

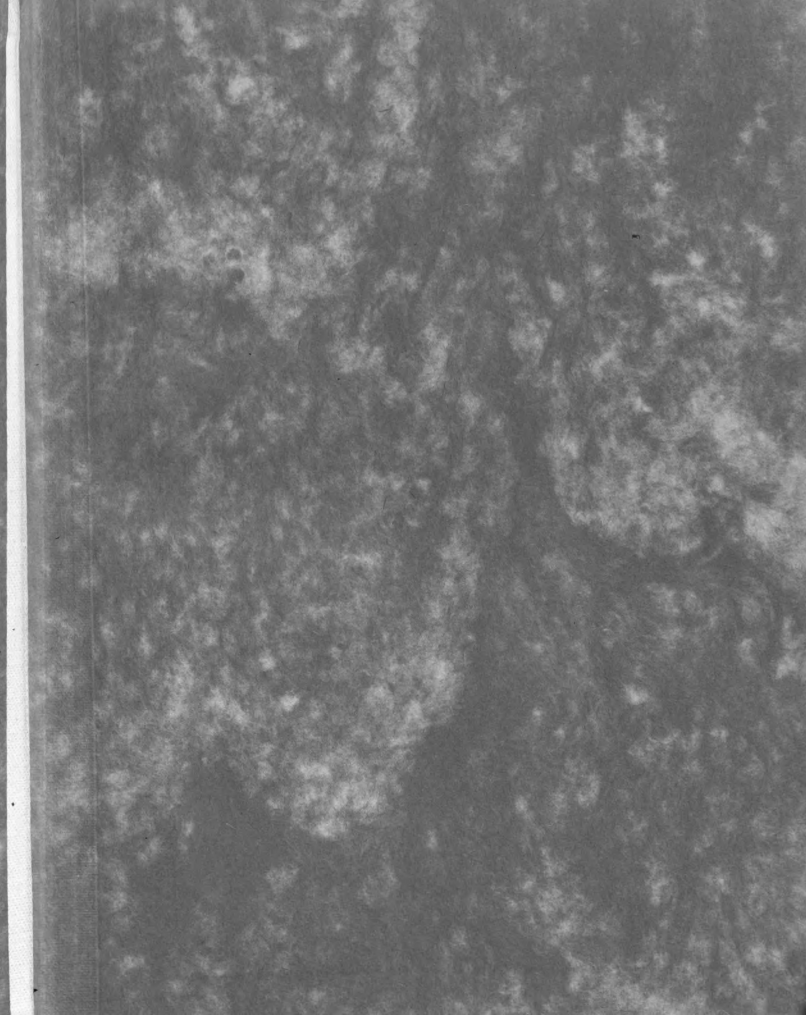


104
845
THS

RESISTANCE OF STRAINS OF
Eberthella typhi AND *Escherichia coli*
TO CHLORINE DISINFECTION

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Constance E. Clack
1938

THESIS



RESISTANCE OF STRAINS OF Eberthella typhi AND Escherichia coli
TO CHLORINE DISINFECTION

Thesis for degree of M. S.
Michigan State College

Constance Elisabeth Clack

1938

THESIS

(Clack)

RESISTANCE OF STRAINS OF Eberthella typhi AND Escherichia coli
TO CHLORINE DISINFECTION.

INTRODUCTION

A bacteriological test that is to demonstrate the safety of a drinking-water supply should prove the freedom of the water from all disease-producing microorganisms. Unfortunately determination of the presence or absence of pathogens in water is not possible for routine work, and since most water-borne diseases are of intestinal origin we are forced to find a more easily isolated test organism of fecal origin. Then if intestinal pollution is shown to exist we may logically reason that dangerous disease germs may also be present in the water supply.

Levine (18.) suggests the following requirements for an organism which is to serve as an index of fecal contamination of a water:

"1. It should be distinctively and characteristically of human or animal intestinal origin.

"2. It should be absent or very rare in nature outside of the intestinal tract.

"3. It must be capable of easy and rapid detection.

"4. Its incidence in water should bear some constant relation to the sanitary survey of our knowledge as to the probability of pollution, particularly with sewage.

"5. It should be distinctly more viable and more resistant in water and to treatment than are the intestinal pathogens, but not excessively so."

The use of Escherichia coli as an index of the bacteriological condition of a water supply has been the accepted practice since 1905 when the first "Standard Methods of Water Analysis" was issued by the American Public Health Association (31.) As early as 1892, Smith (30.) suggested a plan to the New York State Board of Health for the estimation of Esch. coli in water. By 1904 the significance of Esch. coli in drinking water was well established. Prescott and Winslow, in 1904 (27.) seem to voice the general opinion of the day: "Altogether the evidence is quite conclusive that the absence of E. coli demonstrates the harmlessness of water as far as bacteriology can prove it. That when present, its numbers form a reasonably close index of the amount of pollution." In 1914, the United States Treasury Department (35.) in first establishing standards for drinking and culinary water supplied by common carriers in interstate commerce, included a section relating to the bacteriological character which establishes the allowable limits of impurity as measured by the organisms of the coliform group.

The difficulty of isolating the true offender, Eberthella typhi, from water was early realized. Laws and Andrews, 1894 (17.) and Neufeld, 1899 (16.), and Fischer and Flateau, 1901 (9.), met with difficulty in isolating the organism from polluted wells. In the 1912 edition of "Standard Methods" (32.) procedures for isolating E. typhi from water are given, but these were removed in the next edition.

Although Esch. coli is not an entirely faultless index, it has been shown, during the years of its use, to have certain distinct advantages. Levine (18.) points out that although bacteria of the

coliform group are not restricted to the intestinal tract of man (being characteristic also of the intestinal tract of the lower animals), it is nevertheless true that there is a quantitative correlation between Esch. coli and known pollution. Houston (14.) observed that in brooks and rivers which were more or less subject to pollution color bacilli were usually present (61.5%) in small portions of water (1 ml. or less) whereas in unpolluted Loch Ericht the colon group was only occasionally encountered even in 100 ml. samples. Cummings (7.) in his report on the sanitary conditions of the Potomac River watershed found a high incidence of the colon group in the vicinity of sewer outlets. The incidences became less as the distance from the source of pollution increased, until coliform organisms were rare at points remote from contamination. Reports by Clark and Gage (5.), 1898, and by Caird, (4.) are cited by Levine (18.) to show the correlation between the per cent of positive colon tests and the typhoid fever rate.

The ideal index organism should be more viable than the intestinal pathogens, as E. typhi, although not excessively so. Jordan, 1895, (15.) showed that E. typhi gradually died out in a potable water, while Esch. coli at first multiplied rapidly and lived as a rule much longer. Jordan found that his typhoid strain, when recently isolated, lived for 93 days in water, although after 13 months on artificial media, the strain lived for only 12 to 13 days in water. Esch. coli lived as long as 262 days in potable water, but there was variation in the different strains, some strains being viable only a little longer than freshly isolated E. typhi.

Levine, 1921 (18.) states that the colon bacillus is more viable than E. typhi but yet dies off relatively quickly; neither of these organisms find conditions favorable for growth in natural waters. He

1
1850-1851

quotes figures from the work of Hinds, 1916, (13.) on the comparative viability of the two organisms in water at 20°C. A death-rate of 0.094 was found for Esch. coli and a death-rate of 0.87 was found for E. typhi.

Cohen, (6.) 1922, found that the viability of Esch. coli at 0°C. in water to be 67 times greater than E. typhi under the same conditions of pH and temperature. As the temperature was increased by ten-degree intervals the relative resistance of Esch. coli decreased so that at 30°C. it was only eight times that of E. typhi. E. typhi was tolerant to hydrogen ions within a narrow pH range between pH5.0 and 6.4, while Esch. coli had a wider zone of toleration centering around neutrality. These studies were made on strains which had been for a long time on artificial media.

Within the past twenty-five years the use of chlorine compounds has grown in the protection of drinking water supplies, and to-day only exceptional city water supplies are used without chlorination. Under these conditions it is necessary that the organism serving as the index of safety be more resistant, or, at least, no less resistant, to chlorine treatment than the intestinal pathogens. The continuance of the use of Esch. coli as the index organism, after the introduction of chlorine disinfection of water, has been based, to a great extent, on theoretical considerations and on the analogy of its greater resistance to heat and longer survival than the common pathogens which may be present in water. Wesbrook, Whittaker, and Mohler, 1910, (37.) studied the resistance of six strains of E. typhi and Esch. coli to calcium hypochlorite. Varying amounts of hypochlorite solution were added to the suspension of bacteria in water kept at room temperature. These

1

investigators found that different amounts of chemicals were required to sterilize different cultures and strains of both colon and typhoid bacilli. In 2 out of 12 experiments more chemical was required to produce sterility in the E. typhi than in the E. coli suspension. The authors suggest that the value of the colon test of water disinfection should be the subject of further investigation, but that the final test would be "the epidemiological data collected on typhoid infected water supplies before and after treatment."

Tonney, Greer, and Danforth, 1928, (34.) determined the minimal "chlorine death points" of 235 strains of vegetative bacteria, and found Esch. coli to be more resistant to chlorine than the other organisms studied. Twenty-one strains of E. typhi were killed at a concentration of 0.10 p.p.m. of chlorine, while nine strains of Esch. coli were killed by 0.15 p.p.m. of chlorine, ten strains required 0.2 p.p.m., and nine required exposure for 15 seconds to 0.25 p.p.m. of chlorine for their complete destruction. They concluded: "The experiments appear to furnish a satisfactory theoretical basis for the current practice of relying on the consistent destruction of E. coli in water as a criterion of effective chlorination."

A recent paper by Heathman, Pierce, and Kabler, 1936, (12.) suggests that the significance of the coliform group as a bacteriological index of the safety of chlorinated water supplies should be reconsidered. Heathman, Pierce, Kabler observed considerable variation of freshly-isolated strains of Eberthella typhi and members of the coliform group to the disinfecting action of chlorine and

chloramine. They found that some of the newly isolated strains of E. typhi exhibited, under some conditions, greater resistance to the killing action of chloramine and chlorine than recently isolated members of the coliform group.

Breed and Norton, 1937, (3.) quote the above mentioned paper by Heathman and co-workers, in addition to reports by Veldee, 1931, (36.) and Ziegler, 1937 (40.) of water-borne outbreaks of gastro-enteritis occurring when Esch. coli was not demonstrated, as evidence that the use of the coliform group as the sole index of dangerous contamination of water supplies be reconsidered.

Further evidence that this paper has attracted the attention of water bacteriologists is shown by the fact that Beard (1.) mentions it in a recent paper (1938). Beard states "The recent work of Heathman and her associates offers new and startlingly different problems in resistance. They report considerable variation in the resistance of freshly isolated strains of E. typhosus to chlorination, with the further indication that certain strains may be more resistant to such treatment than the coliform groups. This is an exceedingly important pronouncement which should be extensively investigated for confirmation.

As far as we have been able to ascertain, Heathman, Pierce, and Kabler (12.) are the first to make comparative studies on the disinfecting action of chlorine compounds on recently isolated strains of E. typhi and Esch. coli. In the other literature reviewed either stock strains have been used or else the source of the strains has not been stated. Under natural conditions in water supplies we

would encounter fresh strains of these organisms, if any were to be encountered at all. If there is considerable variation in the action of chlorine on recent strains of the colon-typhoid group and on strains which have been on artificial media for a long time, then results obtained with the latter should not be used as the sole basis of comparing the viabilities of Esch. coli and E. typhi in water supplies. Therefore the experiments of Heathman and co-workers, using newly isolated strains of these two species, seem to be particularly significant, and the following attempts have been made to see if their results could be verified in this laboratory.

In view of the far-reaching implications of the work of Heathman, Pierce, and Kabler, their paper has been the impetus for the following critical study. Their technique, as described, has been closely examined, their data analyzed, and their experimental procedure has served as the basis of the laboratory studies here recorded.

EXPERIMENTAL

I. THE EFFECT OF ADDED ORGANIC MATTER (BROTH) ON THE KILLING POWER OF CHLORINE DISINFECTANTS.

In performing their experiments to determine the killing power of chlorine and chloramine solutions, Heathman and co-workers added "a portion" (size of portion not stated) of a 24-hr. broth culture of the test organism to the chlorinated solution. It is not the common practice to use broth cultures in experiments of this type, since the efficiency of chlorine and chlorine compounds is markedly

reduced by the presence of organic matter. Therefore it was thought advisable to determine to just what extent results of speed tests with chlorine compounds would be affected by the presence of a small amount of broth. This was done by first using a technique similar to that of Heathman and co-workers, in which a small portion of a broth culture of an organism was introduced into the test solution. The results of this test were then compared to those obtained by running an experiment on the same strain using a technique in which little or no extraneous organic matter was introduced into the test solution.

Procedure.

The experimental procedure used in making all the tests whose results are recorded in this or later sections of this paper was as follows: Sterile tap water was adjusted to the desired chlorine residual by the addition of the necessary amount of a stock hypochlorite solution, and 400 ml. were added to a sterile liter flask. Immediately before the start of the test and at the end of the test the temperature of the water was noted, and the chlorine residual was tested with ortho-tolidine. The temperatures were kept within the limits of 22 to 24°C. A known number of cells of the strain being tested were added to the flask containing the chlorine solution, and at intervals of one, two, five, ten, and 15 minutes, two 1-ml. portions were removed and plated into plain standard agar. The organic matter in the agar destroyed the residual chlorine present and thus prevented any further germicidal action. When an excessively

high count was expected, the 1-ml. portions were first planted into tubes containing standard nutrient 9 ml. of broth, and appropriate dilutions made from the broth into standard nutrient agar. Plate cultures were incubated at 37° for 48 hours and then counted to determine the number of bacteria surviving each interval of exposure. In order to check the number of organisms in the test flask at the start of the experiment, a control flask containing 400 ml. of sterile tap water was inoculated with the same amount of culture as was added to the chlorinated water, and two 1-ml portions were removed from this control flask and plated in nutrient agar.

In order to simulate the technique of Heathman, Pierce, and Kabler, a series of tests was run in which the test organism was added in the form of 0.1 ml. of a 24 hr. broth culture introduced into the 400 ml. of chlorinated water, and platings made at the intervals as described above. Using the same strains, another set of experiments was carried out in which the test organism was added to the chlorinated solution in the form of 0.1 ml. of a saline suspension of the organism taken from a 24 hr. agar slant culture. In this last method the amount of organic matter introduced into the chlorine solution, aside from the bacterial bodies themselves, was negligible.

Discussion.

In Table I it is clearly shown how even as small an amount of organic matter as 0.1 ml. of broth in 400 ml. of a chlorine solution will decrease the efficiency of the germicidal chlorine. A chlorine residual of 0.45 p.p.m. was unable to effect sterilization in 15 minutes of a suspension of bacteria whose initial count was 1,800

(Clack)

10.

bacteria per ml., when the broth was present in a 1:4000 dilution; but in the absence of organic matter a suspension of only slightly fewer organisms (initial count, 1,475 per ml.) was completely sterilized in one minute by a lower chlorine residual, 0.42 p.p.m. In each of the three other chlorine residual ranges tested the death rate is markedly increased in the absence of organic matter.

(Clack)

TABLE I

EFFECT OF BROTH CULTURES ON KILLING POWER
OF HYPOCHLORITES

Date	Organism	Initial Cl. (ppm.)	Initial no. of bacteria (per ml.)	No. of bacteria remaining after				
				1 min:	2 min:	5 min:	10 min:	15 min:
3-15-37	Ty-157 broth culture)	0.45	1,800	170	160	170	190	100
4/27-37	Ty-157 broth culture)	0.40	2,500	1,500	900	850	950	700
2-17-38	Ty-157 saline suspension)	0.42	1,475	0	0	0	0	0
4-29-37	Ty-157 broth culture)	0.40	700	500	-	-	220	200
2-17-38	Ty-157 broth culture)	0.42	130	106	71	38	33	10
9-23-37	Ty-157 saline suspension)	0.40	420	276	235	150	28	1
9-23-37	Ty-157 saline suspension)	0.40	410	176	177	90	10	1
3-4-37	Ty-157 broth culture)	0.33	1,700	1,000	1,000	600	500	460
6- 2-37	Ty-157 saline suspension)	0.30	30,000	900	50	20	5	6
4-29-37	Ty-157 broth culture)	0.22	300	260	240	230	220	230
2-17-37	Ty-157 saline suspension)	0.20	840	356	310	284	278	241

Since the work of Heathman, Pierce, and Kabler on comparative resistance of E. typhi and Esch. coli was done in the presence of organic matter, using both chloramine and chlorine as disinfecting agents it is of interest here to examine the literature on the effect of organic material on the action of these and related chlorine compounds.

Fabian, Beavens, Bryan, and Jensen, 1931, (8.) found that in the presence of organic matter, as 0.5 per cent of ice-cream mix, sodium hypochlorite rapidly loses its available chlorine. The greatest drop occurred in the first minute, 55 p.p.m. of available chlorine dropping to 31 p.p.m. in 1 min., to 20 p.p.m. in 5 min., and to 12 p.p.m. in one hour.

Tilley, (33.) 1920, studied the effect of blood serum on the killing action of sodium hypochlorite (Dakin's solution), hypochlorous acid, and Chloramine-T. The killing action of all these compounds was definitely impaired in the presence of serum, as the following results show: Eberthella typhi, Staphylococcus aureus, and Ps. aeruginosa were destroyed in 10 min. by 1-4000 sodium hypochlorite in the absence of organic matter, but survived 20 min. exposure to this dilution in the presence of 25 per cent of blood serum. Chloramine-T, at a 1-1000 dilution, killed E. typhi, Staph. aureus, and Ps. aeruginosa in one-half hour in absence of serum, but in presence of 25 per cent of blood serum only Staph. aureus was killed by this dilution in half an hour. Hypochlorous acid (1-4000) killed all three of the above mentioned species in 10 min. even in the presence of serum. However 1-780 hypochlorous acid in the absence of serum killed anthrax spores in four

hours, but in the presence of serum it failed to kill in 24 hours.

The results noted by Tilley (33.) upon the addition of a molecular equivalent of ammonia to a chlorine or hypochlorite solution are particularly significant. First he observed that upon adding a molecular equivalent of ammonia to chlorine water there was a decrease rather than an increase in the germicidal value of the chlorine, in the absence of organic matter, but addition of ammonia did tend somewhat to prevent the depreciation of germicidal activity in the presence of blood serum. On the other hand, the addition of a molecular equivalent of ammonia to Dakin's solution (hypochlorite) not only prevented, to a large extent, the depreciation of germicidal value due to the presence of organic matter, but actually increased the germicidal value against "unprotected" bacteria.

By no means can it be said that the addition of ammonia entirely prevents a drop in the germicidal activity of the resulting compound in the presence of organic matter. That such is not the case is shown by the following figures from Tilley's experiments. In absence of serum, Dakin's solution killed E. typhi in 10 min. at a 1-4000 dilution, and after the addition of a molecular equivalent of ammonia E. typhi was killed in the same time period by a 1-8000 dilution of Dakin's solution. Upon addition of blood serum E. typhi was not killed in 20 min. by a 1-500 dilution of Dakin's solution alone, but in the presence of a molecular equivalent of ammonia the organism was killed in 5 min. by a 1-4000 dilution. As a higher percentage of serum was added, the killing power of the ammonia-Dakin's solution combination dropped appreciably. In the presence of 10 per cent serum a 1-2000 dilution

required 10 min. to kill.

Beard and Kendall, (2.) 1935, observed that the velocity and amount of sterilization of water with a high or low organic load were increased by the use of ammonia with chlorine. However the velocity of sterilization of chloramine slowed down considerably with higher organic loads. A chloramine solution containing 0.2 - 0.3 p.p.m. available chlorine, at pH 6.6, destroyed 100 per cent of the Esch. coli present in 45 min. in the absence of organic matter. A solution of the same strength, with 100 p.p.m. of organic material (peptone) added, removed only 97 per cent of the Esch. coli in one hour. With 300 p.p.m. of peptone, only 32 per cent of the bacteria were removed in one hour. The lag in sterilization was even more marked at lower hydrogen-ion concentrations.

The lowered efficiency of chlorine disinfectants in the presence of organic matter may be the result of the operation of several factors or principles. The mechanical protection afforded bacterial cells by organic matter interferes with the action of all germicides, and even with the lethal action of such a purely physical agent as heat. Mallmann, (21) 1934, points out that in a solution containing organic matter, into which chlorine gas or sodium hypochlorite has been introduced, the chlorine may exist in three states, free, as hypochlorite adsorbed by seemingly inert materials, and as reduced chemically combined chlorine. The adsorbed state is not germicidal. The chemically combined chlorine may become a part of a compound which is inert, or there may be formation of a compound which is less germicidal than the free chlorine. In any case, the disinfectant which has entered into combination with the

1

organic matter has been taken from the solution, leaving it weaker and less effective.

As to whether the compounds resulting from chemical combination of chlorine with organic matter will be inert, or have some germicidal effect, we may refer to the studies of Guiteras and Schmelkes, (10.) 1934. They concluded that the loss of efficiency of chlorine in the presence of organic matter is partially due to the reaction of the hypochlorites with the alpha-amino acids to form chloramino acids which break down more or less readily to aldehydes or to ketones, ammonia, carbonic acid and sodium chloride. In the case of aromatic or heterocyclic amino-acids, such as tyrosine and tryptophane, partial chlorination of the ring takes place in addition to oxidation. Chlorine which is reduced in oxidizing or chlorinating a ring is of no value as a germicide. On the other hand, any chlorine which has substituted a hydrogen atom of the amino group and which is present as $-NCl_2$ or $-NCl$ is still an active germicide. Wright (39.), 1926, has shown that hypochlorites may attack some types of organic matter more readily than others, the quantitative and qualitative results of these reactions varying with the different types and ratios of amino-acids present.

From the foregoing we are forced to conclude that Heathman, Pierce, and Kabler, (12.) by using broth cultures of E. typhi and Esch. coli to test the action of chlorine compounds on these organisms, were introducing a highly variable factor into their disinfection studies. Unless the amount and constituents of the broth entering into each test were determined by exact chemical analysis it would be impossible to say how much effect the broth might have on the available chlorine

present in the test solutions, and on the resulting killing action. At the least, we may be sure that the amount and velocity of sterilization will be considerably decreased.

In addition, we may call attention to the fact that broth has long been used as a dechlorinating agent in laboratory procedures. Mallmann, (22.) 1932, in describing an experimental procedure in which the action of chlorine solutions was being tested against Esch. coli, states "--at intervals --- 1cc. portions were removed and planted into test tubes containing 4 cc. of nutrient broth. The organic matter in the broth destroyed the residual chlorine present and thus prevented any further germicidal action."

Heathman, Pierce, and Kabler (12.) have used another step in their experimental method which is subject to criticism. The residual chlorine concentration was determined by the ortho-tolidine method at the beginning of each run, and after the portion of broth culture had been added to the test solution the residual chlorine of the solution was tested at intervals throughout the course of the experiment. In view of the fact that Mallmann, (21.) 1934, has shown that neither the ortho-tolidine method nor the iodometric method of measuring available chlorine is accurate in determining the germicidal chlorine, in the presence of suspended material, it is very likely that the later chlorine determinations made in each run by Heathman, Pierce, and Kabler are of little value. Both the iodometric and ortho-tolidine test measure in addition to the free chlorine at least a part of the inactive adsorbed chlorine. This is demonstrated by such results as these, recorded in the paper of Heathman and co-workers: at the end of 18 hours; 5 bacteria remaining,

and 0.04 p.p.m. of residual chlorine, 64 bacteria remaining and 0.04 p.p.m. of residual chlorine; and at the end of $2\frac{1}{2}$ hours; 104 bacteria remaining and 0.08 p.p.m. of residual chlorine, 98 bacteria remaining and 0.07 p.p.m. of residual chlorine.

Charlton and Levine (4a.) state "Failure to take proper account of the organic matter present has introduced errors in interpreting and comparing the results of disinfection studies with chlorine. The literature reveals a number of conflicting opinions, -----concerning the relative germicidal power of the several types of chlorine compounds. These conflicting opinions came about partly because proper consideration was not taken of the reaction between the chlorine disinfectant and the organic matter present.

"-----The importance of giving careful consideration to the presence of any type of added substance either in disinfection tests or in the practical application of chlorine compounds cannot be overemphasized."

II. THE RESISTANCE OF *Eberthella typhi* AND *Escherichia coli* to CHLORINATION.

That the significance of the coliform group as a bacteriological index of the safety of chlorinated water supplies should be reconsidered is the conclusion of Heathman, Pierce, and Kabler (12). This is based on their finding that some strains of *E. typhi* were more resistant to the action of chlorine compounds than some strains of the coliform group. In one study of the killing power of chloramine, carried out at room temperature, in 29 instances out of 34, the time required to kill recently isolated strains of *E. typhi* was equal to or in excess

of the time required to kill members of the coliform group studied simultaneously. However, at low temperatures (2-8°C.), a longer time was required to kill members of the coliform group than to kill the E. typhi strains in 18 of the 34 experiments. From these observations Heathman, Pierce, and Kabler conclude "that some strains of E. typhosa may, under certain conditions, exhibit as great (or greater) resistance to the killing action of chloramine as do members of the coli-aerogenes group."

Heathman and co-workers made tests on 13 freshly isolated strains and two old laboratory strains of E. typhi, and on 12 freshly isolated strains and one old laboratory strain of Esch. coli or members of the coliform group. As previously stated, Heathman and co-workers are probably the first to use recently isolated strains in testing the disinfecting action of chlorine compounds on E. typhi and members of the coliform group. Recently isolated strains of these organisms should certainly represent the strains which would be found under natural conditions in water supplies more closely than old laboratory strains of the same species. For this reason, the observations of Heathman, Pierce, and Kabler, that some strains of E. typhi may be more resistant to chlorination than some strains of the index-organism Esch. coli, if correct and based on accurate laboratory work, are of the greatest importance. The purpose of the studies described in this paper has been to see if the results of Heathman, Pierce, and Kabler could be confirmed.

In making the tests of the viability of strains of E. typhi and Esch. coli in the presence of chlorine compounds, the technique described earlier in this paper was used. The test organism was added

to the chlorinated solution in the form of 0.1 ml. of a saline suspension of a 24-hr. agar slant culture, in order that the amount of extraneous organic matter introduced into the test solution would be negligible. Both recently isolated and old laboratory strains of E. typhi and Esch. coli were used in these experiments.

Discussion of Results.

In table II we find by taking the average for each species of the initial counts per ml. and the number of bacteria surviving exposure to a hypochlorite solution in the range of 0.30-0.25 p.p.m. available chlorine that the resistances of E. typhi and Esch. coli are approximately equal. However, in considering the results of individual experiments, we note considerable variation in the different strains of each species, and even in the same strain tested on different days.

In table III we see that for the strains tested, the average resistance of the E. typhi strains to an available chlorine concentration of 0.20-0.22 p.p.m. is considerably less than the resistance of the Esch. coli strains. Here, also, we note differences in the viability of various strains of each species, although tested under the same conditions.

In tables IV and V, we do not have data in such a form that we can attempt to draw any conclusions as to the relative viability of E. typhi and Esch. coli. However, the thing that impresses us in these, as well as the two preceding tables, is the variability of a given strain in its resistance to chlorine, when tested under the same conditions, but on different days. This is in spite of the fact that the chlorine

residual, reaction, and temperature were carefully controlled so that any variation in these factors must have been so small as to be insignificant. This suggests that differences in the death rate of the strains as tested from day to day were due not to chemical factors, but probably to biological changes in the culture of each strain as it was carried from day to day. Table VI shows clearly how some of the strains varied in resistance when tested on different days.

We see in Table V that the Esch. coli C-(RCA) strain tested was more resistant the seventh day after isolation than on the second. After that the resistance decreased somewhat, although it was fairly high again three weeks after isolation, dropping however at the next test. The old laboratory strain of Esch. coli, C-160, was slightly more resistant than the recently isolated strain was on the day that they were tested together. The recently isolated E. typhi strain, Ty-1153, was less resistant when tested on the second day after isolation than the recently isolated Esch. coli strain was at any time.

From Table VI we see that the typhoid strain Ty-7780 was less resistant when first isolated, than it was when tested a month and a half later. With another strain, Ty-8090, the test made within the first week after isolation showed the strain more resistant than it was after being on artificial media for two weeks. Still another typhoid strain, Ty-7525, was less resistant to chlorination when first isolated, than it was five weeks later. The freshly isolated Esch. coli strain, C-Ev.#2, was not so viable in the presence of chlorine as it was 26 days later. About a month later it was a little less

(Clack)

TABLE VI

COMPARATIVE KILLING RATES OF

Eherthella typhi AND Escherichia coli WITH CONSTANT CHLORINE RESIDUALS

Organism	Date	Initial Cl. (ppm.)	Initial no. of bacteria : per ml.	No. of bacteria per ml. remaining after				
				1 min:	2 min:	5 min:	10 min:	15 min:
Ty-7780	4-11-37	0.30	14,000	2	0	0	0	0
Ty-7780	6 -3-37	0.30	8,300	7,700	7,800	-	90	2
Ty-8090	5-28-37	0.30	8,000	6,000	3,800	3,000	4,000	3,600
Ty-8090	6- 3-37	0.30	12,000	7,800	7,000	3,700	2,300	1,250
Ty-7525	5-17-37	0.20	12,600	80	25	0	1	0
Ty-7525	6-25-37	0.20	1,300	48	45	40	26	28
C-Ev.#2	5- 2-37	0.30	60,000	23,000	23,000	18,000	460	700
C-Ev.#2	5-28-37	0.30	12,500	9,000	7,700	7,000	7,000	6,800
C-Ev.#2	6-26-37	0.22	20,000	-	10,000	8,000	8,000	7,500
C-Ev.#2	7- 3-37	0.21	19,000	9,000	6,200	2,000	1,500	870

(Each set of figures represents the average of two runs.)

(Clack)

TABLE V

COMPARATIVE KILLING RATES OF

Eherthella typhi AND Escherichia coli WITH CONSTANT CHLORINE RESIDUALS

Organism	Date	Initial Cl. (ppm.)	Initial no. of bacteria : per ml.	No. of bacteria per ml. remaining after					
				1 min:	2 min:	5 min:	10 min:	15 min:	
C-(RCA)	1-27-38	0.38	1,800	1,500	6	0	1	0	
C-(RCA)	2- 1-38	0.38	2,500	1,940	1,725	1,830	1,735	1,630	
C-(RCA)	2- 1-38	0.36	2,500	1,765	1,625	1,510	1,480	1,450	
C-(RCA)	2- 5-38	0.38	718	68	2	0	0	0	
C-(RCA)	2- 9-38	0.38	2,500	1	0	0	0	0	
C-(RCA)	2-10-38	0.38	4,000	240	11	0	1	0	
C-(RCA)	2-15-38	0.38	3,940	3,700	2,840	390	13	4	
C-(RCA)	3-21-38	0.38	4,150	625	2	4	4	5	
Ty-157 (old lab. strain.)	2-15-38	0.38	860	30	4	0	0	0	
C-160 (old lab. strain.)	3-21-38	0.38	4,100	1,225	50	13	1	1	
Ty-1153	3-22-38	0.38	4,080	0	0	0	0	0	

(Each set of figures represents the average of two runs.)

[illegible]

(Clack)

TABLE IV

COMPARATIVE KILLING RATES OF

Eherthella typhi AND Escherichia coli WITH CONSTANT CHLORINE RESIDUALS

Organism	Date	Initial Cl. (ppm.)	Initial no. of bacteria : per ml.	No. of bacteria per ml. remaining after				
				1 min:	2 min:	5 min:	10 min:	15 min:
Ty-7525	5-17-37	0.42	12,600	14	1	0	0	0
Ty-7634	5-17-37	0.42	37,000	34	6	0	0	0
Ty-7780	6-26-37	0.41	25,000	870	6	0	0	0
Ty-157 (old lab. strain)	9-23-37	0.41	420	276	235	150	28	1
Ty-157	2-17-38	0.42	1,475	0	0	0	0	0
C-Ev.#1	6-25-37	0.42	2,500	0	0	0	0	0
C-Ev.#2	6-25-37	0.42	1,900	0	0	0	0	0

(Each set of figures represents the average of two runs.)

(Clack)

TABLE III

COMPARATIVE KILLING RATES OF

Eherthella typhi AND Escherichia coli WITH CONSTANT CHLORINE RESIDUALS

Organism	Date	Initial Cl. (ppm.)	Initial no. of bacteria : per ml.	No. of bacteria per ml. remaining after				
				1 min:	2 min:	5 min:	10 min:	15 min:
Ty-7758	5-12-37	0.22	166,000	8	1	3	0	0
Ty-7780	5-12-37	0.22	123,000	680	60	40	40	0
Ty-7525	5-17-37	0.20	12,600	80	25	0	1	0
Ty-7525	6-25-37	0.20	1,300	35	43	26	29	22
Ty-7525	6-25-37	0.20	1,300	48	45	40	26	28
Ty-7634	5-17-37	0.20	3,700	1,000	-	7	5	0
C-Ev.#1	6-26-37	0.21	21,000	15,000	10,000	9,000	8,500	8,000
C-Ev.#1	7- 3-37	0.21	17,800	17,200	11,500	8,000	7,500	6,000
C-Ev.#2	6-26-37	0.22	20,000	-	10,000	8,000	8,000	7,500
C-Ev.#2	7- 3-37	0.21	19,000	9,000	6,200	2,000	1,500	870
Average of <u>E. typhi</u> strains			51,316	308	35	13	17	8
Average of <u>Esch. coli</u> strains			19,450	13,733	9,425	6,725	6,375	5,342
Ty-157 (old lab. strain)	2-17-38	0.22	840	356	310	284	278	256

(Each set of figures represents the average of two runs.)

(Clack)

TABLE II

COMPARATIVE KILLING RATES OFEtherthella typhi AND Escherichia coli WITH CONSTANT CHLORINE RESIDUALS

Organism	Date	Initial Cl. (ppm.)	Initial no. of bacteria : per ml.	No. of bacteria per ml. remaining after					
				1 min:	2 min:	5 min:	10 min:	15 min:	
Ty-7758	4-11-37	0.30	17,000	7,000	2	16	10	8	
Ty-7780	4-11-37	0.30	14,000	2	0	0	0	0	
Ty-7780	6- 3-37	0.30	8,300	7,700	7,800	-	90	2	
Ty-8090	5-28-37	0.30	8,000	6,000	3,800	3,000	4,000	3,600	
Ty-8090	6- 3-37	0.30	12,000	7,800	7,000	3,700	2,300	1,250	
C-Ev.#1	5-23-37	0.30	27,000	100	0	0	0	0	
C-Ev.#1	5-25-37	0.25	2,500	0	0	0	0	0	
C-Ev.#2	5-22-37	0.30	60,000	23,000	23,000	18,000	460	700	
C-Ev.#2	5-25-37	0.25	1,900	50	2	0	0	0	
C-Ev.#2	5-28-37	0.30	12,500	9,000	7,700	7,000	7,000	6,800	
C-(RCA)	2- 5-38	0.30	920	730	736	278	153	45	
Average of <u>E. typhi</u> strains			11,830	5,700	3,720	1,679	1,280	972	
Average of <u>Esch. coli</u> strains			17,470	5,480	5,340	4,213	1,402	1,258	
Ty-157 (old lab. strain)	5- 2-37	0.30	30,000	900	50	20	5	6	
Aer-130	8-13-37	0.30	5,000	4,700	4,000	3,600	3,100	2,000	
Aer-130	8-13-37	0.30	5,000	3,400	3,400	3,200	2,700	2,400	

(Each set of figures represents the average of two runs.)

viable than in the preceding test, and still a month later its viability had dropped, but not so low as at the first test.

It is interesting to note that in their studies, Heathman, Pierce, and Kabler found that a longer time was required to kill recently isolated strains of E. typhi than to kill the old laboratory Rawlings strain and another old laboratory strain of E. typhi with which they worked. They state "This appears to indicate that prolonged growth on artificial media materially reduces the resistance of E. typhosa to the disinfecting action of chloramine." This is a different view than that presented by Cohen, (6.) 1922, who worked on the effect of temperature and reaction on the viability of Esch. coli and E. typhi in water. Cohen used cultures of these organism which had been grown for a long time on artificial media, and says "--this does not constitute a defect in the present experimental plan. The undoubted acquisition of higher resistance to external influences altered somewhat the degree of the mortality rate by accentuating and magnifying the retardation during the early phases of the process, precisely the condition desired."

The earlier work of Melick, (25.) 1917 is also of interest in this connection. In studying the survival of E. typhi in soils infected with excreta, he found that young strains were less resistant than old.

In glancing over the data presented in tables II-V it will be seen that the old laboratory strain of E. typhi, Ty-157, was in most cases more resistant than the recently isolated strains tested under the same conditions. However even this strain showed some variation in its viability.

The results presented here suggest that the phenomenon of dissociation, that is, the change of "Smooth" bacterial forms to "Rough" forms, is the most outstanding means of accounting for the variations in resistance here observed. In the Esch. coli strain designated as C-(RCA), some evidence of cultural dissociation was seen. After about three weeks on artificial media, an occasional rough colony was observed on the plates poured with this strain. The colony was only slightly larger than the smooth type, and had pie-shaped wedges which were more opaque than the rest of the colony. The margin was slightly undulate. This colony was observed only six times altogether, but was always the same. On being transferred to sugar media the fermentation reactions were those usually characteristic of Esch. coli and the indol test was positive. Re-plating from this colony always gave rise to "smooth" colonies. No other strains of this species with which we worked showed rough growth on plates or slants. The old laboratory strain of E. typhi, Ty-157, regularly gave rise to about 80 per cent of colonies with slightly undulate margins, although the recently-isolated typhoid strains, in spite of showing considerable variation in resistance, showed only "smooth" cultural characteristics.

In 1927 Hadley (11.) presented a complete and impartial resumé of the existing literature on dissociation. The evidence presented points to the fact that dissociation or variability occurs in all species of bacteria and that under suitable environmental conditions these changes can be induced in a more or less reversible manner. Hadley observed that parallel trends of dissociation are frequent, although exceptions to these character correlations are often found, and gives a list of

twenty-four characteristics in which bacteria may vary from the S type to the R type. The mutational trends that seem most to be the common property of nearly all species are listed as follows: "assumption of a sedimentary or spontaneously agglutinative form of growth; change in size, form, color and consistency of colony; loss or modification in antigenic power and agglutinability; loss or diminution in virulence; and increased resistance to unfavorable conditions of environment, including the action of starvation, antiseptics, heat, desiccation, and the lytic principle."

It has not been within the scope of these studies to observe the other mutational trends in the strains used, but we have met with definite evidence that variation in resistance, one of the characteristic named by Hadley, is a function of the dissociation phenomenon. Whether increased resistance should be associated with the S type or the R type, we have no basis for saying; that variation of this characteristic does occur we are sure.

Mallmann, (20.) in dissociation studies on the Salmonella group, found three types of organisms to exist, as concerns colony stability. They are (1.) the pure smooth type, (2.) the pure rough type, and (3.) the variable rough-smooth type. The pure smooth and pure rough strains did not undergo dissociation changes. Under the influence of dissociation incitants, there might be some slight change, but these changes were not permanent, as evidenced by the fact that the cultures reverted to the original types soon after the incitants were removed. When dissociation incitants were applied to the intermediate types, which indiscriminately produced both smooth and rough colonies on agar plates,

these intermediate cultures could be converted to either rough or smooth colonies at will. They did not retain this induced property long after the specific incitants were removed before reverting to mixed colonies again. From these data, Mallmann concluded that the type of colony formation depended on hereditary factors, and that the pure R and S type organisms are mutants in the strict sense, of the normal RS type organism.

If the analogy is permitted, we may attempt to explain the variation in resistance observed in our studies in the light of Mallmann's observations on colony stability as an index of dissociation. We may consider that in the normal type organism both tendencies exist, and that some of the organisms will be more resistant and some less resistant. The total resistance of a strain on a given day would depend on the numbers of each type of organism present, and if we are dealing with an intermediate type, we could expect this resistance to vary from day to day. The strains of organisms of the colon-typhoid group that would find their way from the human body to pollute a water supply would probably contain a high proportion of these normal, intermediate type organisms. Thus, in working with recently isolated strains of colon-typhoid organisms, we are most likely to be working with strains which are variable in at least some of their characters.

In only two of the strains of the colon-typhoid organisms studied were parallel dissociation characteristics observed along with variation in resistance to the effect of disinfectants. The characteristic observed was a slight change in colonial form, as previously described. Other less obvious variations may have been present at times, but no

tests were made for their presence. Nungester and Jung, (26.) 1932, Mackenzie, Fitzgerald, and Irons, (19.) 1933, observed independent variation of biological characters of bacteria as a result of dissociation. Mackenzie, et al, found twelve different characters all to vary independently of each other. It was even possible to demonstrate the independent variation of such closely allied characters as the agglutinative, absorptive, and agglutinogenic properties.

It is recognized by those who have worked on the problem of formulating bacteriological tests of the efficiency of disinfectants that the variability from time to time of the reaction of the test organism with the disinfectant is an important factor affecting their results. Rideal and Walker, (28.) 1903, in their method of determining the phenol coefficients of agermicidal compounds, took into consideration the variations in vital resistance of the same species of an organism and provided that the resistance of the strain used be checked by means of a standard phenol control in running each test. Rideal mentions that the seasonal variation in the resistance of the organism (Rawling's or Hopkin's strains of E. typhi) appears to be a difficult factor to eliminate. Ruehle and Brewer, (29.) 1931, in presenting the F.D.A. method of determining the phenol coefficient, gave detailed instructions for the frequency of transferring and the media used for growing the test organism, so that a minimum of variation would result. Wright (38.) 1937, found that even minor variations in the reaction or composition of the culture medium influenced the results obtained in the critical testing of disinfectants. The strain of E. typhi used affected the final result, and variations in the phenol coefficient of over 200 per cent could be obtained by the use of different strains of

E. typhi.

Heathman, Pierce, and Kabler (12.) attribute the variations in the resistance of strains with which they worked to variations in the compositions of the test solutions used. They state "There were variations (in the killing power of chloramine) from day to day, even within the same chlorine residual and temperature ranges. These variations were to be expected, since the water used in these experiments were not a reproducible synthetic water, but rather was taken from the municipal water-supply system and consequently was subject to the variations which occur in treated surface waters. -----These observed differences in action indicate the inconstancy of chlorine waters, and also the difficulties encountered in preparing them."

We are willing to grant that the inconstancies in the composition of the chlorine waters used, would account for a proportion of the inconstancies in the results obtained by Heathman and co-workers. Furthermore we suggest that the biological variation in the resistance of the strains used can be fully as important a factor. This is born out by the variations observed by us in experiments where the reaction, temperature, composition, and chlorine residuals of the test solutions used have been carefully controlled. Our contention is that the conclusions drawn by Heathman, Pierce, and Kabler are based on an attempt to compare altogether too many variables, and that these conclusions are carrying more weight with other water-bacteriologists than is warranted.

The method which Heathman, Pierce, and Kabler (12.) have used in obtaining the data on which their conclusions are based is of interest.

Each run consists of five determinations of the number of organisms surviving per m v. after given periods of time, together with the initial number of organisms per ml. of solution at the start of the experiment. These figures were determined by plate counts taken in the usual manner. If rapid killing was expected, tests were made at intervals of 5, 10, 20 and 30 minutes, and one hour. Frequently there were still bacteria surviving at the final test. If less rapid killing was expected, tests were made at intervals of 30 minutes, 1, 1½, 2, 2½, and 18 hours. Sterility was almost always found at 18 hours, but there were usually organisms surviving at the 2½ hour interval. These tests were usually not run to completion (sterility) for, although the 18-hour tests usually showed no organisms surviving, the interval between this and the preceding (2½-hour) test was so long that no clue to the actual time of killing of the strain was furnished. Nevertheless, Heathman and co-workers wished to know the killing-time for each strain under a given set of conditions. The method by which they arrived at the killing-time is best described by quoting directly from the paper of Heathman, Pierce, and Kabler.

"When the plate counts for the various periods of exposure in an experiment were plotted on semi-logarithmic paper it was found possible to project through the point representing the initial concentration a straight line which would pass through or close to practically all of the plotted points.

"It will be seen that all the plotted points do not lie on the line drawn. However, the points lie within the zone of experimental error. From the line slope as indicated on the resulting curve, the time required to kill 99.9 percent of the bacteria was computed."

CHLORAMINE DEATH CURVE

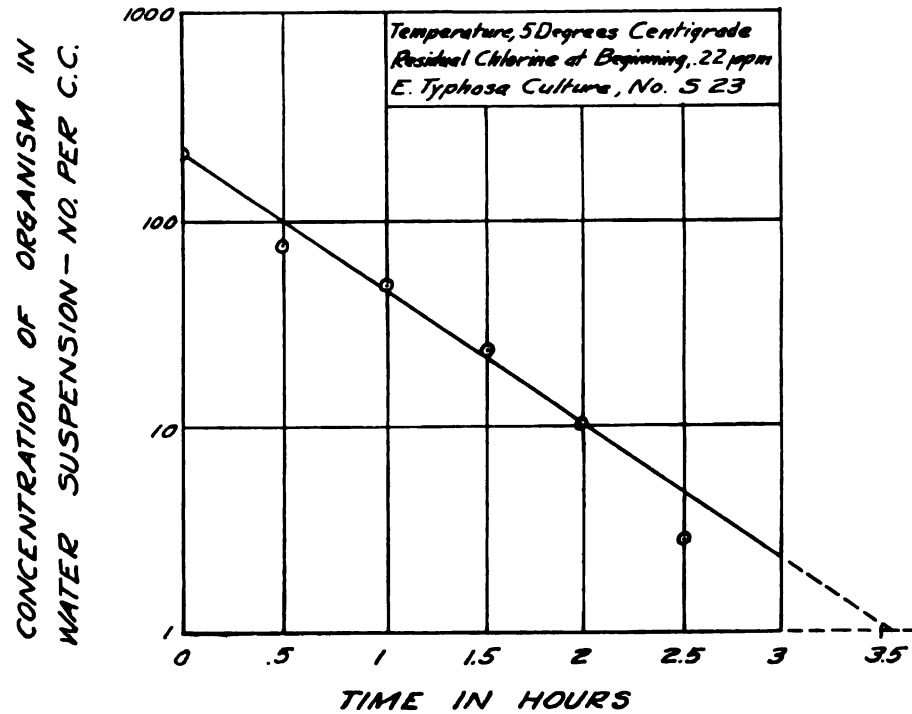


Figure I. Reproduction of graph from paper of Heathman, Pierce and Kabler showing method of plotting killing-time. Killing-time as plotted here 3.6 hrs. Killing-time (for same run) reported in same paper 5.35 hrs.

No theoretical basis was offered by these authors to justify the use of this curve to fit data of this type. Nor did they explain why only five points, all subject to plus or minus 25 per cent error, near the beginning of a death-rate curve should be considered sufficient to determine the course of the entire curve, which in most cases had to be projected for a considerable distance before the base-line was struck.

In order to determine how well Heathman, Pierce, and Kabler's treatment of their data is justified, we have attempted to reconstruct many of the graphs by means of which their figures for killing-times were computed from the results of plate counts. A mass of data (132 runs) was presented by these authors, from which the figures used in these graphs have been chosen at random. Careful inspection of the rest of the data assured us that these figures were entirely representative of all the data presented by Heathman and co-workers.

In Figure II. is presented a case where a strain of E. typhi (Ty S-129) was tested on two different days, at the same chlorine residual. The killing-time as recorded by Heathman et al, and as determined by them from graphs drawn in the manner described above, is 6.38 hours for both tests. It is plainly seen from the graph presented of each run that the curves formed by the six points determined in each case do not coincide or even closely resemble each other. The curves drawn by Heathman et al pass through only the first three points in each case, and disregard the positions of the last three points. Since it is the killing-time that was to be determined, and since the later plate counts are the ones which are nearer sterility, it would seem that these later counts would be at least as important in determining

the course of disinfection as the earlier counts.

Nearly all of the graphs presented indicate that the killing-times recorded by Heathman, Pierce and Kabler are probably greater than the time that actually was taken to attain sterility, or, as they put it, "the time required to kill 99.9 percent of the bacteria." This appears to be the case in Graph a. of Figure III., where the last three points on the graph fall below the curve drawn by Heathman et al, just as they do in the graphs shown in Figure II. The same is seen in Graph a. of Figure IV., Graph a. of Figure VI., and in both Graph a. and b. of Figure VIII. In both the runs recorded graphically in Figure VIII, the 2 $\frac{1}{2}$ -hour test showed only one organism surviving per ml. of test solution. This showed that more than 99 per cent of the bacteria were already killed at 2 $\frac{1}{2}$ hours, since the initial counts were 119 bacteria per ml. for the Nov. 26 run, and 186 bacterial per ml. for the Nov. 19 run. Yet Heathman and co-workers give the killing-times as 11.05 hours and 11.9 hours respectively.

A similar, but even less excusable, discrepancy is shown in Figure III, graph b. Although Heathman, Pierce, and Kabler report sterility in the 18-hour test of this particular run, in another table which summarizes the killing-times as computed by the method described, they report a killing-time of 22.18 hours for this same run. In this case the curve from the point representing the initial number of organisms per ml. to the point representing the killing-time (22.18 hours) passes through or close to practically all the other plotted points. However, there is no explanation given by the authors as to why they consider a killing-time thus computed more accurate than an actual test showing bacteriological sterility. Graph b. of Figure IV. shows the

curve from which a killing-time of 18.05 hours was computed, although the 18-hour test showed sterility. In Graph b. of Figure V. is a curve plotted by Heathman et al to show a killing-time of 28.91 hours, a computed time of more than 10 hours after the 18-hour plate test which showed that all organisms were destroyed. Likewise Graph b. of Figure VII., and Graph b. of Figure IX. show curves plotted to killing-times of 20.06 hours and 26.42 hours, respectively, when in both cases the 18-hour test plate was sterile. As a matter of fact, Heathman and her associates report a killing-time of more than 18 hours in 29 cases, in 25 of which the 18-hour plate was reported sterile.

When the amount of culture and the chlorine residual were such that rapid killing was expected Heathman and her associates plated samples from the test-solution at intervals of 5, 10, 20, and 30 minutes and one hour. Figures X.-XIII. show some examples of the points obtained by plotting counts from platings, taken at these intervals, against the time, together with the death-curves indicated by the killing-times as reported by Heathman, Pierce, and Kabler. In Figure X. the results are shown of an E. typhi strain (initial count 261 per ml.) and an Esch. coli strain (initial count - 214 per ml.) both acted upon by the same chlorine residual, and at the same temperature. Heathman and co-workers report a slightly longer killing-time for the Esch.coli strain in this test, although from the position of the points plotted from the plate-counts it appears as if the Esch. coli strain was being killed more rapidly than the E. typhi strain. In neither of the two graphs presented in Figure X. nor in those presented in Figures XI. and XII. does a straight line appear to be the type of death-curve that should be used to fit the points plotted from the experimental data.

The straight-line curves used in these cases by Heathman and associates do not pass even close to the points representing the determinations made at the one-hour interval. Since the one-hour test was the last one made in these cases, it should be most important in determining the course of the death curve.

In the last two graphs of this series, presented in Figure XIII., the plotted points all lie on, or close to the straight line, but, although it can be seen that the course of disinfection for both strains is practically identical, Heathman, Pierce, and Kabler report a killing-time of 6.3 hours for the E. typhi strain, and 16.2 hours for the Esch. coli strain. There is nothing in the data presented, which give nearly identical figures for the plate-counts of both runs, to show why a difference of ten hours is recorded in the killing-times of these strains.

The graphs presented in Figures III. to XIII. show that a straight line does not truly represent the type of death curve that results from the action of chlorine on strains of the coliform group under the conditions of the experiments of Heathman et al. The graphs presented in Figure II. and Figures XIV. to XVI. show that this type of curve fits the facts of the case no better for chloramine disinfection.

The plotting of death curves is good practice in disinfection studies. The usual procedure, however, is to determine the bacterial count at the start of the test and at frequent intervals thereafter until the disinfectant ceases to act or until all the organisms are destroyed. The figures representing the number of bacteria surviving after each interval may then be plotted against the time, and a curve drawn through the points thus obtained. In this way the approximate time of the death

of all the organisms has been found before the curve is plotted, and the curve results from the killing-time determined by experiment, instead of determining a theoretical killing-time.

In contrast to this usual procedure we have the method used by Heathman and associates, where the killing-time is the result of a curve drawn arbitrarily through a few points determined by tests made early in the course of the experiment. During a long period in the later part of the experiment (between the 2½ and the 18-hour intervals) no tests were made. Although the 18-hour tests in all but four experiments showed sterility, there is no way of knowing at just what time during this long interval the last surviving organisms were killed. In fact, since the result of the 18-hour tests were so often disregarded by Heathman, Pierce and Kabler in computing killing-time these tests might just as well have been omitted entirely.

It has been seen in the experiments of Heathman et al that when plate counts for the various periods of exposure were plotted on semi-logarithmic paper attempts made to project a straight line through a point representing the initial concentration of bacteria and these subsequent points were not highly successful. In fact there was no justification found for using a straight line so plotted as the type of death curve to express the course of chlorine and chloramine disinfection under the conditions of the experiments of Heathman and associates. Killing-times determined by actual bacteriological tests might be valuable in comparing the resistances of strains of E. typhi and Esch. coli to chlorine disinfectants. But killing-times roughly determined by projecting curves through a few points at the beginning of the curve, especially when

these points are all subject to plus or minus 25 per cent of error, cannot be considered valid for this purpose.

Since we are forced to conclude that the killing-times recorded by Heathman, Pierce, and Kabler are valueless in comparing the resistances of strains of E. typhi and members of the coliform group to the action of chlorine disinfectants, some attempt has been made to interpret their data by examining the plate counts recorded for each individual experiments. It was found next to impossible to draw any definite conclusions from these figures as so many variables were present. The nature of these variables may be enumerated as follows:

- 1.) Introduction of extraneous organic matter into the test solutions by use of broth cultures.
- 2.) Variations in the resistances of the strains to the disinfectants, due both to variations in the constituents of the test solutions, and to dissociation of the strains tested.
- 3.) Rather large numerical differences in the initial concentrations of organisms of the E. typhi strain and the coliform strain, when tested at the same chlorine residual.
- 4.) Duplicate tests-, in regards to using same strain, same initial bacterial concentration, same chlorine residual, and same temperature, --- not run.

From the foregoing critical analysis of the paper of Heathman, Pierce, and Kabler, the decision is reached that the data which they present does not support their conclusions; namely (1.) that there is danger of viable E. typhi persisting in waters treated with chlorine

and chloramine longer than members of the coliform group, and (2.) that the significance of the coliform group as a bacteriological index of the safety of chlorinated water should be reconsidered. There is no reason why the conclusions presented in the paper of Heathman and her associates should be made the basis of discrediting previous studies on the value of Esch. coli as an index of pollution of water supplies.

CONCLUSIONS

1.) The use of broth cultures in test on the germicidal activity of chlorine caused a reduction of the residual chlorine in the test solution due to the extraneous organic matter introduced in the broth. This loss of chlorine reduced the amount and velocity of disinfection.

2.) In comparing the resistances of E. typhi and Esch. coli to the action of chlorine disinfectants, it was noted that the differences in resistance of the two species were no greater than the differences in resistance of strains of the same species. Furthermore the variations in resistance observed in any given strain from day to day were as great as any differences observed in the resistances of the two species.

The phenomenon of dissociation was advanced to account for the variations in resistance observed in the strains as they were tested from day to day.

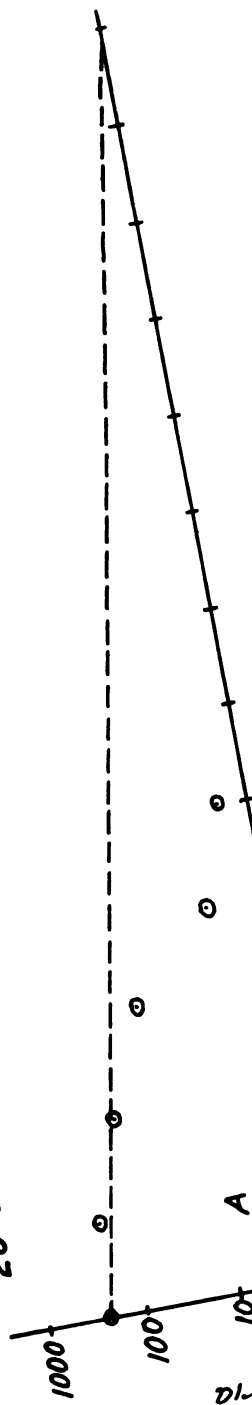
3.) No evidence was found to support the statement of Heathman and associates that "prolonged growth on artificial media materially reduces the resistance of E. typhosa to the disinfecting action of chloramines." Both recently isolated and stock strains of E. typhi were subject to so much variation in resistance that it was difficult

to compare one strain to another.

4.) The conclusions drawn by Heathman, Pierce, and Kabler that E. typhi may persist in chlorine-treated waters as long, or longer, than members of the coliform group, and that the significance of the coliform group as the bacteriological index of the safety of chlorinated waters should be reconsidered, were not warranted by the data which they presented.

CHLORAMINE DEATH CURVES

TyS 129 Oct. 9
20-25°C. C1 = 0.13 ppm



TyS 129 Oct. 15
19-24°C. C1 = 0.13 ppm

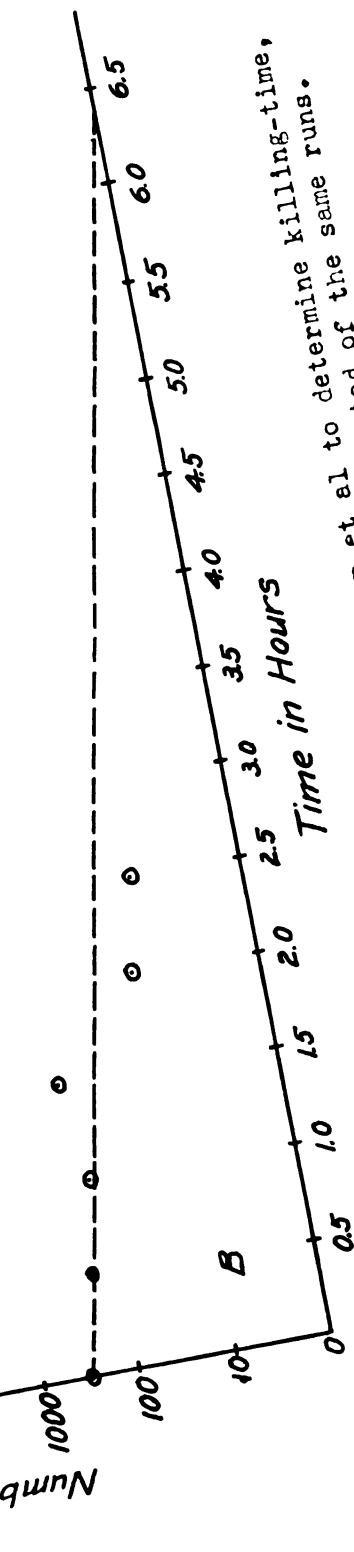


Figure II. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

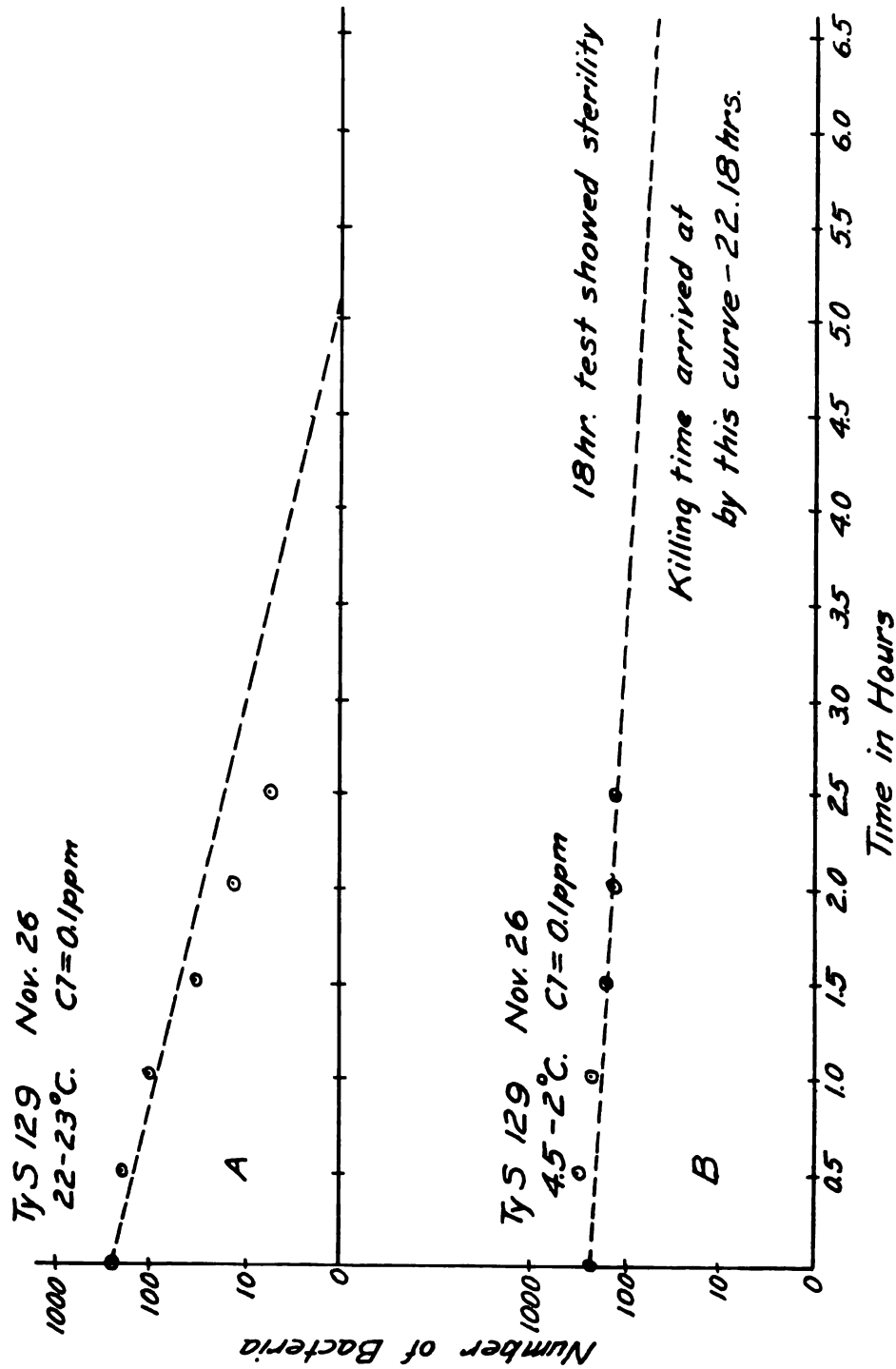


Figure III. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

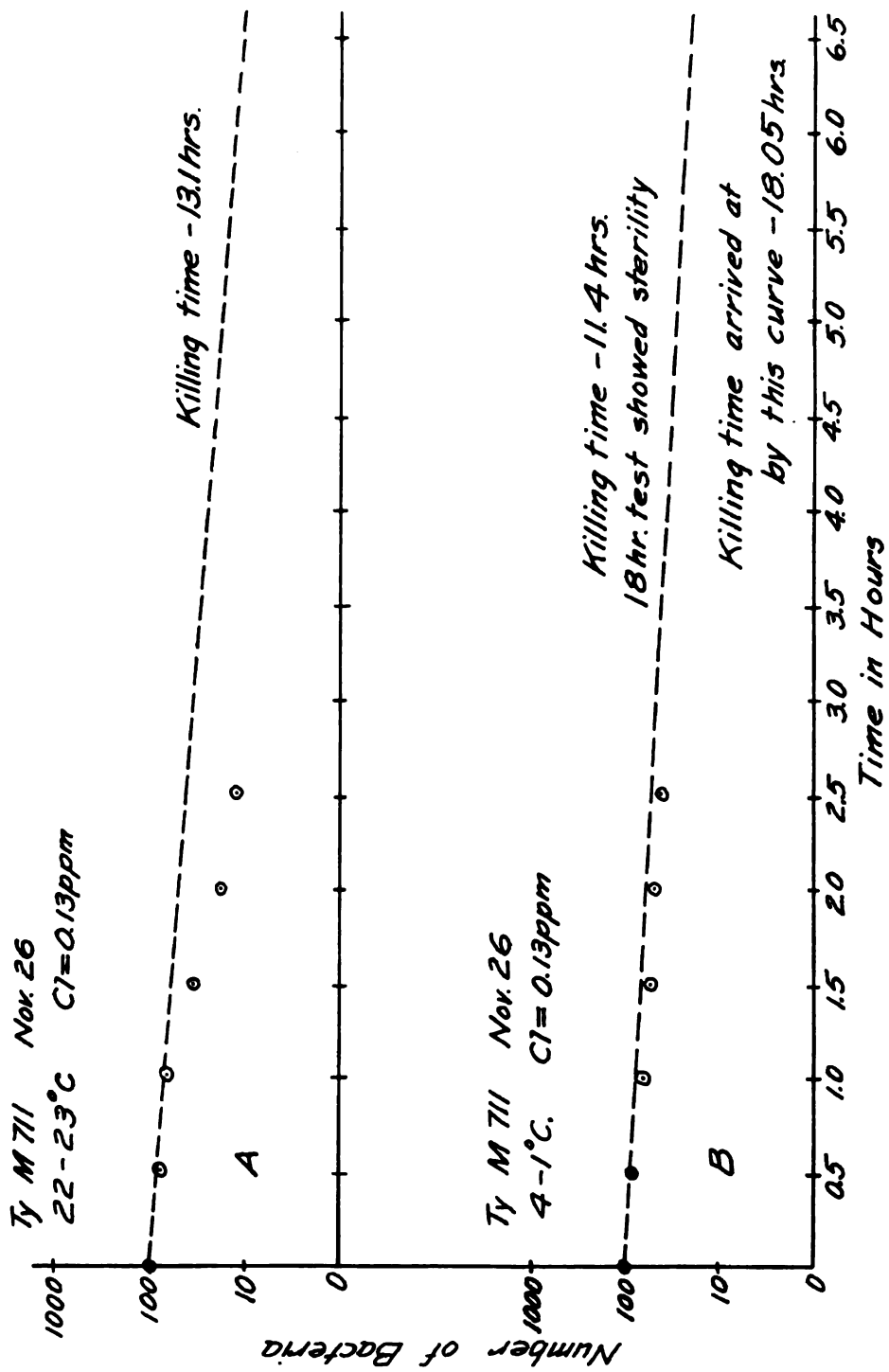


Figure IV. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

CHLORINE DEATH CURVES

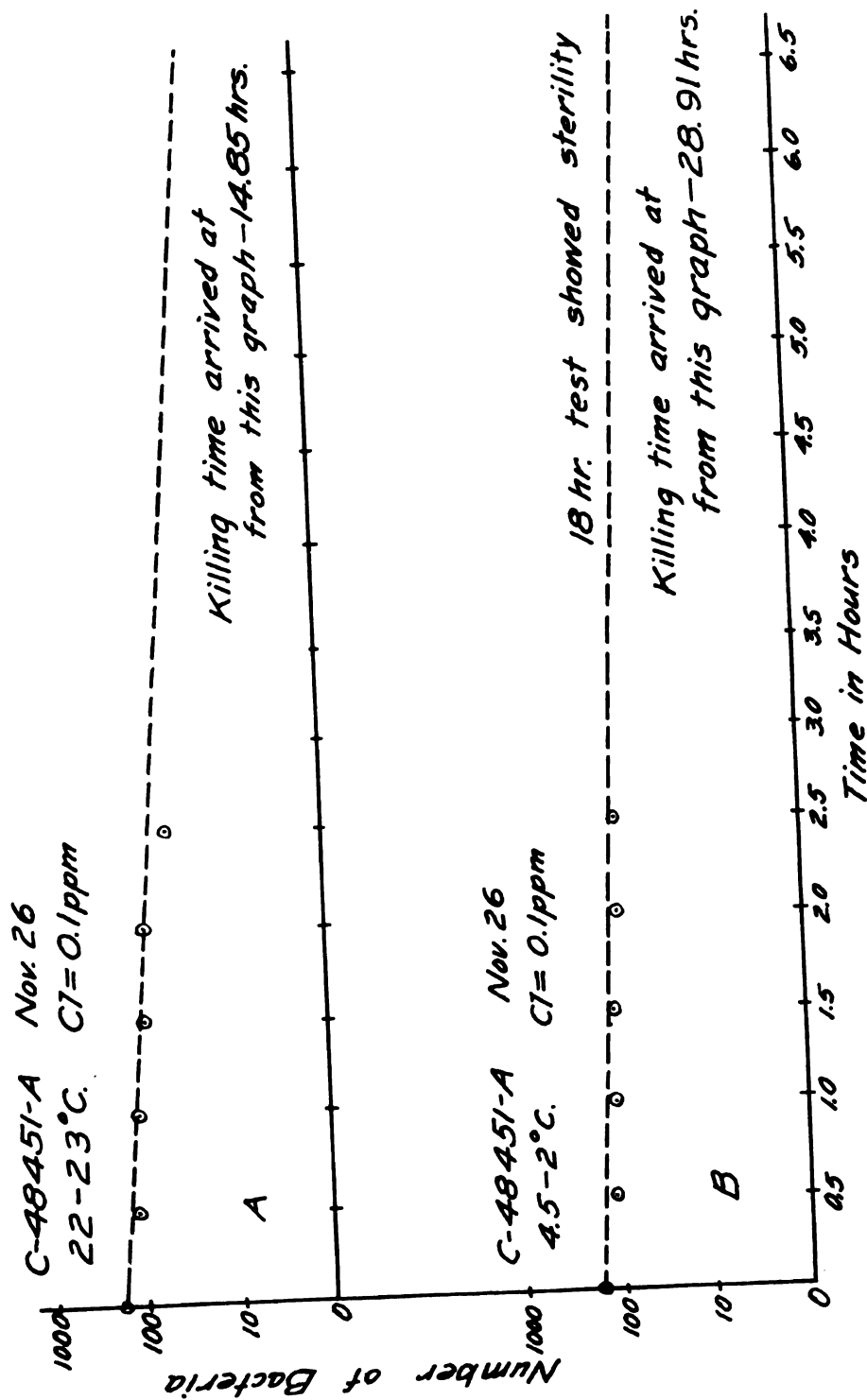


Figure V. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

1000+ C-1-48451-A Oct 29
22-24°C. CI = 0.12 ppm

CHLORINE DEATH CURVES

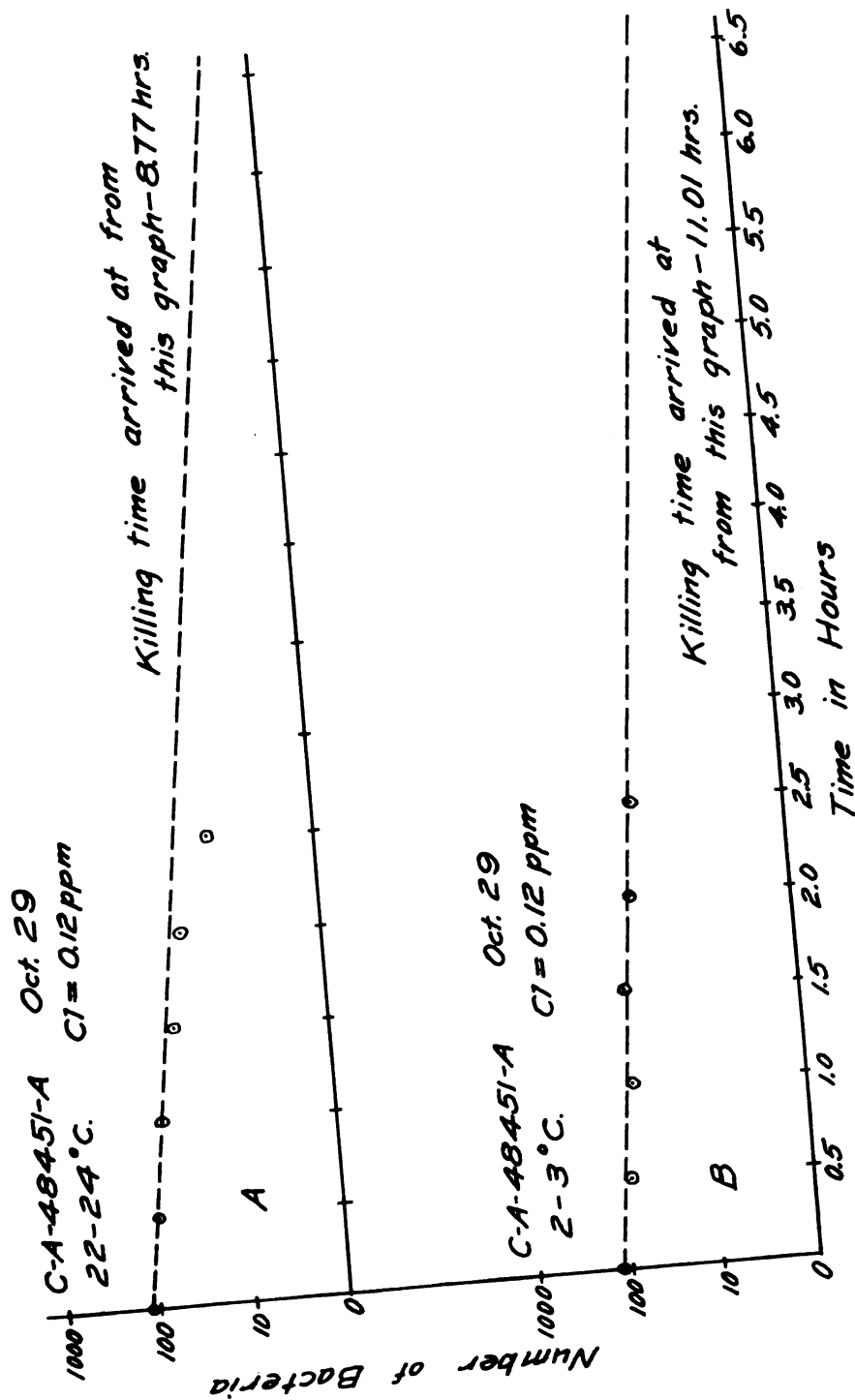


Figure VI. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

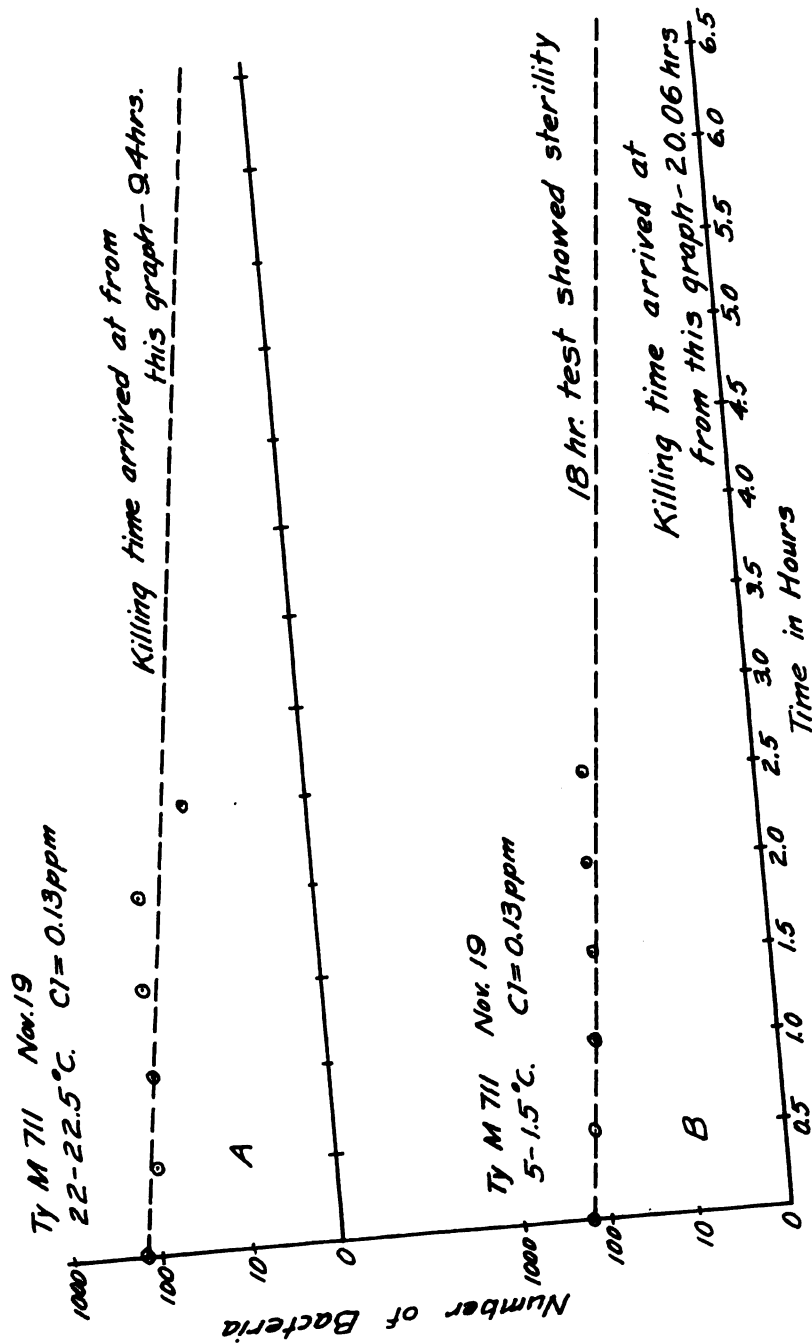


Figure VII. Compiled from curves plotted by Heathman et al to determine Killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

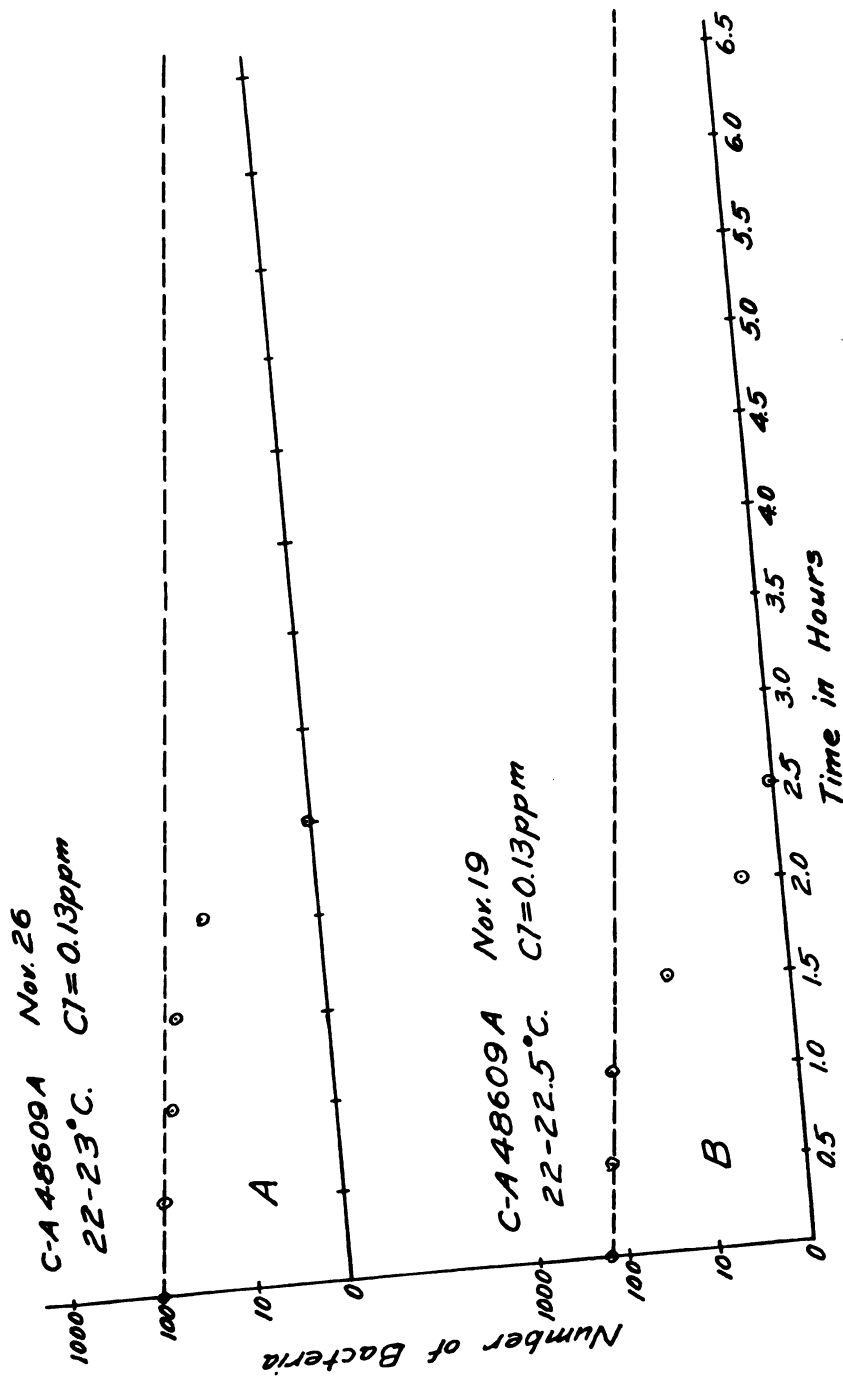


Figure VIII. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

100% 71.5 12.9
22-24°C. Cl = 0.12 ppm

Oct 29

CHLORINE DEATH CURVES

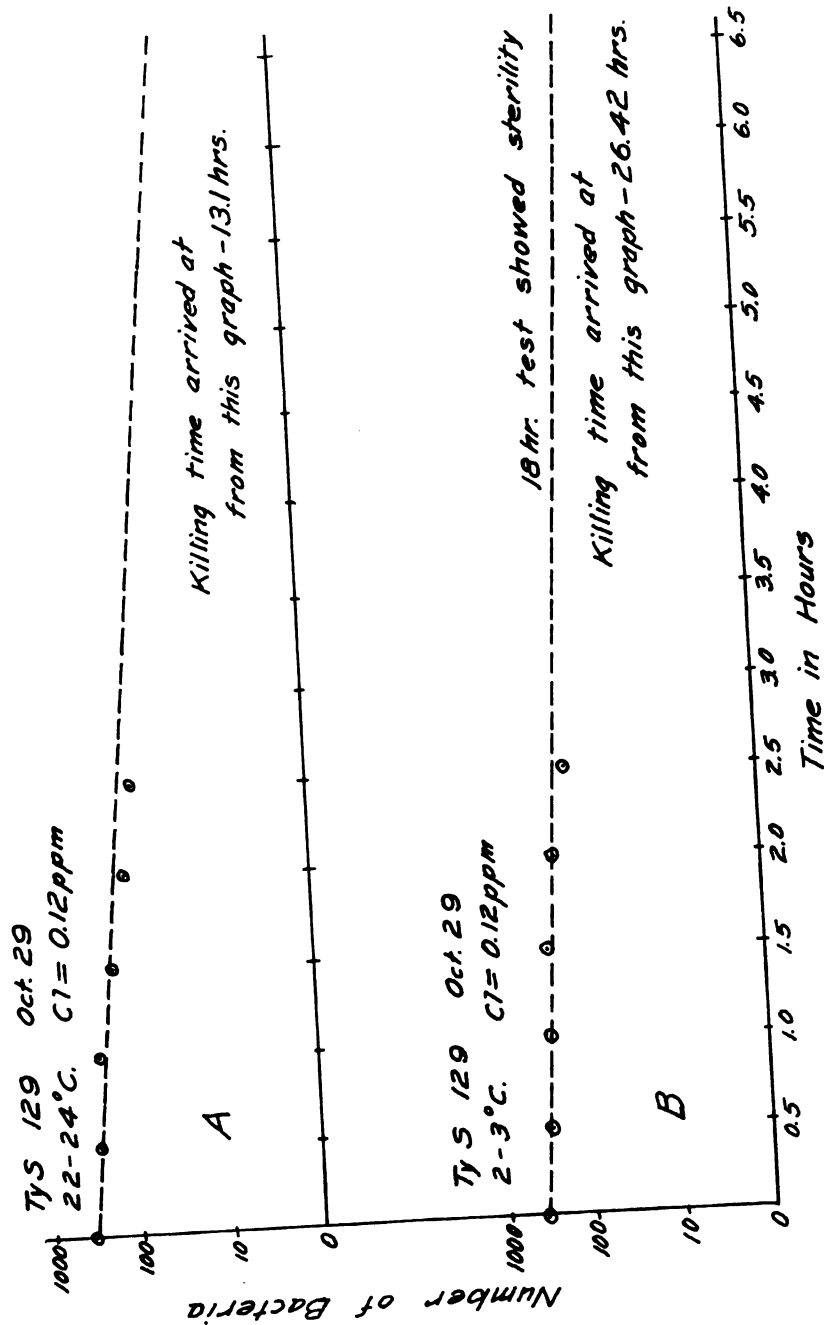


Figure IX. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

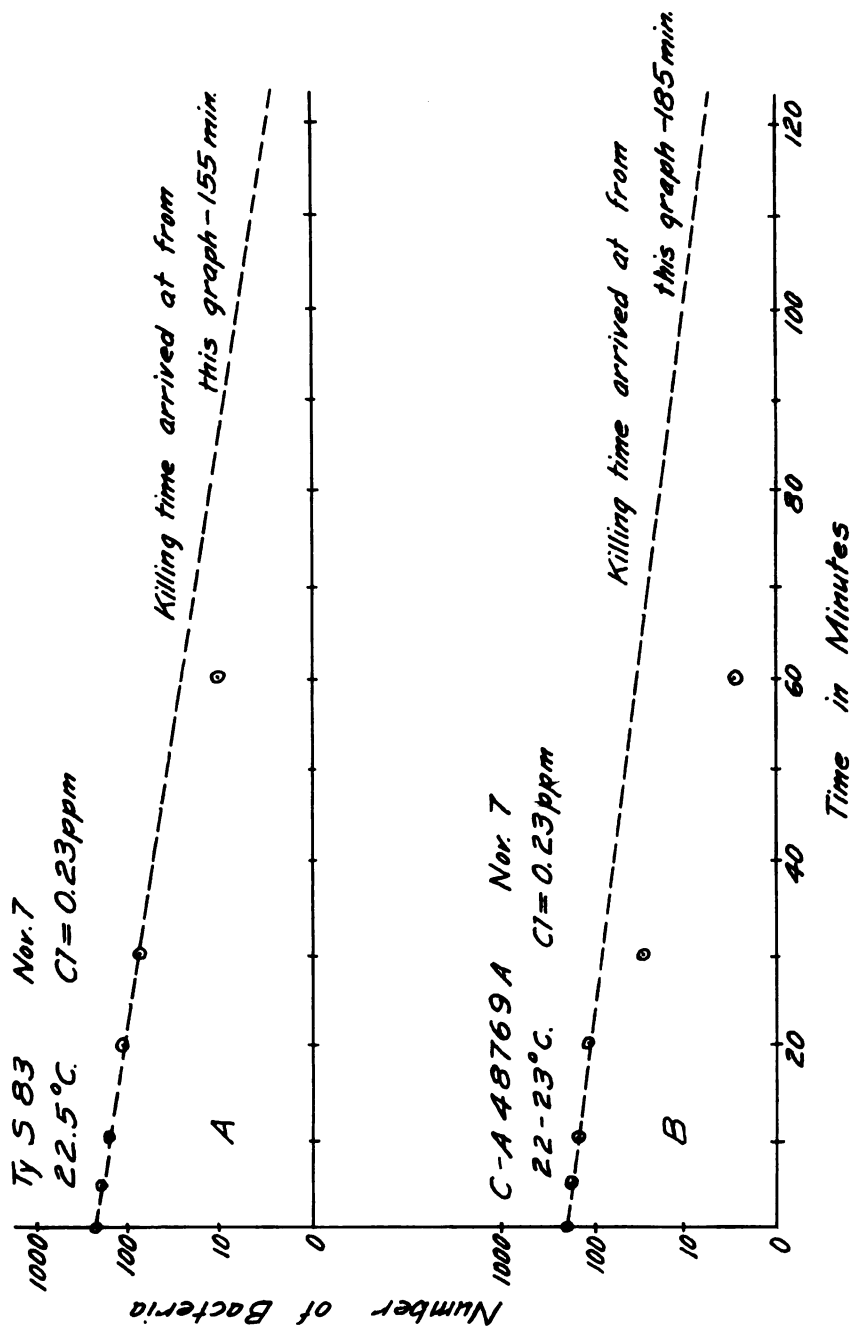


Figure X. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

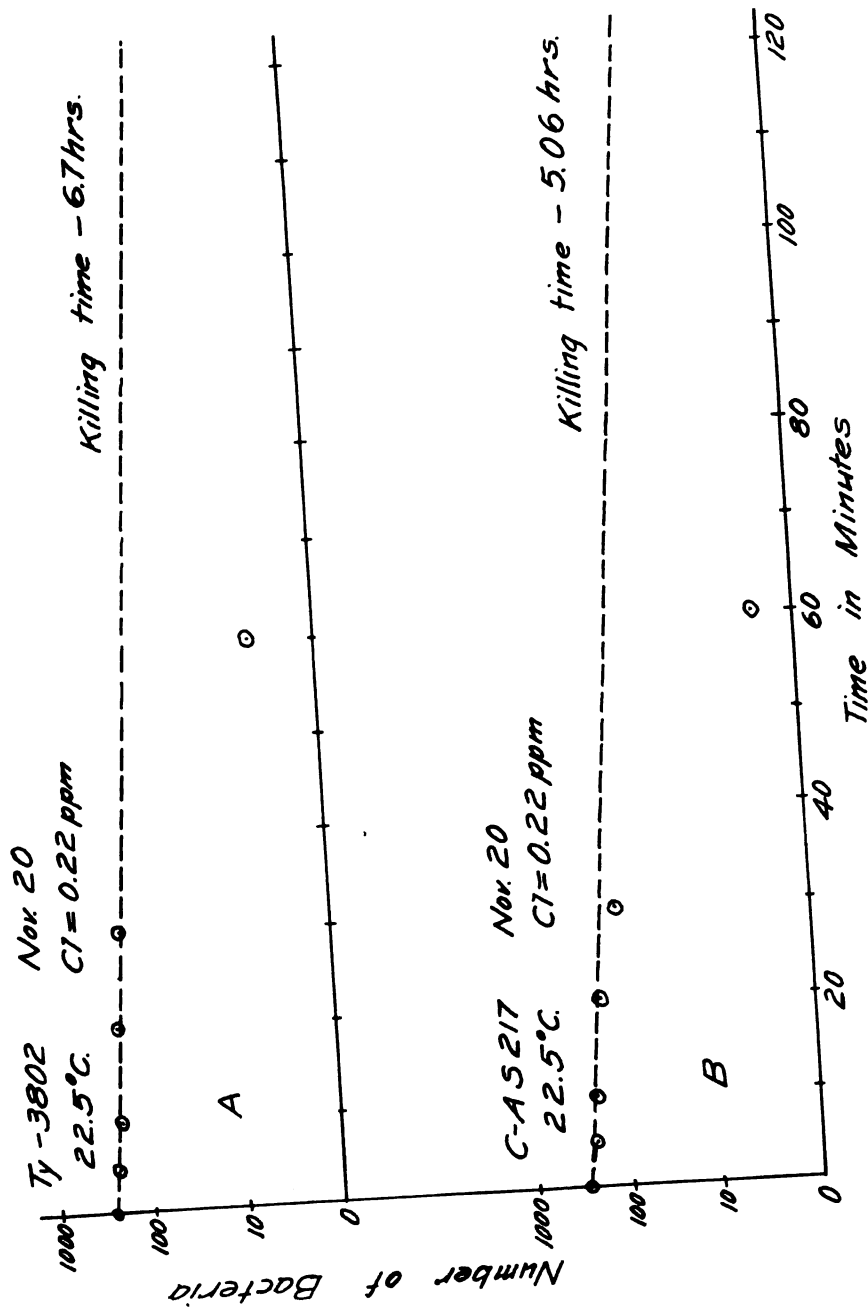


Figure XI. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORINE DEATH CURVES

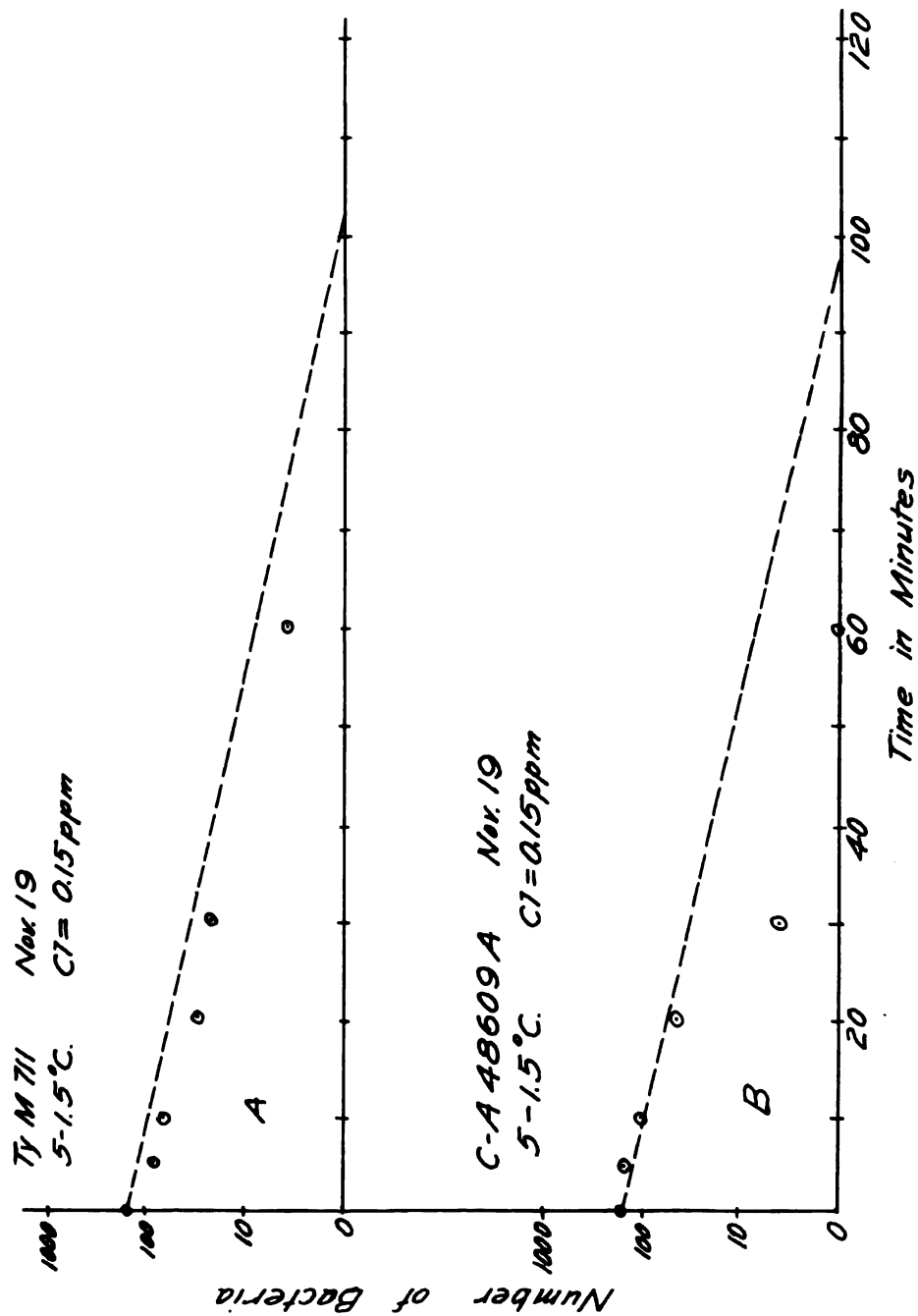


Figure XII. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

1000
7.5 B.3
1-15-91
CHLORINE DEATH CURVES
Nov. 7
CI = 0.23 ppm

CHLORINE DEATH CURVES

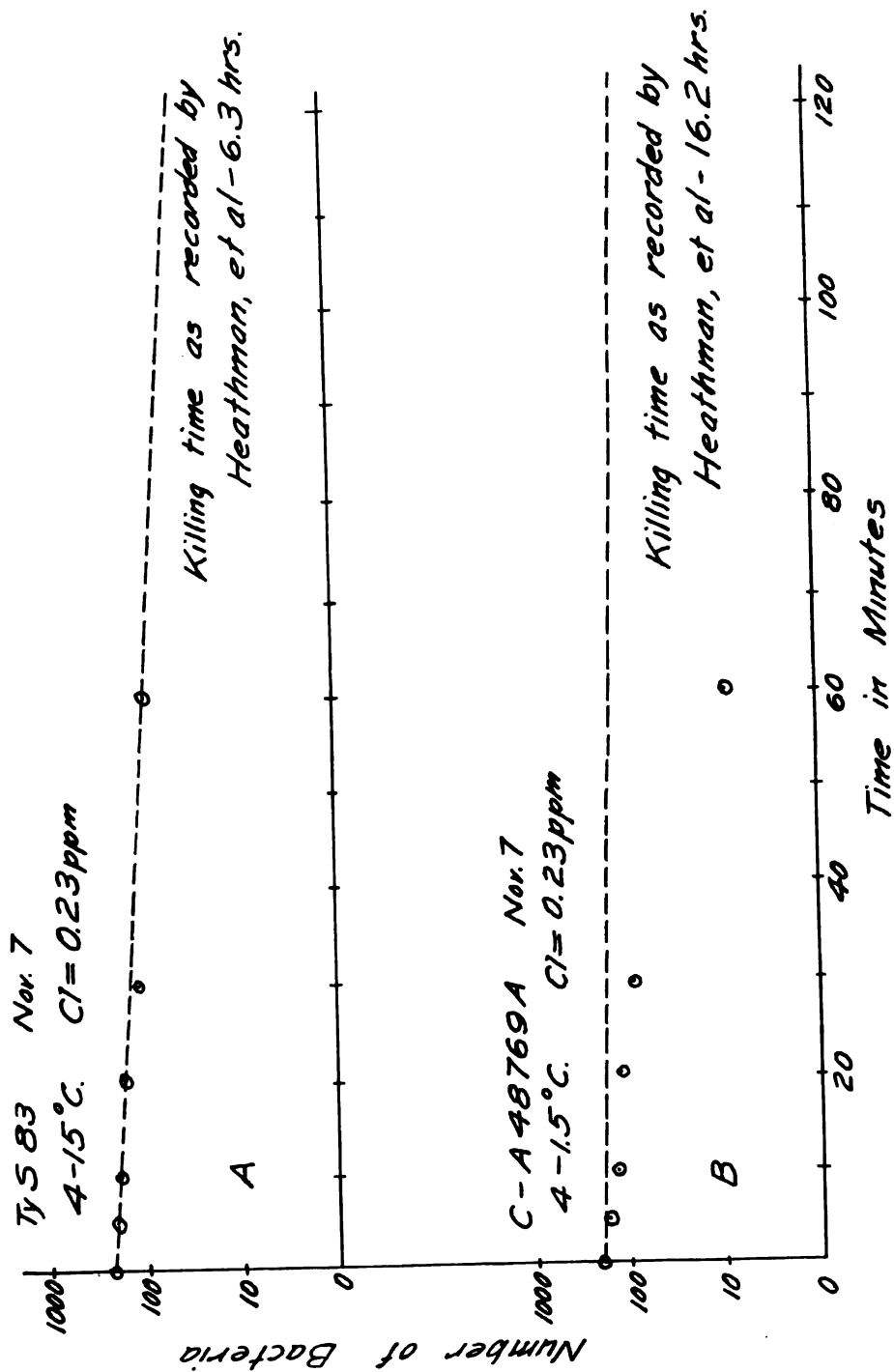


Figure XIII. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORAMINE DEATH CURVES

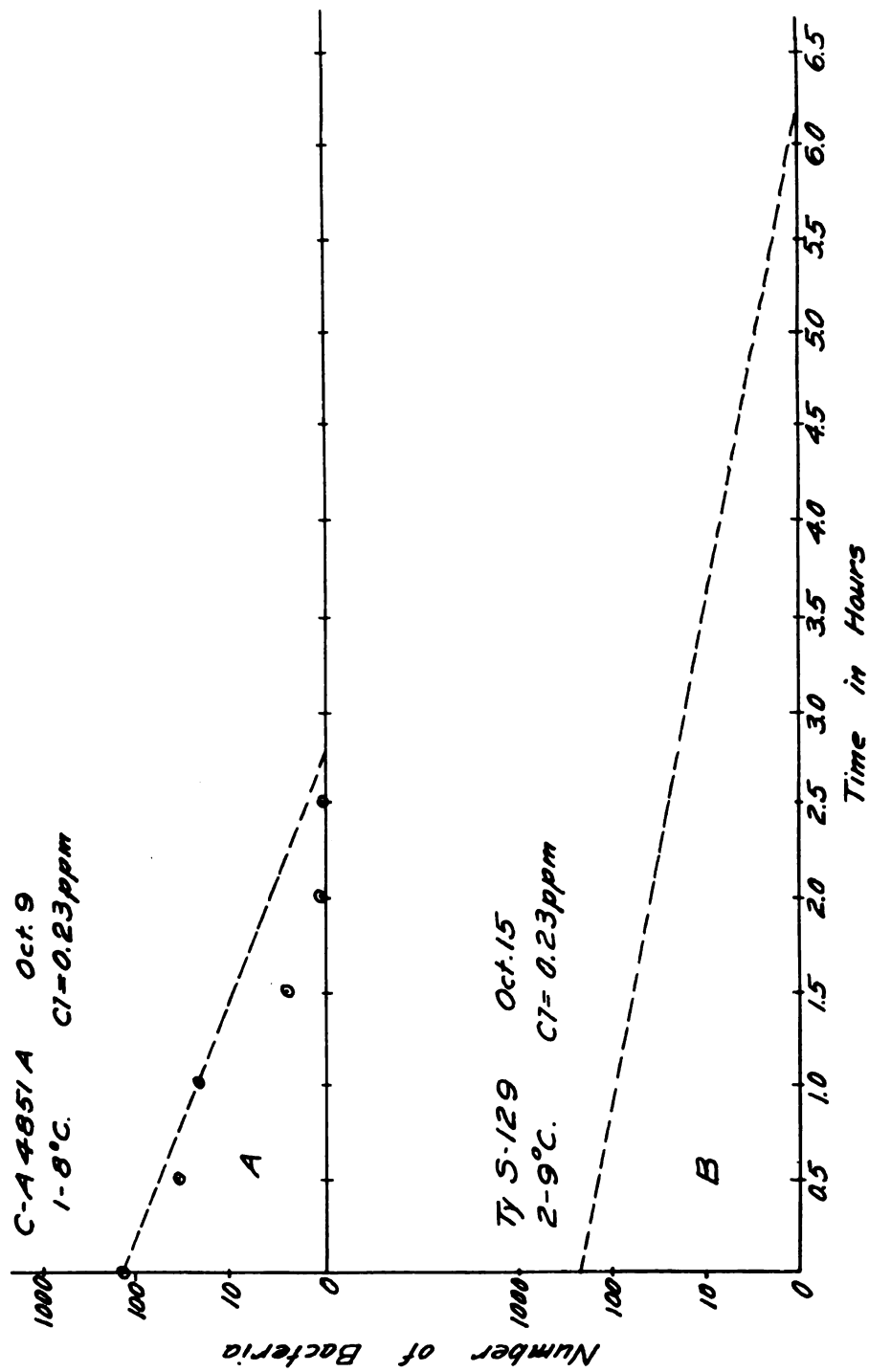


Figure XIV. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORAMINE DEATH CURVES

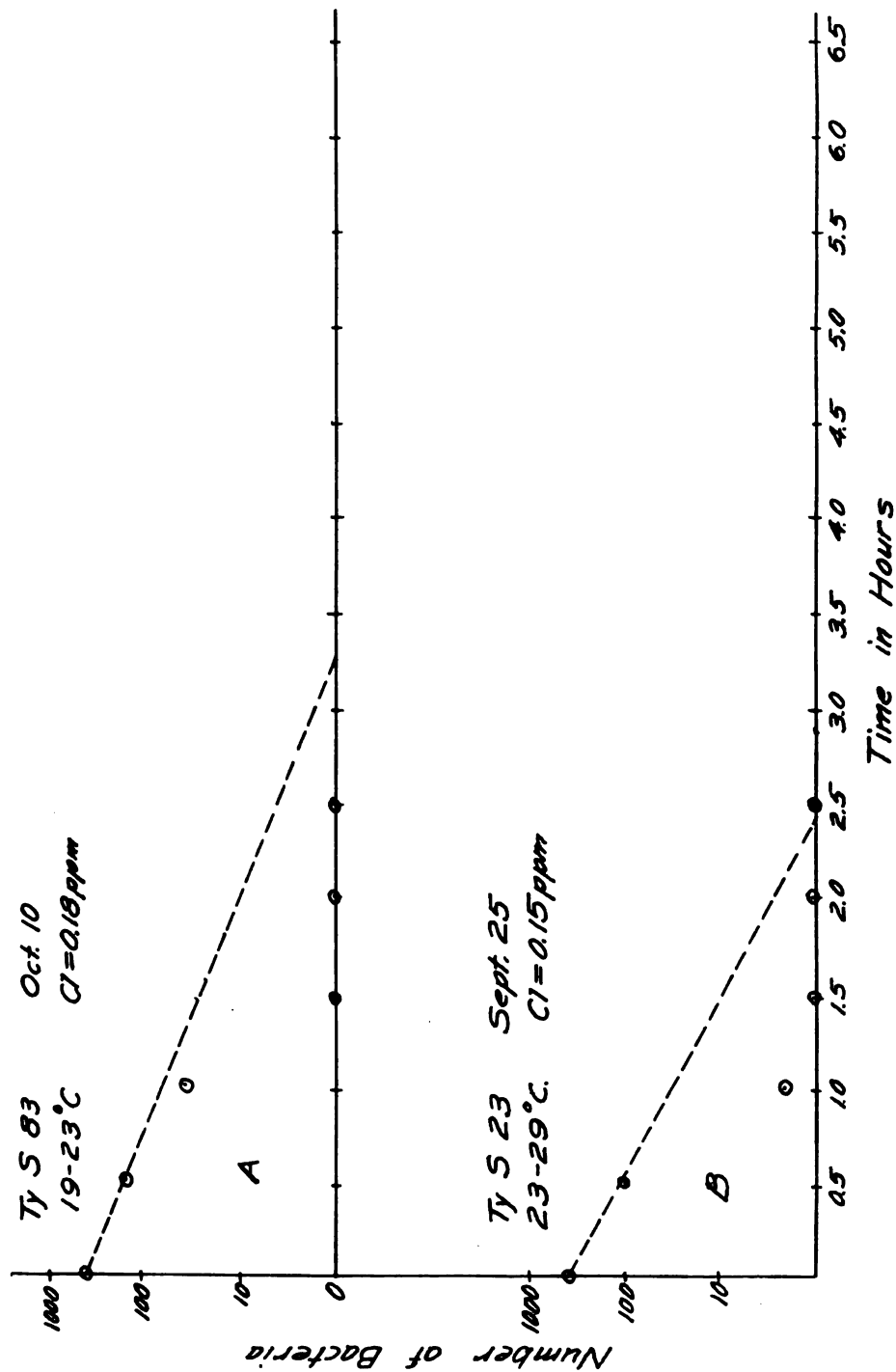


Figure XV. Compiled from curves plotted by Heathman et al to determine killing-time, together with points plotted from the plate counts reported of the same runs.

CHLORAMINE DEATH CURVES

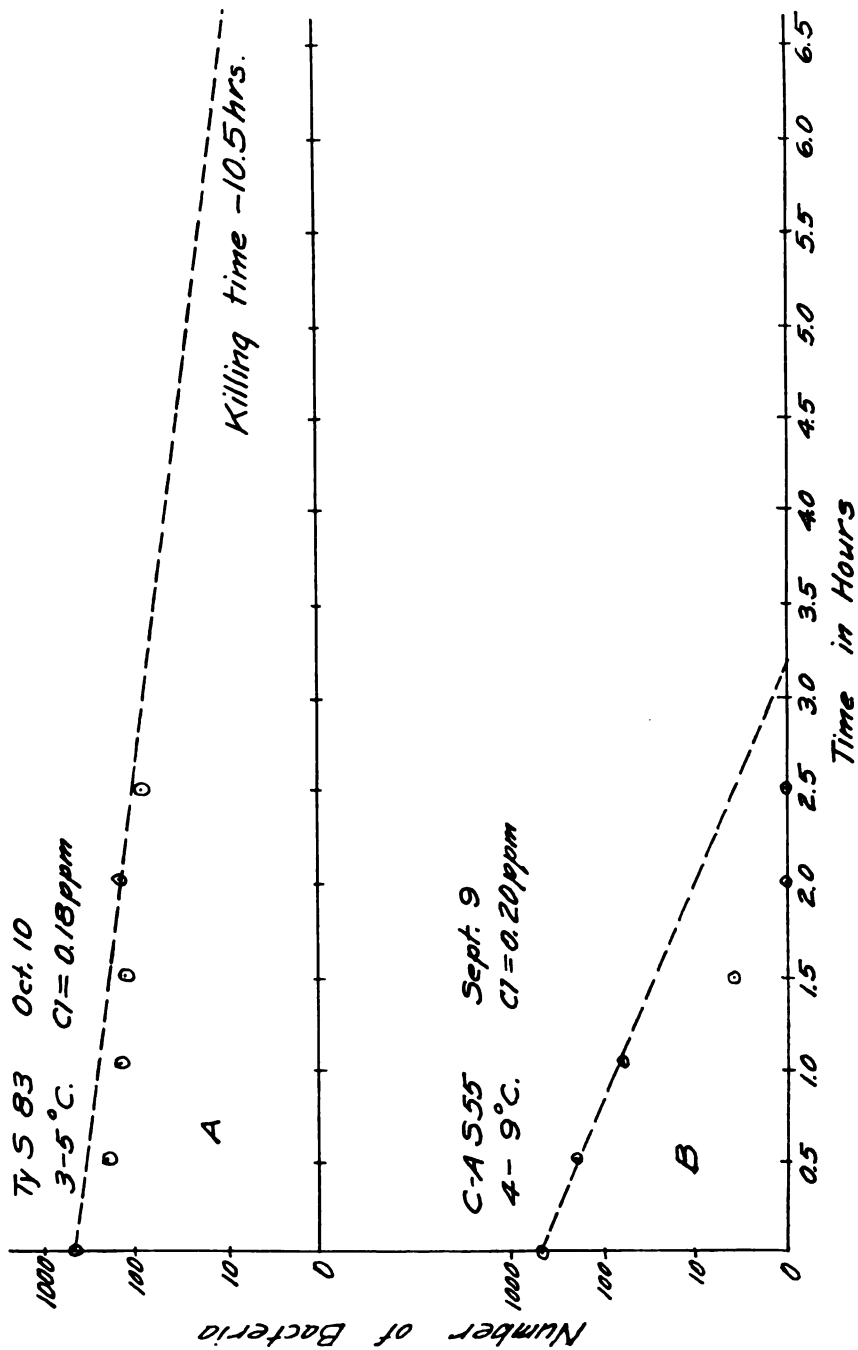


Figure XVI. Compiled from curves plotted by Heathman et al to determine killing-time together with points plotted from the plate counts reported of the same runs.

(Clack)

LITERATURE CITED

1. BEARD, Paul J. "The Survival of Typhoid in Nature." Jour. Am. Water Works Assn., 30:124-130. 1930.
2. BEARD, P. M., and N. J. KENDALL. "Sterilizing Velocities of Chlorine and Chloramine under Varying Conditions of Organic Load and pH." Jour. Am. Water Works Assn., 27:876-887. 1935.
3. BREED, R. S., and J. F. NORTON. "Nomenclature for the Colon Group." Am. Jour. Pub. Health, 27:560. 1937.
4. CAIRD, James M. "The Operation of an American or Rapid Water Filtration Plant for Twenty Years at Elmira, New York." Jour. Am. Water Works Assn., 6:409-21. 1919.
- 4a. CHARLTON, D., and M. LEVINE. "Germicidal Properties of Chlorine Compounds." Iowa State College, Engr. Exp. Sta. Bul. 132. 1937.
5. CLARK, H. W., and GAGE, S. DeM., "Significance of the Appearance of E. coli communis in Filtered Water." Jour. of Boston Med. Research, Iv, 178. 1900.
6. COHEN, B. "Disinfection Studies. The Effects of Temperature and Hydrogen Ion Concentration upon the Viability of Bact. coli and Bact. typhosum in Water." Jour. Bact., 7:183. 1922.
7. CUMMINGS, H. S. "Investigations of the Pollution and Sanitary Conditions of the Potomac River Water-shed." Bull. 104, Hyg. Lab. Wash. 1916.
8. FABIAN, F. W., E. A. BEAVENS, C. S. BRYAN, and J. M. JENSEN. "Influence of Alkalies on Available Chlorine and on Germicidal Effect of Sodium Hypochlorite in the Presence of Organic Matter as Ice Cream Mix." Ind. and Engr. Chem., 23:1169-74. 1931.
9. FISCHER, B., and G. FLATAU. Centralblatt fur Bakteriologie, 29:329. 1901.
10. GUITERAS, A. F., and F. C. SCHMELKES. "The Comparative Action of Sodium Hypochlorite Chloramine-T, and Azochloramide on Organic Substrates." Jour. Biol. Chem., 107:235-239. 1935.
11. HADLEY, P. H. "Microbic Dissociation." Jour. Inf. Dis. 40: 1927.
12. HEATHMAN, Lucy S., PIERCE, G. O., and KABLER, P. "Resistance of Various Strains of E. typhi and Coli-Aerogenes to Chlorine and Chloramine." Pub. Health Reports., 51:1367 (Oct. 2.). 1936.
13. HINDS, M. E. "The Factors which Influence Longevity of B. coli and B. typhosus in Waters." Univ. of Ill. Bull. Water Survey Series No. 13, 225-33. 1916.

(Clac)

14.

15.

16.

17.

18.

19.

20.

21.

22.

23.

24.

25.

26.

27.

28.

29.

30.

(Clack)

14. HOUSTON, A. C. "The Vitality of the Typhoid Bacillus in Artificially Infected Samples of Raw Thames and Lee River Waters." First Report on Research, Metropolitan Water Board, London. 1908.
15. JORDAN, E. C. Jour. Hyg., 1:295. 1901.
16. KUBLER, R. and F. NEUFELD. Zeitschrift fur Hygiene, 31:133. 1899.
17. LAWS, J. F. and F. W. ANDREWES in "Elements of Water Bacteriology" by Prescott, S. C., and Winslow, C-E.A., P. 51. 1904.
18. LEVINE, Max. "Bacteria Fermenting Lactose and Their Significance in Water Analysis." Iowa State College Engr. Exp. Sta. Bull. 62. 1921.
19. MACKENZIE, G. M., H. FITZGERALD, and V. IRONS. "Independent variation of Biological Characters of Bacteria as a Result of Dissociation." Proc. Soc. Exp. Biol. and Med., 30:536. 1933.
20. MALLMANN, W. L. "Stadieson Bacterium Pullorum." Doctor's Thesis: Univ. of Chicago. 1924.
21. MALLMANN, W. L. "The Germicidal Activity of Available Chlorine as Measured by the Ortho-Tolidine and Iodometric Tests for Chlorine." Mich. State College, Engr. Exp. Sta. Bull. 59. 1932.
22. MALLMANN, W. L. "Hydrogen Ion Concentration in Disinfection by Chlorination." Jour. Am. Water Works Assn., 24:7. 1932.
23. MALLMAN, W. L., and D. S. GELPI. "Chlorine Resistance of Colon Bacilli and Streptococci in a Swimming Pool" Mich. Eng. Expt. Sta. Bull. 27. 1930.
24. MALLMAN, W. L., and O. B. WINTER. "Loss of Residual Chlorine in a Swimming Pool." Municipal Sanitation, 1:382. 1930.
25. MELICK, C. O. Jour. Inf. Dis., 21:28. 1917.
26. NUNGESTER AND JUNG. Jour. of Exp. Biol.
27. PRESCOTT, S. C., and WINDSLOW, C-E.A. "Elements of Water Bacteriology." John Wiley and Sons. 1904.
28. RIDEAL, S., and J. T. A. WALKER. "The Standardization of Disinfectants." Jour. of Roy. Sanitary Inst., 24:424. 1903.
29. RUEHLE, G. L. A., and C. M. BREWER. U. S. Dept. of Agr. Circ., No. 198. 1931.
30. SMITH, T. 13th Annual Meeting of the State Board of Health of N. Y. p. 712. 1892.

(Clack)

31. STANDARD METHODS of Water Analysis, Am. Pub. Health Assoc. 1905.
32. STANDARD METHODS of Water Analysis. Am. Pub. Health Assoc. 1912.
33. TILLEY, F. W. "Investigations of the Germicidal Value of Some of the Chlorine Disinfectants." Jour. Agr. Res., 20:85-110. 1920.
34. TONNEY, F. O., F. E. GREER, and T. F. DANFORTH. "The Minimal 'Chlorine Death Points' of Bacteria: I. Vegetative Forms." Am. Jour. Pub. Health, 18:1259. 1928.
35. UNITED STATES TREAS. DEPT. Bulletin. 1914.
36. VELDEE, M. V. "An Epidemiological Study of Suspected Water-Borne Gastroenteritis. Am. Jour. Pub. Health Assn., 21:1227. 1931.
37. WESBROOK, E. F., H. A. WHITTAKER, and B. M. MOHLER. Am. Jour. pub. Health Assoc., 1:123. 1910.
38. WRIGHT, J. H. "The Importance of Uniform Culture Media in the Bacteriological Examination of Disinfectants." Jour. Bact., 2, 315. 1917.
39. WRIGHT, N. C. "The Action of Hypochlorites on Amino-Acids and Proteins." Biochem. Jour., 20:524-532. 1926.
40. ZIEGLER. Am. Jour. Pub. Health Assn., 27: 1937.

ROOM USE ONLY

ROOM USE ONLY.

Jan 4 '50

T

MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 03046 4865