A CONTRIBUTION TO THE DYNAMICS OF TOXICITY AND THE THEORY OF DISINFECTION

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I. INTRODUCTION

It has long been known that the effects of a toxic agent acting upon bacterial or other forms of protoplasm become manifest only after the lapse of a shorter or longer period of time. Some thirty years ago Abbott (1891), studying the disinfection of *Staphylococcus pyogenes-aureus* by mercuric chloride, concluded that the explanation for the time curve must lie with differences in the resistance of individual cells in a bacterial population to the lethal agent. Since then numerous workers have studied the course of disinfection processes by various quantitative methods with the hope that such studies might cast some light upon the nature of the reaction between toxic agent and cell and lead to an explanation of the shape of the time curve of the process. Admittedly, the physical and chemical systems involved in the structure of protoplasm are extremely complex and the reactions between a toxic agent and the constituents of these systems may be equally complex. None the less, it has been the hope of many investigators that some simple physical or chemical reaction between disinfectant and protoplasm would be found to suffice as an explanation for the dynamics of the lethal process. And some have considered that these hopes have been attained.

In recent years there have been published several reviews of the extensive literature on the theory of disinfection dynamics. Hence it is not our intention to present more than a few comments on earlier papers and only such as are especially pertinent to the purposes of the present study. Critical discussions may be

consulted in the papers of Brooks (1918–1919), Eisenberg (1918–1919), Cohen (1922), Reichenbach (1922), Falk (1923) and Fulmer and Buchanan (1923).

In their classic contribution, Krönig and Paul (1897) reported the results of careful studies on disinfection by mercuric chloride and succeeded in demonstrating conclusively that the process of disinfection is a gradual and not an instantaneous one, and that it follows an orderly sequence. From their data, Ikéda (1897) found that the curve prepared by plotting the logarithms of surviving organisms against elapsed time is a straight line and that the rate of the disinfection process is in agreement with the rate calculated from the equation of an unimolecular reaction. In a like manner, Miss Chick (1908, 1910, 1912, 1913) was able to calculate a similar, essential concordance with the logarithmic rate from the data of Clark and Gage on the disinfectant action of sunlight and from her own experiments with silver and mercury salts, phenol, heat, blood serum, etc. Numerous other workers (Madsen and Nyman, Cohen, etc.) have obtained essentially the same results. However, nearly all these workers have observed occasional deviations in the logarithmic curve from a straight line, such deviations being especially common in the earliest and latest periods of the disinfection process. Bellei, Loeb and Northrop, Eijkman, Hewlett, Reichel, Reichenbach, Brooks, Peters and others have raised objections to an interpretation of these observations in terms of a simple unimolecular reaction. Indeed, some workers have contended "that such resemblances as have been found between such curves and unimolecular reaction or logarithmic curves are superficial and fortuitous. Any method therefore of evaluating disinfecting power based upon such a concept must prove misleading."[1] With these few introductory remarks for orientation we may proceed to an examination of some new experimental data and then return to a more careful reconsideration of the theoretical aspects of the problem.

The experiments here reported were originally designed as part of an extensive study to determine the effects of certain inorganic

[1] Fulmer and Buchanan, 1923, 88.

salts upon bacterial viability and to cast some light upon the dynamics of the processes of cellular death and of disinfection. It is our plan to present for analysis here only a few of hundreds of similar experiments on the rates of dying of *Bacterium coli* in salt solutions, choosing for our present purposes the data from typical findings upon the mortality in aqueous sodium and calcium chloride solutions of various concentrations and in distilled water at various pH values.

II. EXPERIMENTAL METHODS

Our experiments were conducted with a single strain of *Bacterium coli* which was isolated from a polluted stream near New Haven in the autumn of 1916. It was strain 38 of the original collection of Winslow and Cohen (1918). This strain of *Bact. coli* maintains itself in distilled water at a favorable pH value without material decrease in numbers for a period of nearly twenty-four hours, actual increases being not uncommon during the first few hours. Occasionally, however, a particular suspension will show a marked decrease due to some cause which we have not yet determined. The details of technique used in these studies have been reported in earlier papers (Winslow and Falk, 1923a, 1923b) and need not be repeated.

III. GENERAL RESULTS

In table 1 we cite the data taken from a typical experiment, 37-C, to indicate certain characteristics of the viability curve of *Bacterium coli* in a comparatively dilute solution of $CaCl_2$ at 37°C. The velocity constants of the mortality processes have been calculated from the well-known equation for an unimolecular chemical reaction:[2]

$$0.434\, K_1 = \frac{1}{t} \log \frac{a}{a - x} \qquad (1)$$

where a = the original number of viable bacteria
a-x = the number of viable bacteria after time t

[2] Throughout this paper we have utilized the equations for chemical reactions which are direct and isolated, on the assumption that the changes which finally lead to loss of viability are irreversible. Cf. the theoretical treatment of the dynamics of reactions in homogeneous systems by Rice (1924).

t = time in hours
K_1 = velocity constant
log = logarithms to the base 10

Here we observe that the average value of K_1 is 0.232 and that the individual values of the velocity constant range between 0.213 and 0.256, with a probable error (P.E.) of ±0.011, or approximately 5 per cent.[3] For a biological experiment in which the errors of method involved in various of the procedures used in the technique of bacterial quantitation are appreciable, such a constancy of K_1 as is indicated by a P.E. of less than 5 per cent is

TABLE 1

The viability of Bact. coli in 0.145 M $CaCl_2$ solution

Experiment 37-C

INCUBATION PERIOD—*t*-HOURS	NUMBER OF VIABLE BACTERIA	LOGARITHM OF VIABLE BACTERIA	VELOCITY CONSTANT—K_1
0	15,200,000	7.182	
1.83	9,500,000	6.978	0.256
5.33	4,700,000	6.672	0.220
7.83	2,390,000	6.378	0.237
21.33	163,000	5.212	0.213

Average K_1 = 0.232
P. E. = ±0.011

striking. From the uniformity of the values of K_1 it follows that if the course of bacterial mortality is illustrated by a graph of logarithms of surviving organisms plotted against time the curve will approximate a straight line. That this is the case is evident from inspection of figure 1. There can be no doubt that such an experiment as this furnishes a general confirmation of the findings of Krönig and Paul, Madsen and Nyman, Chick, Cohen and others who have described the concordance between a bacterial mortality curve and the logarithmic straight line of the unimolecular mass action equation. It is also significant to observe that the mortality rate was comparatively low, approximately two-thirds of the bacteria having survived for a period of nearly two

[3] The probable error of the average was calculated on the basis: P. E. = 0.6745 $\Sigma d^2 n$ where Σd^2 = the sum of the squares of the deviations from the average; and n = the number of values averaged.

hours. Experiments of this kind were specifically designed to meet the issue which has been raised by Loeb and Northrop (1917) and others that in the initial stages of a disinfection process, there is a lag period and that the logarithmic law is not followed if the rate of the process is measured in the early stages of the reaction.

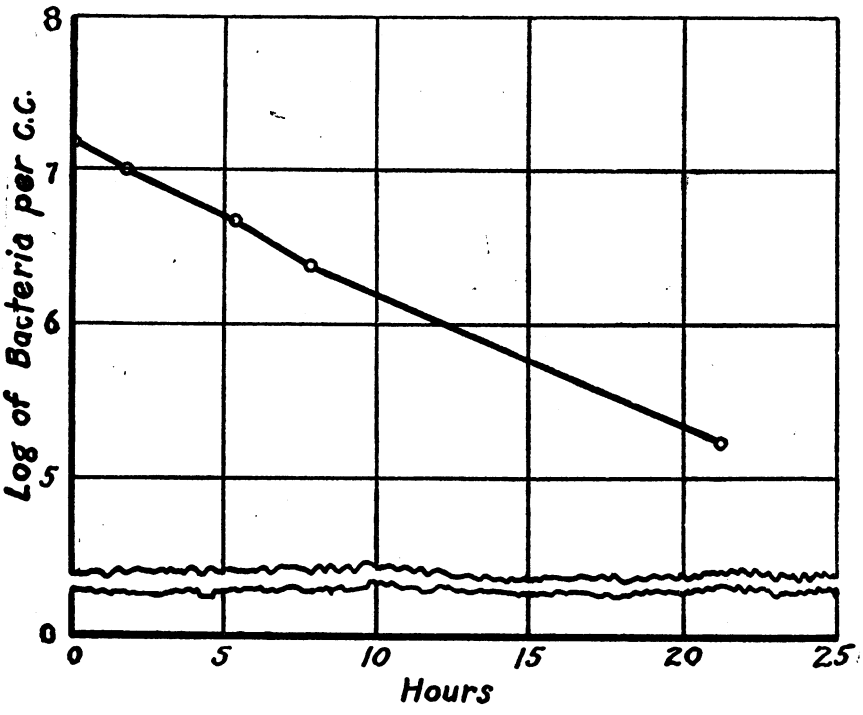

FIG. 1. BACT. COLI IN 0.145 M $CaCl_2$ SOLUTION AT 37°C.

In table 2 we have presented the results of typical experiments on the course of mortality in a bacterial population exposed to various concentrations of calcium chloride. (All of these tests were made at the same time, under similar conditions and with the same technique. Solution C and the data in column (4) are identical with experiment 37-C of table 1.)

From an examination of column (2) of the table it appears that in the presence of a very low concentration of calcium chloride

(0.00145 M) there was a slight multiplication of the bacteria.[4] It also appears that there was no multiplication of the bacteria in the first time interval (0 to 1.83 hours), an appreciable multiplication in the second interval (1.83 to 5.33 hours) a maximal multiplication in the third interval (5.33 to 7.83 hours) and a lesser multiplication in the final period (7.83 to 21.33 hours). From column (3) it appears that there was an even more marked multiplication of the bacteria in the presence of a ten-fold higher

TABLE 2

The viability of Bact. coli in $CaCl_2$ solutions of various concentrations at 37°C.

INCUBATION PERIOD—t-HOURS (1)	VELOCITY CONSTANTS (K_1) IN: A 0.00145M (2)	B 0.0145M (3)	C 0.145M (4)	D 0.725M (5)	E 1.450M (6)
0					
1.83	0	0.101	0.256	1.93	3.36
5.33	−0.178*	−0.384	0.220	1.07	1.93
7.83	−0.203	−0.636	0.237	1.14	∞†
21.33	−0.036	−0.037	0.213	0.766	....
Average.....	−0.104	−0.0239	0.232	1.23	2.65‡
P. E.....	±0.059	±0.199	±0.011	±0.43	

* A minus sign (−) before a value of K_1 indicates that the bacteria had *increased* in numbers.

† An infinity value (∞) indicates that the reaction went to completion in the time interval (i.e. no viable bacteria remaining). Such a value, calculated by setting (a−x) = 0 is inconsistent with the mass action law. Calculated values of K_1 will range from $\left(\frac{2.3}{t} \log a\right)$ to (∞) according as the bacterial count is taken between *1* and *o* per cc.

‡ Average calculated from the two finite values.

concentration of the salt (0.0145M), the rate of growth again attaining a maximal value in the third interval.[5] Columns (4),

[4] It is recognized that the application of the unimolecular equation to the tests in which there are observed *increases* in the number of viable bacteria is not strictly sound. It is carried out for the sake of uniformity of procedure and in order to provide in the $(-K_1)$ values a convenient measure of the rate of multiplication.

[5] The stimulating effect at this salt concentration was unusual. As will be shown later on a concentration of 0.01 M $CaCl_2$ generally proves slightly toxic.

(5) and (6) representing data from viability experiments with solutions of higher salt concentrations (0.145M, 0.725M and 1.450M) show no multiplication of the bacteria, but, instead, progressively higher mortality rates.

It would appear, then, from these data that in very dilute aqueous solutions $CaCl_2$ stimulates *Bact. coli* to multiply and that in higher concentrations it is highly toxic. Now, it is especially significant to observe that in one of the three toxic solutions (column (4)) the course of mortality follows that of an unimolecular reaction, witness the sensible constancy of the values of K_1. If only this particular concentration of $CaCl_2$ had been studied and had been presented alone as in table 1, it would have implied a confirmation of the unimolecular disinfection theory. However, examination of the last two columns of table 2 brings out clearly that with more toxic concentrations of $CaCl_2$ the values of K_1 are not even approximately constant. They show, instead, values which decline steadily for successive intervals of time. If the constancy of K_1 in solution C means that the reaction between $CaCl_2$ and *Bact. coli* is essentially unimolecular,[6] it becomes impossible to reconcile the data in column (4) with those in columns (5) and (6) without making additional assumptions.

If the course of a disinfection process be such as to give the logarithmic straight line of an unimolecular reaction and if it be assumed that the fundamental reaction is unimolecular, we might consider that of the reacting agents (1) disinfectant, and (2) living cell, one or the other is present in excess and that its concentration is not changing significantly during the course of the reaction. Inasmuch as the constant K_1 obviously changes with a change in salt concentration it must be assumed that the reacting substances of the bacterial cell (whether those cells are dead or alive) must always be present in excess if anything approaching the monomolecular curve is to be attained.

[6] Assuming that one of the reacting agents is present in excess and that hence its concentration remains essentially constant. In such a case, the reaction may really be of a higher order, but it will *appear* to be of the first order.

IV. DEVIATIONS FROM THE LOGARITHMIC CURVE

We may now proceed to consider the deviations from a regular logarithmic curve which are manifest on a closer analysis of the results of such experiments as are under discussion. Almost all the observers who have studied this problem report such deviations. At the beginning of the observation period marked and more or less irregular mortalities are manifest and during the later course of an experiment there is a rather regular tendency for the rate of dying to become progressively less. Even in the work of Chick this tendency is clearly manifest.

TABLE 3

Viability of Bact. coli in distilled water

INCUBATION PERIOD—t-HOURS	VELOCITY CONSTANTS (K_1) AT pH = :						
	4.0	5.0	6.0	6.5	7.0	7.5	8.0
0							
1	0.14	0.14	0.18	0.09	0.39	0.25	0.23
3	0.31	0.11	0.10	0.14	0.21	0.47	0.21
6	0.54	0.12	0.07	0.20	0.24	0.42	0.35
9	0.51	0.04	0.02	0.29	0.17	0.34	0.23
24	(*)	0.12	0.01	0.16	0.06	0.15	0.10
Average K_1.....	0.37	0.11	0.08	0.18	0.21	0.33	0.23
Number of experiments...	2	2	4	2	10	2	4

* See table 2, footnote †, for explanation.

In all viability studies there are marked irregularities, such as we are accustomed to find in biological investigations,—which make the elucidation of the more subtle influences at work exceedingly difficult. The only way to overcome such difficulties is by the use of averages obtained from the results of large numbers of experiments, and we have therefore attempted to throw some light upon the problem in hand by working out such averages for a considerable number of experiments. These data are presented herewith in tables 3 to 5 and figures 2 and 3. In these tables the values for K_1 have been expressed only in two decimal places since

the chance errors are such as to rob the third decimal of any practical significance.[7]

From tables 3 to 5 it is obvious that there is a rough general approximation to a constancy of K_1 with a given concentration of a given toxic substance. In distilled water solutions near

TABLE 4

Average reaction velocities of mortality of Bact. coli in various concentrations of $CaCl_2$, and after various periods of exposure

CONCENTRATION $CaCl_2$ NORMALITY	NUMBER OF TESTS	HOURS					
		1	3	6	9	24	Average
0.001–0.002	1–4	−0.18	0.04	−0.10	−0.05	−0.02	−0.06
0.01–0.03	28–31	0.32	0.10	0.09	0.05	0.01	0.114
0.07–0.14	20–24	0.16	0.18	0.17	0.12	0.09	0.144
0.4	2–11	0.84	0.80	0.73	0.60	0.48	0.690
0.7	3–15	1.30	1.34	1.01	1.17	0.82	1.128

TABLE 5

Average reaction velocities (K_1) of mortality of Bact. coli in various concentrations of NaCl and after various periods of exposure

CONCENTRATION NaCl NORMALITY	NUMBER OF TESTS	HOURS					
		1	3	6	9	24	Average
0.01–0.03	7–8	0.00	0.00	−0.03	−0.02	−0.02	−0.002
0.07–0.14	9–11	0.11	0.09	0.10	0.09	0.08	0.094
0.4	6–8	−0.05	0.14	0.17	0.24	0.03	0.106
0.7	19–20	0.04	0.24	0.24	0.22	0.23	0.194
0.9–1.0	14–16	0.12	0.34	0.29	0.20	0.09	0.201
1.4	5–15	0.44	0.71	0.81	1.22	0.48	0.732

neutrality and in dilute salt solutions K_1 is very small, that is the rate of bacterial mortality is low. A very dilute solution of either NaCl or $CaCl_2$ is definitely favorable to survival, causing in general an actual increase in numbers ($-K_1$). With an acid concentration equivalent to pH 4.0 or an alkaline concentration equivalent to pH 7.5 the mortality constant increases as it does

[7] The averages have been obtained arithmetically,—a procedure not perhaps justified on mathematical grounds, since we are dealing with logarithmic terms, but indicating general trends with an accuracy which has been found by calculation to be sufficient for the present discussion.

with NaCl concentrations of 1.0 M or over and with a $CaCl_2$ concentration of 0.4 M and over.

Closer analysis of the figures shows, however, rather definite deviations from a constant value of K_1. Even with the distilled water data in table 3 it is apparent that the K_1 values are generally

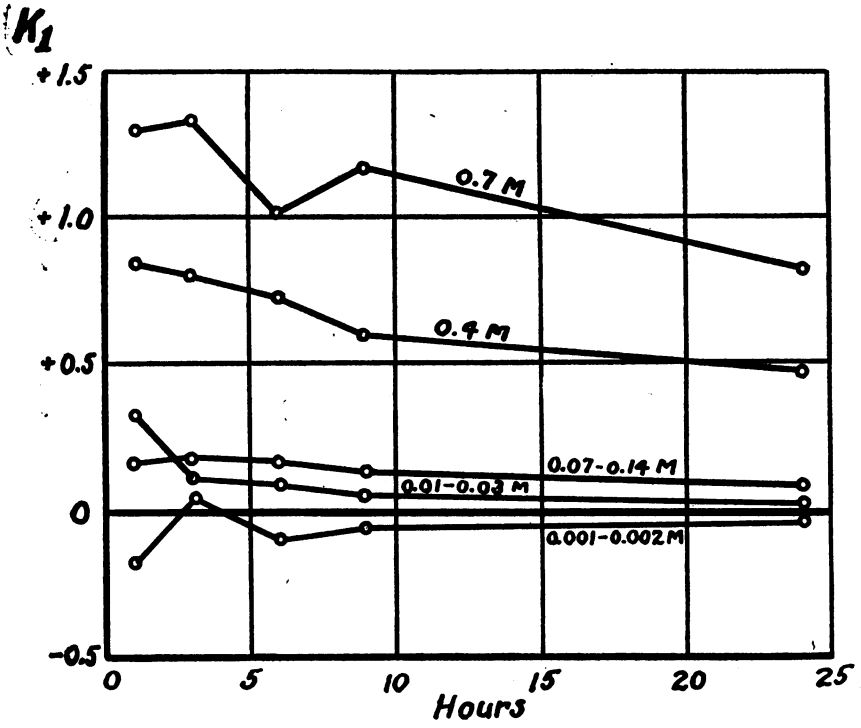

FIG. 2. BACT. COLI IN $CaCl_2$ SOLUTIONS OF VARIOUS MOLARITIES

lower after twenty-four hours than at earlier periods. This fact is brought out much more clearly by the averages based on more abundant data in tables 4 and 5 and figures 2 and 3.

In the $CaCl_2$ solutions (see fig. 2) every one of the five curves shows an almost steady decrease in K_1 with the progress of the experiment. In the NaCl solutions (fig. 3) there is apparently an increase in K_1 during the first few hours with a subsequent decrease. In the 1.4 M concentration the variation is so great as to eliminate almost all semblance to the curve of a monomolecu-

lar reaction. This may in part be due to the fact that while the averages for one, three and six hours are based on 15 to 16 observations, those for nine and twenty-four hours are based only on 5 observations. Within the series of 5 experiments carried through the whole twenty-four hours the same relation is, however, manifest; the average K_1 values for these five experiments

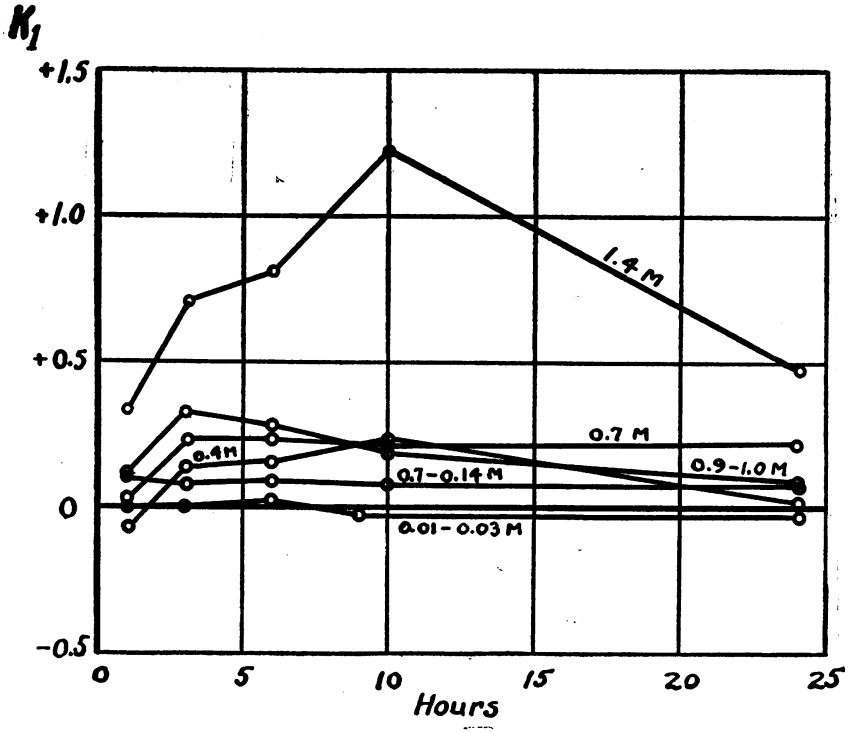

FIG. 3. BACT. COLI IN $NaCl_2$ SOLUTIONS OF VARIOUS MOLARITIES

being as follows: 1 hour, 0.37; 3 hours, 0.57; 6 hours, 0.88; 9 hours, 1.22; 24 hours, 0.48. It appears to us probable that there is a real increase in K_1 values during the first few hours of exposure to NaCl solutions; and there is certainly a progressive decrease in K_1 during the later hours in most of the solutions studied.

V. CRITICISMS OF THE VIEW THAT MORTALITY FOLLOWS A LOGARITHMIC CURVE

Most of the investigators who have studied the dynamics of disinfection,—Krönig and Paul (1897), Ikéda (1897), Madsen and Nyman (1907), Chick (1908) and Cohen (1922),—have been chiefly impressed with the broad general similarity between the curve of viability and that of a monomolecular reaction. Their general conclusions have been essentially as follows:

a. Disinfection is a gradual and not an instantaneous process.

b. Under the prescribed conditions, it generally follows an orderly sequence.

c. The course of the process under these conditions is generally logarithmic and is described by the equation of an unimolecular reaction.

Among these workers there have been differences of opinion on the interpretation of the findings but not on the nature of the findings themselves. Three other investigators on the other hand have specifically questioned the validity of conclusions *b* or *c* (or both) listed above.

First of all, Loeb and Northrop found in their work with Drosophila (fruit fly) that the mortality curve at higher temperatures simulates a probability distribution curve and is not of the nature of a mass action logarithmic curve. As has been pointed out in another connection, these authors in discussing the work of Miss Chick, overlooked the fact

> that Miss Chick's unimolecular reaction curve is not an assumption (as they state) but a deduction, and that there is no inherent reason why there should be an essential similarity between the equation which describes the rate of mortality of bacteria subjected to a large excess of toxic substances and the rate of mortality of fruit flies whose span of life—according to Loeb and Northrop—is probably limited by the production within the animal, in the course of its own metabolism, "of a substance leading to old age and natural death or by the destruction of a substance or substances which normally prevent old age and natural death" (Falk, 1923, pp. 95–96).

It appears, also, that there may be but scant validity in the comparison of mortality data for *Drosophila melanogaster*—an

animal form which shows bisexuality and differentiation between soma and germ plasm, and bacteria—plant forms in which neither sexual nor somatic specilizations are demonstrable.

The second of these critics of the monomolecular reaction hypothesis, Brooks (1918) showed that if erythrocytes are suspended in an indifferent medium and are subjected to a hemolytic agent (radiation from a mercury vapor arc or specific hemolytic antibody) the curve of liberated hemoglobin plotted against sampling time gives not the curve of a mass action reaction but of an asymmetrical sigmoid, i.e., showing an initially slow rate of hemolysis, an increasing rate to a maximum, a gradual retardation and the attainment of an equilibrium. Without going into the details of his arguments, we may indicate that Brooks interprets this finding by postulating the existence of differences in resistance of the cells to the hemolytic agent. Brooks further distinguishes between the resistances of the cells to the hemolytic agent which determine the "course of the process" (i.e., the time curve) and the "fundamental reaction" (i.e., "the physicochemical processes in the protoplasm") and treats each as an independent variable. Hence, he concludes that the time curve cannot directly indicate the nature or order of the fundamental reaction.

Finally, Fulmer and Buchanan (1923) have reported the results of a series of experiments on the toxicity of phenol and phenol and alcohol for yeast cells. Their measurements of toxicity were made by staining periodic aliquot portions taken from a yeast cell suspension exposed to the toxic agent. They say:

> It has been shown that all cells which take up the stain are dead, that is, they no longer can reproduce. However, it was found that the ability to reproduce was lost *before* the cell acquired the ability to stain. In other words, cells which stain are dead, but a dead cell does not of necessity immediately acquire the ability to stain. The two phenomena are closely related, so that cell staining is a satisfactory criterion of toxicity (p. 79).

Of twenty-seven sets of experiments which they cite, they find that in four there was no period with a maximal rate of dying,

in nineteen there was one and that in four there were two well marked maximal periods. They conclude, therefore, that their time curves do not in general prove to be logarithmic; that such resemblances as have been found between such time curves and unimolecular or logarithmic curves are superficial and fortuitous; and that variations in resistance of individual cells and the distribution of such variations must be regarded as of fundamental importance in accounting for rates of death of microörganisms.

The finding of Fulmer and Buchanan that the time curves of their yeast disinfections are not even in a general way logarithmic, and their conclusion that such resemblances as have been found are "superficial and fortuitous" demands critical evaluation.

It is recognized that staining with dilute methylene blue may be—as Fulmer and Buchanan state—an adequate test for the mortality of the cell. It must also be recognized, however, that such a technique may serve as an adequate criterion of completed toxicity, i.e., of lethality, without providing a criterion for measurement of *rate* of lethality, unless it be first demonstrated that the time interval which must elapse after the death of a cell before it displays avidity for the stain is (1) constant for all cells in a test suspension, (2) is invariate with different toxic agents, and (3) is sensibly small by comparison with the time requisite for the effective action of the toxic agent. The reasons for conditions (1) and (2) are self evident. Condition (3) arises out of the consideration that—assuming fulfillment of (1) and (2)—the rate curve would show anomalous inflections, especially at the beginning and end, if the time for development of avidity for the stain is appreciable by comparison with the time necessary for the disinfectant to exert toxicity. Thus, if the first interval of time on a lethality rate curve were equal to the time which elapses between the establishment of the status which is measured by loss of reproductive capacity and the development of avidity for the dye, the rate of dying in this first period—as measured by the staining reaction—would be zero. In the next interval of time it would be something greater than zero,—in the direction of a positive value. In successive intervals the rate would increase and approximate the true rate of dying. As the end of the dis-

infection was approached, the rate would in a similar manner decline from the true value for the rate of mortality. Fulmer and Buchanan picture curves of just the kind which could be deduced from this analysis. (A precise, mathematical treatment of consecutive reactions is presented by Mellor, 1904, pp. 113 ff. and is reconsidered in its biological applications by Osterhout, 1922.) It is also pertinent to note that if initial and final lag deviations from a logarithmic straight line are attributable to the catenary nature of the fundamental reactions involved in the development of dye avidity by yeast cells, there should be no inflection in the direction of terminally accelerated rates. Nor are any clearly marked inflections of this kind observed by Fulmer and Buchanan.

VI. CONCLUSIONS IN REGARD TO THE REGULARITY OF DISINFECTION PROCESSES

It appears from a general review of the subject that while Fulmer and Buchanan find no regularity in the disinfection process as measured by the stainability of yeast cells, all of the other workers with microbic viability report a general correspondence with the logarithmic curve but with more or less distinct deviations toward the beginning and the end of the process. Cohen's curves are, on the whole, the most regular with which we are familiar. Even in his studies, however, many of the curves for mortality at 30° show a lessened rate of reduction toward the end. Chick's figures reveal much greater deviations from the monomolecular straight line, although she is inclined to minimize them in her discussion. Our own studies, as pointed out above, show a marked and definite tendency toward a decreasing rate for K_1 as the process of disinfection continues.

It seems, in general, that while the rate of disinfection follows a logarithmic curve it does not, as a rule, follow it strictly; and the degree of deviation from a monomolecular reaction varies with the particular type of disinfection which is going on. The death of bacteria in water at low temperature follows the monomolecular curve very closely (Cohen); the destruction of bacteria by standard disinfectants (Chick) deviates slightly from it; the mortality

in presence of NaCl and $CaCl_2$ (our own data) shows greater differences; the mortality of Drosophila (Loeb and Northrop) and the hemolysis of red blood cells (Brooks) differ still more; while the stainability of yeast cells after exposure to phenol and alcohol exhibits very little correspondence with the monomolecular law. It appears to us that this is exactly what might be expected, since it seems inherently very improbable that all these various processes destructive of protoplasm should be of the same simple chemical nature.

VII. POSSIBLE EXPLANATIONS OF DEVIATIONS FROM THE LOGARITHMIC CURVE

Where deviations from the logarithmic curve are actually present, we are by no means convinced that the assumption of variability on the part of the reacting cells is a necessary or an altogether satisfactory one. It is significant to recall that "resistance" of a cell to a lytic or to a lethal agent is not an independently *measurable* factor. "Resistance" is known only as a function of time. Thus, a cell is "more resistant" to a lytic or a disinfectant agent if a longer period—and is "less resistant" if a shorter period —of time must elapse before it is lysed or killed. We are not aware that "resistance" is known in any other terms or is measurable in any other units. It represents, therefore, a state whose existence is *assumed* but not *proved.*

In the field of chemical phenomena it is not clear why molecules, atoms or ions which eventually take part in a reaction do not all react to the completion or equilibrium of the reaction in a single unit of time.[8] In the terms of the kinetic theory, it was customary to consider that the interactions were determined by the occurrences of collisions or bombardments and that these were determined by chance (that is, by the operation of a large number of factors), and by the nature and the concentrations of each kind of substance. On this ground, however, it became difficult to account for the fact that the temperature coefficients of most chemical reactions are much greater than postulates of the ki-

[8] Cf. Rice (1924, p. 898, et seq.).

netic theory would warrant. In terms of the quantum theory, as Cohen (1923) has indicated, the explanation for the time curve would lie, presumably, with the chance distribution of variable energy quotas in the molecules of a substance. For the time curve of a disinfection process, Chick (1908) was unable to conceive the existence of such differences in the "resistance" of the cells to the disinfectant as could account for its shape. She considered instead, from analogy with the dynamics of heat coagulation of proteins, which closely resemble the destruction of bacteria by hot water, that "an explanation has been sought in temporary changes in the energy of the molecules, as a consequence of which all molecules do not possess the same sensibility to attack at the same moment. Some such property is therefore attributed to the molecules (or aggregates of molecules) of the constituent protein of the bacteria, whereby at a given moment only a certain proportion is liable to attack; the amount being dependent upon the concentration at the moment of unaltered protein, in other words the number of bacteria surviving in unit volume."[9] It is at once evident that these views are essentially the same as those deduced from general chemical or thermodynamical considerations. From this standpoint while the curve for an array of bacteria of uniform chemical composition should show a monomolecular curve of mortality, a population of variable composition (resistance) might be expected to yield a curve made up of several different logarithmic curves, each component curve corresponding to a different degree of resistance.

Some years ago, we pointed out (Winslow and Falk, 1920) that the postulation of such a specific resistance factor was unnecessary and that it needlessly complicated the concepts of disinfection dynamics. We also pointed out that the results obtained from quantitative experiments can be explained if it be assumed that the death of a cell be due not to the results of a single

[9] Similarly, Arrhenius (1915) had clearly recognized the discrepancy between a postulation of different resistances in a bacterial population and the observation of a logarithmic course in disinfection. He concluded that "the different lifetime of the different bacteria does not, therefore, depend in a sensible degree on their different ability to resist the destructive action of the poison" (pp. 78–79).

reaction but to a chain of reactions (i.e., conceiving the fundamental reaction of Brooks as a catenary reaction). Brooks himself considered that "the fundamental reaction may be either a simple process, or the expression of a complex series of changes whose rate is at all times governed by that of the slowest of the series" (p. 79).

In the field of chemical dynamics it is known that a reaction between reagents may be of one order with one set of concentrations and of another with a second. Thus it appears that the reaction between potassium ferricyanide and potassium iodide works out to be unimolecular with respect to the former component, whereas at other concentrations it is shown to be bimolecular (Mellor, 1904, p. 64; also, Rice, 1924, p. 883). In table 6 we cite the results of our Experiment 47-B on the course of mortality for *Bact. coli* in 0.145 $CaCl_2$ solution, showing the velocity constants (K_1, K_2, K_3, and K_4) which were calculated from the following equations for uni-, bi-, tri-, and quadri-molecular reactions:[10]

$$K_1 = \frac{2.3}{t} \log \frac{a}{a - x} \tag{2}$$

$$K_2 = \frac{1}{t} \frac{x}{a(a - x)} \tag{3}$$

$$K_3 = \frac{1}{t} \frac{x(2a - x)}{2a^2 (a - x)^2} \tag{4}$$

$$K_4 = \frac{1}{t} \frac{1}{3} \left(\frac{1}{(a - x)^3} - \frac{1}{a^3} \right) \tag{5}$$

From column (2) it appears at once that the disinfection did not follow the logarithmic course illustrated in a similar experiment cited in table 1 (the same concentration of $CaCl_2$ was used in the two cases) as is evident from the inconstancy of K_1. The steadily decreasing values of K_1 indicate that the reaction between disinfectant and viable cell was proceeding at a rate higher than that

[10] It should be clearly understood that these equations represent only first approximations for the present purposes. The actual concentrations of the two, three or four reacting molecules cannot be determined and are therefore taken as equal merely for the sake of making these first approximations.

indicated by the so-called logarithmic law. Further, if the first and last two values of K_2 (column 3) be disregarded, the reaction may be considered to have proceeded in accordance with the course of a bimolecular chemical reaction. The approximately constant values of K_3 in column (4) suggest, however, that after the first three or six hours, the reaction proceeded in the manner of a chemical reaction of the *third* order. These observations are confirmed by the progressively increasing values of K_4, listed in the last column, which indicate that the course of the disinfection rate was lower than that postulated from the equation of a *fourth* order reaction. In brief, then, the data of table 6 indicate that when the disinfection of *Bact. coli* by 0.145 M $CaCl_2$ does not fol-

TABLE 6

The viability of Bact. coli in 0.145 M $CaCl_2$ solution at 37°C.

INCUBATION PERIOD—t-HOURS (1)	VELOCITY CONSTANTS			
	K_1 (2)	K_2 ($\times 10^8$) (3)	K_3 ($\times 10^{16}$) (4)	K_4 ($\times 10^{24}$) (5)
1	1.56	2.14	3.39	6.50
3	0.65	1.16	2.59	7.01
6	0.46	1.39	6.48	39.3
9	0.34	1.30	8.23	68.9
12	0.27	1.17	8.50	83.9
24	0.15	0.85	8.92	127.0
125	0.034	0.36	8.00	236.0

low the logarithmic course of a unimolecular reaction in a particular experiment, it may be following the course of a reaction of a higher order through a considerable period of time (i.e., *second* order, 1 to 12 hours; *third* order, 9 to 125 hours in table 6). Further, we may even consider that by the method of best fit of the equations the course of disinfection in experiment 47-B was chiefly that of a third order chemical reaction with a lag in the early stages which approximated the course of a second order reaction.

We come now to the consideration of a phase of disinfection theory which is suggested by our experimental findings and which hitherto appears to have been neglected. More than thirty

years ago, Richet (1892) pointed out that for each of a considerable series of salts whose influences on lactic fermentation of milk whey were studied it is possible to demonstrate that there are concentrations which are:

a. Indifferent
b. Stimulating
c. Inhibitive
d. Toxic

His series included salts of Na, K, Li, Mg, Ca, Sr, Ba, Fe, Pb, Zn, U, Al, Cu, Hg, Au, Pt, Cd, Co, and Ni. His findings, especially with respect to the existence of the stimulating concentrations, are the more noteworthy because of the inclusion in his series of salts with such highly toxic cations as those of cadmium, cobalt, nickel, copper, gold, platinum and mercury. Recently, Dr. Hotchkiss (1923), unaware of Richet's work, came to essentially the same conclusions from more carefully conducted experiments on the stimulating and inhibiting influences of a series of salts upon the growth of *Bact. coli.*[11] She was able to find for 15 of 23 chlorides studied a concentration which stimulated growth—as indicated by the production of a turbidity greater than that in the control cultures to which no salt had been added. The stimulating salts in her series included not only chlorides of K, Na, NH_3, Li, Sr, Mg, Ca and Ba, but also of such toxic metals as Ti, Sn, Ni, Pb, Co and Hg; and she was persuaded that stimulating concentrations for the other eight salts could have been established by more exhaustive study. Madsen and his associates have recenty reported[12] similar stimulating effects of minute amounts of Cu and Mn upon the growth of the tubercle bacillus.

Accepting these conclusions as indicative of a general biological principle,[13] it is of considerable interest to reëxamine from this point of view the course of mortality of *Bact coli* in the presence of various concentrations of calcium chloride. In a more detailed report (Winslow and Falk, 1923a) and in tables 4 and 5

[11] The same strain as that used in our studies.
[12] Meeting, Laboratory Section, A.P.H.A., Detroit, November, 1924.
[13] Further evidence is cited at length in a review by Falk (1923).

we have indicated that either sodium chloride or calcium chloride, in suitable concentrations, may be stimulating as well as toxic to *Bact. coli* suspended in non-nutrient menstrua. If from our data average velocity constants of mortality (K_1) are calculated (assuming, tentatively, the applicability of the unimolecular reaction equation), we obtain (see last columns of tables 4 and 5) a series of values which pass from negative to positive values and which give a reasonably smooth curve for both salts (fig. 4). We are not aware that any chemical theory of disinfection which has previously been presented is in harmony with the fact that these curves are approximately continuous, above and below the

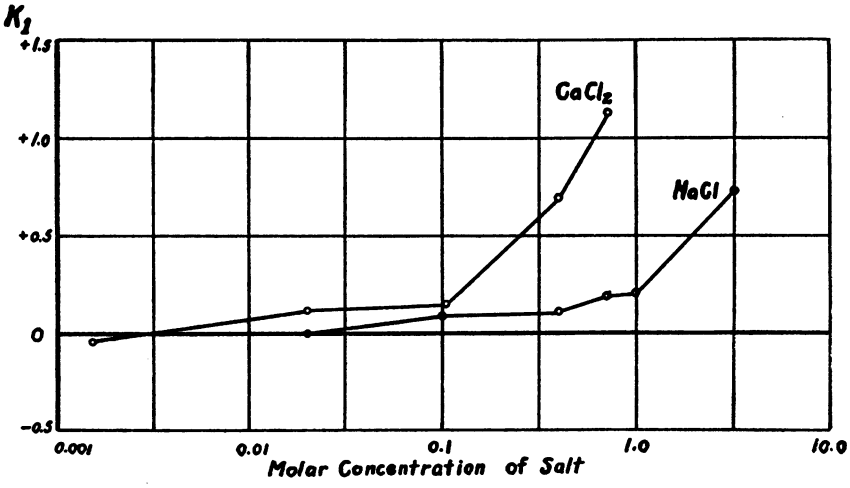

FIG. 4. VIABILITY OF BACT. COLI IN SODIUM AND CALCIUM CHLORIDE SOLUTIONS

base line. We can scarcely assume that the velocity of a lethal reaction is inhibited by certain concentrations and accelerated by other concentrations of one of the reacting substances. We are not aware that chemical dynamics of homogeneous systems can be harmonized with such conditions. The curve, presumably with an origin when both x and y are 0,[14] suggests smooth and continuous gradations for values of K_1 from 0, through negative to progressively increasing positive values. It therefore implies

[14] Because in pure water (x = 0) the bacteria maintain themselves in practically undiminished numbers for twenty-four hours.

that there is no sharp discontinuity between such effects of a reagent as result in acceleration of the reproductive rate to such as markedly accelerate the rate of mortality. It might be assumed that the effect of a toxic agent on *Bact. coli* becomes manifest because mortality is the consequence of a stimulation of reproduction to a degree which is incompatible with maintenance of viability (i.e., if the fundamental reactions of stimulation and toxicity are identical); but such a view would be extremely far-fetched except perhaps in connection with the influence of temperature. If it be considered that this assumption is unwarranted, it becomes necessary to consider that the action of a toxic substance upon a bacterial cell is not explicable in terms of a simple type of chemical reaction.[15] The course of a disinfection process is the algebraic resultant of stimulating effects of the toxic agent upon reproduction and of truly toxic effects measured by accelerations of mortality rates. Considered in this light, the time curve of a mortality process may lie at any position between the extremes, i.e., the time curves of the stimulating and of the intoxicating chemical reactions. Variations in the shape of this resultant curve are therefore to be expected with the same as well as with different concentrations of toxic agent.

Certain data which we have cited from our experiments indicate a general concordance between the courses of disinfection by calcium chloride and of bi- or tri-molecular reactions. It is not our aim, however, to contend that the course of a disinfection process necessarily or even generally simulates that of a bi- or tri-molecular chemical reaction. We wish merely to emphasize

[15] By making additional assumptions, especially by assuming reaction in a heterogeneous system in which the number of phases is variable, it may be possible to deduce the equation of such a curve. If the disinfection of a bacterial suspension by $CaCl_2$ is treated as a reaction in a heterogeneous instead of a homogeneous system and if the course of the process is found in accord with the logarithmic law, the significance of the finding becomes radically different from that which has been generally placed upon it. It may mean that in the changes which lead to loss of viability a number of reactions (physical or chemical or both) are involved and that the slowest (and determining) one may be of the nature of a diffusion reaction or of any one of many kinds of multi- or inter-phase reactions (vide Taylor, 1924). The precise treatment of these conditions cannot be conducted with our data.

that although disinfection processes generally give the logarithmic curve and are approximately described by the equation of a unimolecular reaction, under certain conditions—particularly in the presence of certain mild disinfectants—the course of the process may more closely approximate that of a multimolecular reaction.[16]

In the present state of knowledge, the time curve of a disinfection process remains as a valuable tool in the measurement of toxicity but not as a guide to the chemistry or physics of the fundamental reaction between cell or cell constituents and toxic agent. Conclusions to the effect that the time curve is always, necessarily, logarithmic appear not to be in accord with experimental findings. The exceptions from the logarithmic course which have been reported here lead to the conclusion that the mechanism of a disinfection process is probably highly complex. On the other hand the observed variation can be quite plausibly explained on chemical grounds without the introduction of the assumption of biological variability.

VIII. GENERAL CONCLUSIONS

1. The mortality of *Bact. coli* in solutions of NaCl and $CaCl_2$ and in distilled water of varying pH follows a generally logarithmic course and may be roughly described by the equation of a unimolecular chemical reaction, as has been shown to be the case for other processes of disinfection.

2. This relationship is not, however, a close or an exact one. Like most other students of this problem, we find, on close analysis, more or less marked deviations from the logarithmic curve. With NaCl the rate of reaction seems to increase at first. With both salts, and with acid and alkali, it gradually decreases as the experiment proceeds through its later stages.

3. Where disinfection does not follow a logarithmic course and is not to be described by the unimolecular equation, the course of the process may sometimes be described by the equation of a

[16] It is significant to recall that from a recalculation of Chick's data on phenol disinfection, Watson (1909) concluded that the reaction was of at least the seventh order.

bi-, tri-, or higher multimolecular reaction. Such an explanation may render unnecessary the assumption of variability in biological resistance to account for deviation from a logarithmic mortality curve.

4. When used in sufficiently low concentration, NaCl and $CaCl_2$ may be not only without toxic properties for *Bact. coli*, but may even stimulate the organisms to increased growth and reproduction. This observation is in harmony with the conclusions of Richet, Hotchkiss, Madsen and others and appears to be an illustration of a general biological principle. For a series of concentrations of $CaCl_2$ acting upon suspensions of *Bact. coli* a curve may be plotted which shows continuous gradations between intoxicating and stimulating effects.

5. From an analysis of the time curves of disinfection processes it appears that the reaction between disinfectant and cell or cell constituents is probably highly complex; and that while the logarithmic curve best expresses its general rate, the correspondence is not, and cannot be expected to be, always a close one.

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