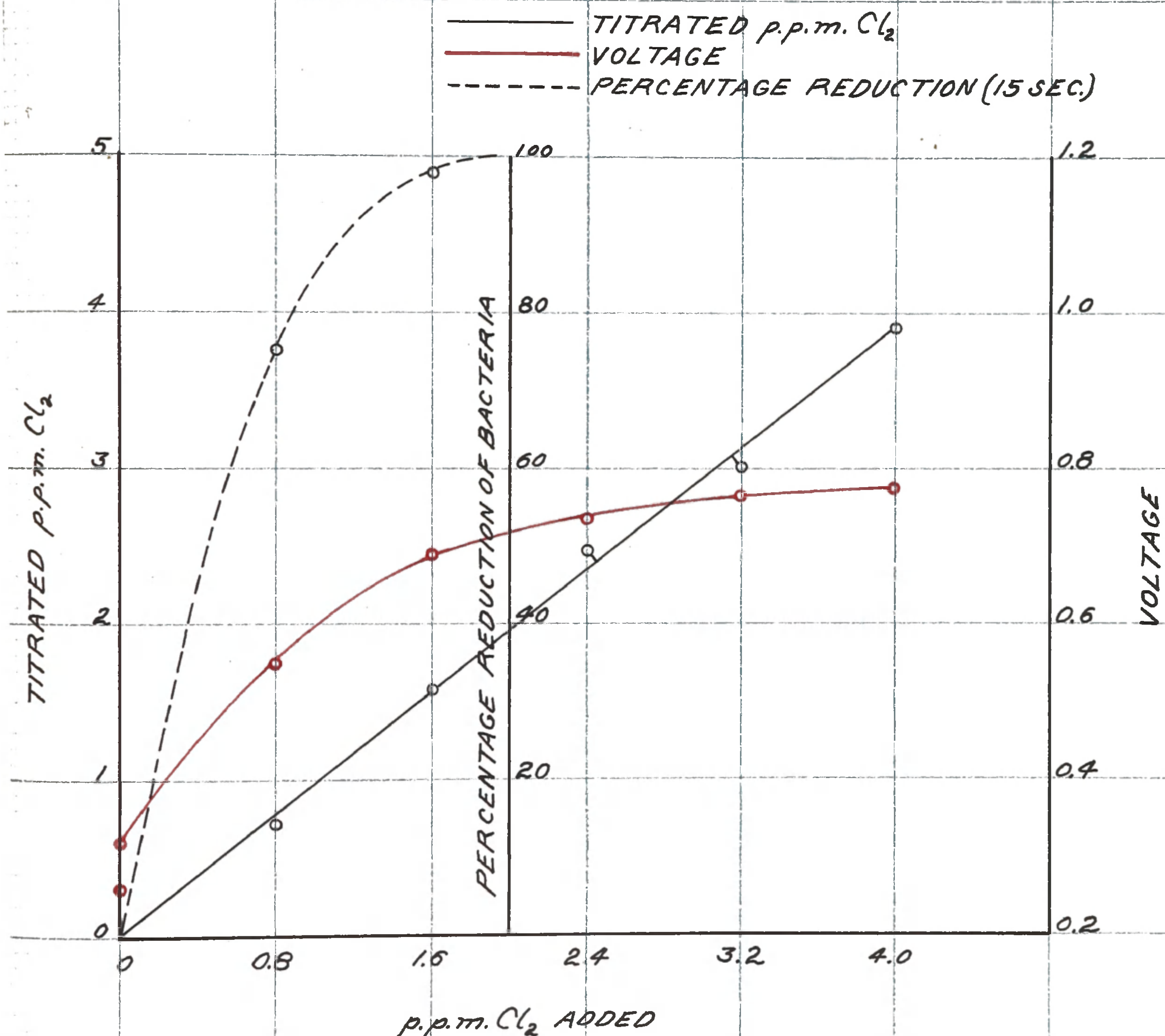
The Relationship of the Oxidation-Reduction Potential to the Germicidal Activity of Chlorine at pH 7.00 in the Presence of 0.1 Percent Agar*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2		Time of Exposure in Seconds						
		Starch-iodide	Ortho-tolidine	15	30	45	60	90	120	180
				Percentage Reduction of Bacteria						
0.260	0.	0.	0.	0.						
0.550	0.80	0.71	0.70	75	90	99	100	100	100	100
0.690	1.60	1.59	1.50	98	100	100	100	100	100	100
0.735	2.40	2.48	2.50	100	100	100	100	100	100	100
0.765	3.20	3.01	3.00	100	100	100	100	100	100	100
0.775	4.00	3.90	4.00	100	100	100	100	100	100	100

*3 min. contact between chlorine and agar
25,000 organisms per ml.

FIGURE XIV

THE RELATIONSHIP OF THE OXIDATION-REDUCTION POTENTIAL TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.00 IN THE PRESENCE OF 0.1 PER CENT AGAR



rise in the titratable chlorine. The starch-iodide and ortho-tolidine titrations agree very closely in all cases instead of diverging in the higher concentrations. Likewise with the voltage curve there is no rise and fall as was the case when the chlorine and agar were first allowed to come to equilibrium. Instead the voltage rose immediately into the germicidal range and then tended to level off, producing a curve much like the one formed in the complete absence of organic matter except that the voltage readings were higher due to the larger amounts of chlorine added. Here again the curve derived from the percentage reduction of bacteria tended to follow in a general way the oxidation-reduction curve and not the titration curve, in that it rose steeply at first and then leveled off whereas an inspection of the titration curve would lead one to suspect that there would be twice as much germicidal action on the addition of 1.60 p.p.m. of chlorine as was shown with 0.80 p.p.m. of chlorine.

Table XVI and Fig. XV show these same relationships using 0.01 percent peptone in the place of agar. Here again the picture was quite different from that where the chlorine and peptone were first allowed to come to equilibrium. Although the starch-iodide and ortho-tolidine titration curves present much the same relationship as they did after the chlorine and peptone had been allowed to come to equilibrium, the voltage curve and the curve derived from the

Table XVI

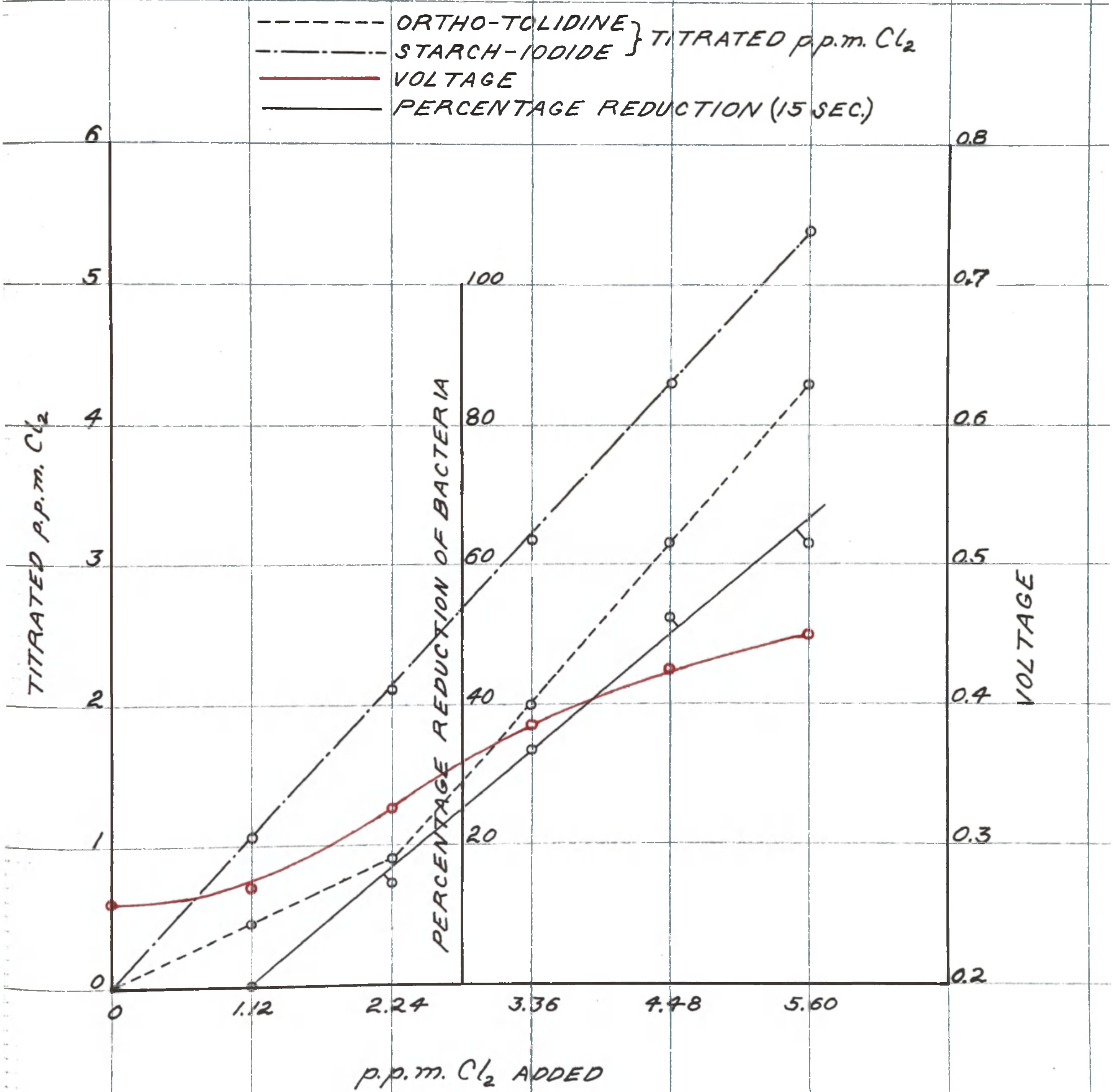
The Relationship of the Oxidation-Reduction Potential to the Germicidal Activity of Chlorine at pH 7.00 in the Presence of 0.01 Percent Peptone*

Voltage	P.P.M. Cl_2 added	Titrated p.p.m. Cl_2		Time of Exposure in Seconds						
				15	30	45	60	90	120	180
		Starch-iodide	Ortho-tolidine	Percentage Reduction of Bacteria						
0.260	0.	0.	0.	0.						
0.270	1.12	1.06	0.45	0.	0.	0.	0.	0.	0.	0.
0.325	2.24	2.12	0.90	14.3	20.0	25.8	33.4	27.2	43.9	62.4
0.385	3.36	3.17	2.00	33.4	42.9	49.6	57.2	69.6	75.3	88.6
0.425	4.48	4.30	3.15	52.4	62.9	71.5	77.2	89.6	97.2	100
0.450	5.60	5.38	4.30	62.9	78.1	90.5	96.2	100	100	100

*3 min. contact between chlorine and peptone
10,500 organisms per ml.

FIGURE XV

THE RELATIONSHIP OF THE OXIDATION-REDUCTION POTENTIAL TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.00 IN THE PRESENCE OF 0.01 PER CENT PEPTONE



percentage reduction of bacteria present an entirely different picture. After the first two additions of chlorine up to 2.24 p.p.m. the starch-iodide and ortho-tolidine curves ran parallel to each other, rising steadily with each addition of chlorine. The ortho-tolidine titration, however, as before gave a much lower value than that obtained with the starch-iodide titration. Here as in the previous cases cited the oxidation-reduction potential seemed to give a more accurate estimation of the relative amounts of germicidal activity which may be expected than did either the starch-iodide or ortho-tolidine titrations. The voltage reading remained almost constant up through the addition of 1.12 p.p.m. of chlorine, rising only from 0.260 to 0.270. Upon successive additions of chlorine the voltage rose rather sharply and steadily up to 0.450 on the addition of 5.60 p.p.m. of chlorine. The curve derived from the percentage reduction of bacteria at 15 seconds followed this voltage curve very closely. Up through the addition of 1.12 p.p.m. of chlorine there was no reduction in the number of bacteria. From this point on the curve rose steadily to 62.90 percent reduction on the addition of 5.60 p.p.m. of chlorine. Judging from the starch-iodide and ortho-tolidine titrations which gave values of 5.38 and 4.30 p.p.m. of chlorine respectively on the addition of 5.60 p.p.m. of chlorine, it would be expected that 100 percent reduction would be obtained in 15 seconds.

Tests were next made to determine the relative value of the starch-iodide titration, ortho-tolidine titration, and oxidation-reduction potential as means of determining the germicidal activity of chlorine (NaOCl) in the presence of sewage. The sewage was well mixed by shaking to insure even distribution of organisms and organic matter and 500 mls. measured out into flasks. Sufficient chlorine was then added to each of the flasks to give final concentrations of 0.84, 1.68, 2.52, 3.36 and 4.20 p.p.m. of chlorine. At the end of 3, 5, 10, 15 and 20 minutes one ml. samples were withdrawn into 2 percent peptone solution and plated out as before. Oxidation-reduction potentials, starch-iodide and ortho-tolidine determinations were made at the three and 20 minute periods. Table XVII and Fig. XVI are representative of the results obtained in this work.

The data show that here again there was a very definite correlation between the oxidation reduction potential and the germicidal activity of the chlorine added. Rudolfs and Ziemba (16) have shown that high reductions in the number of bacteria in sewage may be obtained by chlorinating to different percentages of the ten-minute chlorine demand. The data presented here give this same picture and also show the probable explanation for it. On the first two additions of chlorine up to 1.68 p.p.m. the voltage curve rose sharply whereas on the next three additions of chlorine up to 4.20 p.p.m. it rose less rapidly but nevertheless tended to rise steadily. Fig. XVI shows that the curves derived from the percentage

Table XVII

The Relationship of the Oxidation-Reduction Potential

to the Germicidal Activity of Chlorine at pH 7.70

in the Presence of Sewage

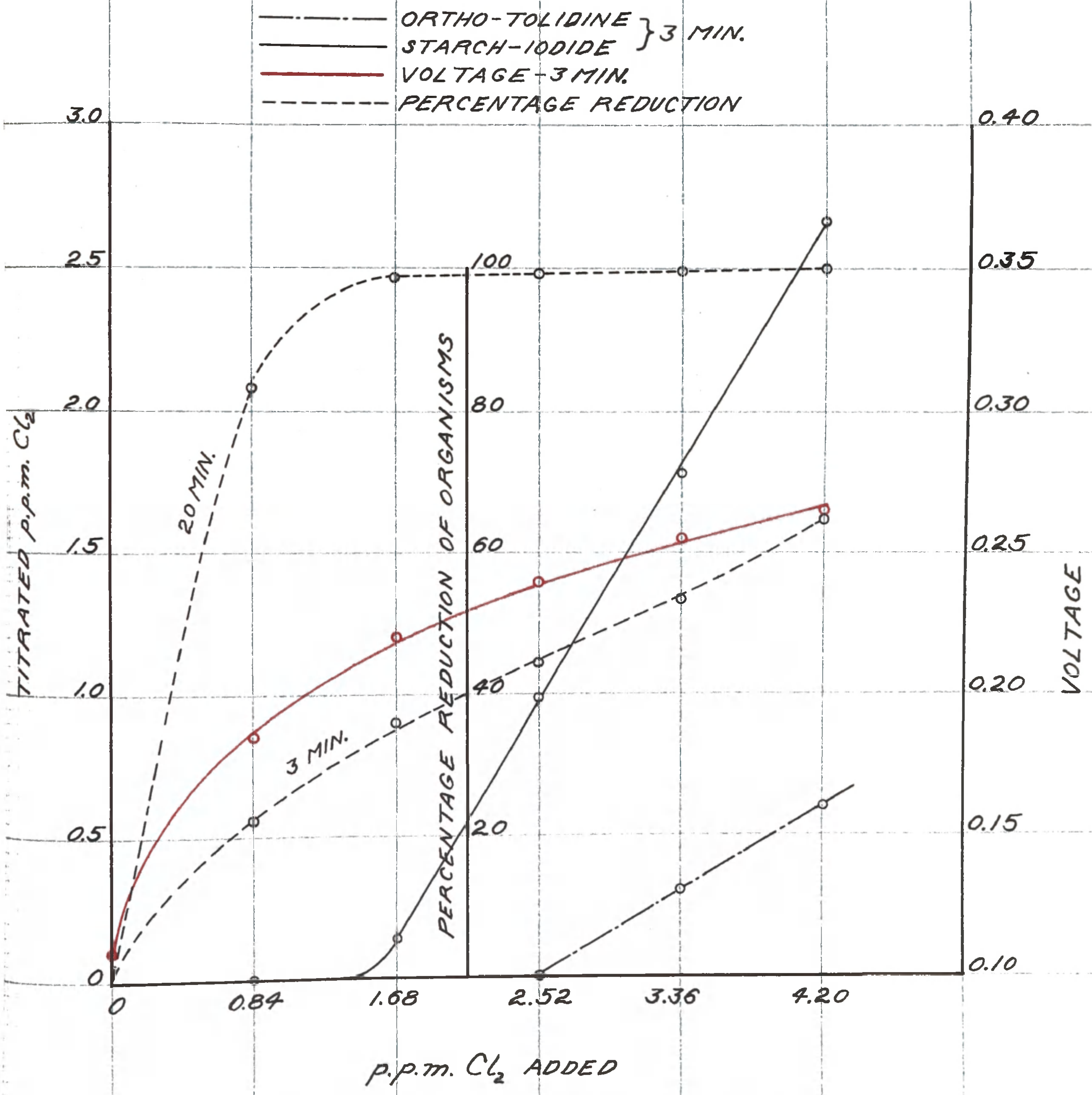
10 min. chlorine demand {Starch-iodide 1.86 p.p.m.; Ortho-tolidine 3.15 p.p.m.}

P.P.M. Cl_2 added	3 minutes			20 minutes			Time in minutes				
	Voltage	Titrated p.p.m. Cl_2		Voltage	Titrated p.p.m. Cl_2		3	5	10	15	20
		Starch-iodide	Ortho-tolidine		Starch-iodide	Ortho-tolidine	Percentage Reduction of Organisms				
0.	0.110	0.	0.	0.110	0.	0.	0.				
0.84	0.185	0.	0.	0.180	0.	0.	22.23	40.75	51.86	68.52	83.34
1.68	0.220	0.14	0.	0.215	0.	0.	36.12	48.15	92.60	98.41	98.60
2.52	0.240	0.99	0.	0.235	0.35	0.	44.45	77.78	96.30	98.86	99.12
3.36	0.255	1.77	0.30	0.250	1.24	0.12	53.71	83.34	97.41	99.50	99.85
4.20	0.265	2.66	0.60	0.260	2.13	0.40	64.81	88.89	98.97	99.70	99.93

540,000 organisms per ml.

FIGURE XVI

THE RELATIONSHIP OF THE OXIDATION-REDUCTION POTENTIAL TO THE GERMICIDAL ACTIVITY OF CHLORINE AT pH 7.70 IN THE PRESENCE OF SEWAGE



reduction of bacteria tended to follow this voltage curve very closely, as in every case the greatest relative amount of reduction took place on the first two additions of chlorine up to 1.68 p.p.m. and then increased gradually up through the addition of 4.20 p.p.m. of chlorine. As before, there appears to be no correlation between the starch-iodide titration and the ortho-tolidine titration nor between these two tests and the amount of germicidal activity produced. The starch-iodide titration gave a three-minute chlorine demand of 1.47 p.p.m. of chlorine and the ortho-tolidine titration a demand of 2.52 p.p.m. From this it would be inferred that little or no germicidal activity would take place till after the addition of 1.47 or 2.52 p.p.m. of chlorine whereas it is seen that the greatest percent reduction took place before the addition of 1.68 p.p.m. of chlorine. This is probably due, as pointed out by Rudolfs and Ziemba (16) to the formation of certain "chloro-products" which are very germicidal and yet are not demonstrable by either the starch-iodide titration or the ortho-tolidine titration.

Summary

With increasing concentrations of chlorine below 1.50 p.p.m. in the absence of organic matter the oxidation-reduction potential indicates the relative amount of germicidal activity which may be expected for each concentration. With increasing concentrations both the oxidation-reduction potential and germicidal activity tended toward a step formation, rising sharply at first, leveling off and then rising sharply again. Neither the starch-iodide nor the ortho-tolidine titrations demonstrated this relationship between different concentrations but gave steadily increasing values with each successive amount of chlorine added. The same type of relationships existed at pH values of 5.00, 7.00 and 9.00.

When increasing amounts of chlorine were added to solutions containing 0.1 percent agar and allowed to stand for 24 hours to come to equilibrium the titratable chlorine first increased, then decreased sometimes to 0. p.p.m., and then increased again. The oxidation-reduction potential and the percentage reduction of bacteria tended to follow this same type of curve. However, the starch-iodide and ortho-tolidine titrations gave too high values before the disappearance of chlorine and too low values afterwards. The oxidation-reduction potential in each case gave a true evaluation of the amount of germicidal activity which may be expected. The starch-iodide and ortho-tolidine titrations agreed with each other up to the point where the chlorine disappeared but from

this point on the ortho-tolidine titration gave a lower value than did the starch-iodide titration.

Solutions containing 0.1 percent agar and chlorinated beyond the point where the chlorine disappeared were found to be much more germicidal than solutions containing no agar; whereas solutions which had just been chlorinated up to this point were much less germicidal than solutions containing no agar.

When increasing amounts of chlorine were allowed to come to equilibrium for 24 hours in the presence of 0.01 percent peptone there was found to be a poising effect on the oxidation-reduction potential, tending to hold it down in a non-germicidal range. When sufficient chlorine had been added to overcome this effect, the voltage rose sharply and was accompanied by a sharp increase in the percentage reduction of organisms. There was found to be no correlation between the starch-iodide and ortho-tolidine titrations and the amount of germicidal activity. The ortho-tolidine titration gave a much lower value than the starch-iodide titration but was still much too high for the corresponding germicidal activity. Apparently some inert "chloro-products", without germicidal activity but still able to show up on titration, were formed, since no reduction in bacteria was observed even with time periods up to five hours as long as the voltage remained constant.

On adding increasing amounts of chlorine to solutions of agar and peptone containing organisms without allowing the chlorine and organic matter to first come to equilibrium the oxidation-reduction potential gave a better relative idea of the amount of germicidal activity than did either the starch-iodide or ortho-tolidine titrations. In the case of agar there was not a rise and fall in titratable chlorine as was the case where chlorine and agar were first allowed to come to equilibrium. In the presence of agar the starch-iodide and ortho-tolidine titrations were the same but in the presence of peptone the ortho-tolidine titration again gave a lower value than did the starch-iodide titration.

In the presence of sewage the oxidation-reduction potential again tended to measure the relative germicidal activity of different chlorine concentrations whereas there was no correlation between the starch-iodide and ortho-tolidine titrations and the amount of germicidal activity. Likewise there was no correlation between the starch-iodide titration and ortho-tolidine titration in the measurement of the chlorine demand, the starch-iodide titration in every case giving a much lower demand than the ortho-tolidine titration.

From these results it is obvious that the present methods of measuring available or germicidal chlorine are of little value, especially in the presence of organic matter. The oxidation-reduction potential gives a true indication of the relative germicidal activity of different concentrations of chlorine whether in the presence or absence of organic matter.

Conclusions

1. The oxidation-reduction potential gives an accurate indication of the relative amounts of germicidal action which may be expected between different concentrations of chlorine (NaOCl) both in the presence and absence of organic matter.
2. Neither the starch-iodide nor the ortho-tolidine titrations give an accurate indication of the relative amounts of germicidal action between different concentrations of chlorine (NaOCl) either in the presence or absence of organic matter.
3. There is little or no correlation between the values obtained by the starch-iodide and ortho-tolidine titrations in the presence of organic matter.
4. The chlorine demand of solutions containing organic matter is much higher as measured by the ortho-tolidine than by the starch-iodide titration.
5. With solutions containing identical amounts of chlorine (NaOCl) at different pH concentrations, the oxidation-reduction potentials indicate the increased germicidal activity with increasing acidity whereas the starch-iodide and ortho-tolidine titrations give the same values at different pH concentrations.
6. With the addition of increasing amounts of chlorine (NaOCl) to solutions containing agar, a point is reached where the agar exerts an increased germicidal effect over that of

solutions containing the same amount of titratable chlorine but in the absence of agar.

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