THE SIGNIFICANCE OF THE VARIATION IN SHAPE OF TIME-SURVIVOR CURVES

By E. R. WITHELL, B.Sc., B.Pharm., Ph.C., A.I.C.

From the Department of Pharmacy and Biology, Central Technical College, Birmingham

(With Figs. 1–27 in the Text, and Figs. 28–46 in Appendix I)

CONTENTS

I. Introduction

Since the work of Krönig & Paul (1897), Madsen & Nyman (1907), Ikéda (1897) and Chick (1908*a*, 1910), on disinfectant action, a large number of observations have been made to determine the shape of time-survivor curves. Many observers have based their theories of disinfection on the shape of the time-survivor curves they have obtained. When the logarithm of the survivors plotted against time gave a straight line, mechanistic theories have been generally applied (Chick, 1908*a*, 1910; Rahn, 1929, 1930; Lee & Gilbert, 1918).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

When, on the other hand, the logarithm of the survivors (or the percentage survivors) gave sigmoid curves or curves with a 'lag' or induction period, then theories involving the distribution of resistances in a bacterial population were advanced (Brooks, 1918; Smith-Henderson, 1921, 1923). Some of the results and theories of different observers are collected in Table 1. This shows the shape of time-survivor curve obtained with both vegetative organisms and spores, together with the results when more highly organized individuals were used.

Table 1

Author	Bactericide	Organism	Remarks
Krönig & Paul (1897)	Mercuric chloride and hot water	Anthrax spores	Exponential rates
Madsen & Nyman (1907)	Mercuric chloride and phenol	Anthrax spores	Observed exponential rates themselves and showed Krönig & Paul's work the same
Chick (1908*a*)	Mercuric chloride, silver nitrate, phenol and emulsified disinfectant 'A'	Anthrax spores and *Bact. paratyphosum*	Anthrax spores—exponential, *Bact. paratyphosum*—*k* fell throughout reaction
	Phenol	4th generation of young cultures of *Bact. paratyphosum*	*k* fell throughout, but to a much smaller degree than above
Chick (1910)	Phenol	Anthrax spores	Exponential curves
		Bact. paratyphosum (4th generation)	Exponential curves
		Bact. coli, *Staph. aureus*	Short lag period then exponential rate
		Bact. typhosum	Exponential curves
	Hot water	*Bact. typhosum*	Exponential curves
		Bact. coli	Exponential curves
		P. pestis	Exponential curves
		Bact. paratyphosum	Short period when reaction quicker than theoretical, then, exponential
		Staph. aureus, *Staph. albus*	Exponential, lag phase, and as *Bact. paratyphosum* above
Chick (1912)	—	—	Résumé
Chick (1913)	Normal rabbit serum	*Bact. coli*	Exponential
	Normal goat serum	*Bact. typhosum*	Exponential
Chick & Martin (1908)	Emulsified tar acids	*Subtilis* spores	Exponential rate
Clark & Gage (see Chick, 1910)	Sunlight	*Bact. coli*	Exponential rate
Paul (1909)	Drying	*Staphylococcus*	Exponential rate
Paul, Birstein & Reuss (1910)	Drying and acid	*Staphylococcus*	Exponential and sigmoid
Paul & Prall (1907)	Drying	*Staphylococcus*	Exponential rate
Cohen (1922)	Weak acid	*Bact. coli*, *Bact. typhosum*	Exponential rate
Harvey (1909)	Hydrochloric acid	*Chlamydomonas*	Exponential rate
Burbury (1928)	Heat	Virus	Exponential rate
Eijkmann (1912)	Phenol and heat	Yeast	Sigmoid curves
Eijkmann (1908, 1909)	Phenol	*Bact. coli*	Sigmoid, logarithmic rate in middle for short period
	Hot water	*Bact. coli*	
Smith-Henderson (1921)	Phenol	*Botrytis* spores	Sigmoid, and as conc. of phenol increased—exponential
Smith-Henderson (1923)	Heat	*Botrytis* spores	Sigmoid curves
Peters (1920)	Mercuric chloride	*Colpidium*	Sigmoid curves
Boycott (1920)	Hot water	Tadpoles	Sigmoid curves
Falk & Winslow (1926)	Calcium chloride solution	*Bact. coli*	Sigmoid and exponential
Fulmer & Buchanan (1923)	Phenol, and phenol in alcohol	Yeast	Irregular—(no counts)
Phelps (1911)	—	—	Résumé
Reichenbach (1911)	Hot water	*Bact. paratyphosum*	Both logarithmic, sigmoid, 'and concave', curves
Arrhenius & Madsen (1903)	—	Haemolysis	Exponential rate
Henri (1905)	—	Haemolysis	Exponential rate
Von Liebermann & Von Fenyvessey (1912)	—	Haemolysis	Exponential rate
Wyckoff (1932)	Ultra-violet light	'Colon bacilli'	Exponential rate
Wyckoff & Rivers (1930)	Cathode rays	'Colon bacilli'	Exponential rate
Wyckoff (1930)	X-rays	*E. coli*, *Bact. typhi-murium*	Exponential rate

Table 1 (*continued*)

Author	Bactericide	Organism	Remarks
Hollaender & Duggar (1936)	Ultra-violet light	Plant virus	Exponential rate
Heřčík (1936)	Ultra-violet light	*B. megatherium*, both spores and vegetative	Exponential rate
Coblentz & Fulton (1924)	Ultra-violet light	—	Sigmoid curves
Baker & Nanavutty (1929)	Ultra-violet light	*Bact. coli*	Sigmoid curves
Gates (1929*a*, *b*, 1930)	Ultra-violet light	*Staph. aureus*	Slightly sigmoid, flat part in the middle
Hollaender & Claus (1936)	Ultra-violet light	*E. coli*	Slightly sigmoid, flat part in the middle
Lea & Haines (1940)	Ultra-violet light	*Bact. coli*, *Ch. prodigiosum*, *B. mesentericus*	Exponential rate
Lea, Haines & Coulson (1937)	α and β rays	*Bact. coli*, *B. mesentericus*	Exponential rate
Lea, Haines & Bretscher (1941)	X-rays, neutrons and radioactive radiations	*Bact. coli*, *B. mesentericus*	Exponential rate
Axelrod, Aebersold & Spear (see Lea, Haines & Bretscher, 1941)	Neutrons	*B. mesentericus* spores	Exponential rate
Habermann & Ellsworth (1940)	X-rays	*Staph. aureus*	Exponential rate
Salmonsen & Dreyer (1907)	—	Haemolysis	Exponential rate, but lag period at beginning
Dreyer & Hanssen (1907)	Ultra-violet rays and radium	Haemolysis	Exponential rate, but lag period at beginning
Darwin & Blackmann (1909)	—	Death of seeds	Exponential rate
Arrhenius (1915)	—	—	Résumé
Geppert (1889)	—	—	Résumé; rate of disinfection due to individual variation
Mioni (1905)	—	—	Criticized Henri's work on the ground that he neglected individual variation
Dienes (1911)	—	Haemolysis	Suggested degrees of resistance were distributed in accordance with Quetelet's Law
Brooks (1918)	Ultra-violet light	Haemolysis	Spirited défence of the variation of resistances argument
Hewlett (1909)	Mercuric chloride	Mustard seeds	Exponential rate, but disagrees with the application of the monomolecular law to process
Loeb & Northrop (1917)	—	—	Résumé
Ballner (1902)	Saturated steam	Anthrax spores	Exponential rate; Q for saturated steam
Porodko (1926, 1927)	Hot water	Wheat grains	Exponential rate and decrease in k as reaction proceeds
Woerz (see Rahn, 1930)	Hot water	*Torula cremoris*	Exponential for higher temperatures
Holweck (1929)	X-rays	*Staph. aureus*	Logarithmic rate due to clumping
		Ps. pyocyanea	Logarithmic rate with 4 A° not so with 8 A°.
Myers (see Rahn, 1930)	Alkali	'Bacillus 25'	All experiments but one showed an increasing death-rate
Sattler (1928)	Heat	'Pink yeast'	Some experiments showed decreasing rate
Groves (1917)	Heat	Dry wheat seeds	Sigmoid curves
Lloyd (1920)	Lead arsenate	Tomato-moth larvae	Sigmoid curves
Falk (1923)	—	—	Résumé, when material homogeneous—logarithmic, when not—sigmoid
Tamman (1895)	Heat	Enzymes	Logarithmic curves
Heller (1941)	Drying	*Str. pyogenes*, *Bact. coli*	Logarithmic curves
Withell (1938)	Phenol	*Bact. coli*	0·5 % phenol—lag phase, then exponential; higher strengths exponential
Lee & Gilbert (1918)	Ultra-violet light	*Bact. coli*	Exponential rate
Oothuisen (1935)	Moist heat	The confused flour beetle	Sigmoid curves

From this table it can be seen that observers have obtained different results even when they have used the same organisms and lethal agents. Difficulty has been experienced in assessing the value and accuracy of the methods employed and the results obtained, in some cases, because observers have given no counts. It is doubtful whether the present-day counting methods are accurate to more than $\pm 5\%$, and in the absence of experimental evidence as to a particular observer's accuracy of counting organisms, variations greatly in excess of this figure can be suspected. If this is so, then the correct inter-

pretation of the type of time-survivor curve by quoting mathematical formulae derived from single experiments is almost impossible. This is because variations of even 5% in the counts mean that fundamentally different formulae can be applied to the same set of figures. Clark (1933) showed this well when he made calculations to indicate how many different formulae could be used to interpret the same set of figures if a variation of $\pm 5\%$ was allowed. He found that the following relations between concentration (x) and action (y) could be fitted to the same set of figures:

(1) $kx = \frac{y}{100-y} (k=1)$.

(2) $ky = \log(ax+1)$ ($k = 0{\cdot}0166$, $a = 5{\cdot}3$).

(3) $kx^n = y$ ($k = 49$, $n = 0{\cdot}5$).

From similar data in bacteriology it is obvious that bacterial counts can also be interpreted in several different ways.

In many of the papers listed in Table 1 the conclusions as to the course of the reaction mechanism have been derived from a few experiments. Often the statement is made that other experiments yield 'similar' results. How far this latter statement is true and how far the other experiments were 'similar' can never be ascertained from the published works. In my experience with vegetative organisms and phenolic bactericides a considerable variation occurs in the type of time survivor curve when experiments are made as nearly identical as possible. The object of the second part of this paper is to show the variation that does occur when strictly comparable experiments are performed. In the third part the results are considered as a whole and an analysis of the curves made with the idea of constructing an average time-survivor curve. In the fourth part it is shown that this variation of types of curve, which some would consider fundamental, does not occur if the curves are plotted on the assumption that the logarithms of the survivor times are normally distributed. In that case all the variations observed can be explained by a variation in the way the resistances are distributed.

None of the experiments has been omitted. The counts from which the graphs are drawn, and on which the arguments are based, will be found in Appendix II (p. 174).

II. VARIATIONS IN TIME-SURVIVOR CURVES FROM SINGLE EXPERIMENTS

(a) *Technique employed*

The roll-tube capillary dropping pipette method was used throughout this work. The technique has been previously described (Withell, 1938; Wilson, 1922). Withell (1938) made a statistical inquiry into the accuracy of the method when applied to *Bact. coli* and arrived at the conclusion that if the suspension was diluted serially three times and the mean roll-tube count was 200 the standard error was approximately 5%. The technique in the cases

quoted included three serial dilutions of the suspension. If fewer dilutions are used the standard error is reduced. Jennison & Wadsworth (1940) have recently applied somewhat the same method to the problem of the accuracy of viable counts and have arrived at similar figures.

Throughout the work the dilution of the suspension in the bactericide was of such an order that all bacteriostatic effects were nullified. The phenolic bactericides used in this work have a very high concentration exponent (Watson, 1908) and were easily diluted out. The smallest dilution that was used with success for phenol 0·5% and para-chlor-meta-cresol 0·05% corresponded to a dilution of approximately one to two thousand, and at this concentration (phenol 0·00025% and para-chlor-meta-cresol 0·000025%) no bacteriostatic effect of any sort was observed.

The organisms used

One of the most important attributes of any organism used in experiments involving counting is that of forming a suspension which is free from clumps, and which will remain free from clumps when inoculated into the bactericide solution. The importance of this factor has been emphasized by Glynn, Powell, Rees & Cox (1913), Knaysi (1935), Ziegler & Halvorsen (1935) and Jennison (1937). A strain of *Staphylococcus* from the Lister Institute proved unsuitable from this point of view, but a Gram-positive *Micrococcus* which was isolated from the atmosphere was used successfully for counting experiments with no evidence of clumping when microscopic observations or viable counts were made. A strain of *Bact. coli* (Type I), recently isolated, and kindly supplied by Prof. G. S. Wilson, was also found very suitable. These were the two organisms used throughout in the work quoted in this paper, and in every case a 24 hr. culture was used.

The medium

For some of this work a 6 hr. pancreatic digest of lean bullock buttock beef was used, but when supplies of this were exhausted 'C.C.Y.' medium (Gladstone & Fildes, 1940) was used with success. There was no significant difference in the counts of a suspension when dilutions were seeded into pancreatic digest medium and C.C.Y. medium.

The diluent

For the *Micrococcus*, Ringer's solution (Wilson, 1922), previously filtered through a Doulton filter and filled aseptically into sterile screw-cap bottles, was found satisfactory. When this solution was autoclaved in screw-cap bottles, fitted with rubber washers, it was found to kill the *Micrococcus* rapidly. This effect had been noticed by Withell (1938) in experiments with *Bact. coli*. Davis (1940) could not repeat the observation and indeed found that filtered Ringer solution was more toxic than autoclaved Ringer. Davis filtered but 50 c.c. of the Ringer solution, and it is probable that adsorption occurred from this small quantity and disturbed the balance of the solution

(he himself suggests the possibility). Fig. 1 shows the same effect as observed by Withell (1938), but this time using the *Micrococcus*. It will be seen that filtered Ringer solution was markedly superior to autoclaved Ringer. The effect in this instance was finally ascribed to the rubber washers which hermetically sealed the screw-cap bottle. Ringer solution autoclaved in the same bottles, but plugged with wool, showed no bactericidal activity over the period observed.

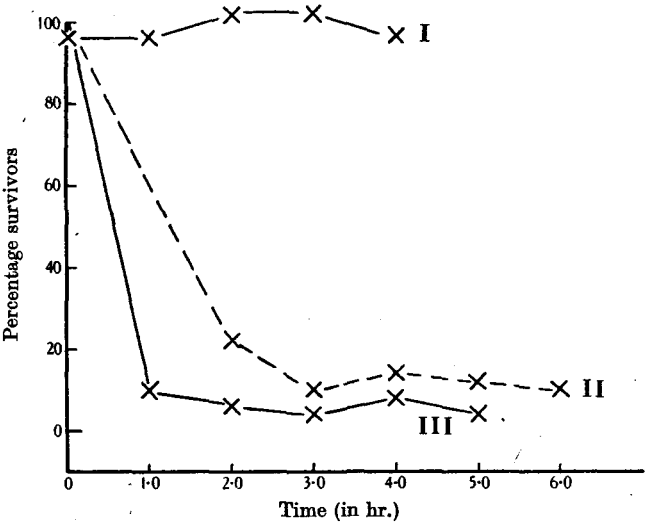


Fig. 1. Death-rate of a *Micrococcus* in filtered, autoclaved and steamed Ringer solution. I, Ringer's solution filtered (Exp. 215). The counts are calculated as deviation per cent. from the mean. II, Ringer's solution autoclaved 30 min. at 10 lb. pressure, in contact with rubber washers (Exp. 217). III, Ringer's solution steamed 60 min., in contact with rubber washers (Exp. 217). The counts from which the figure has been drawn will be found in Appendix II.

For *Bact. coli* autoclaved tap water was found suitable. It is noteworthy that no toxic effects were observed with this organism even though the tap water was autoclaved in screw-cap bottles fitted with rubber disks. These were disks which had previously been autoclaved, and there is reason to believe that freshly vulcanized rubber liberates substances which are bactericidal to some organisms.

The water

The water for every solution used in this work was prepared in the following way: On the day before each experiment tap water was first distilled in an alembic still with a tinned copper receiver and then immediately redistilled in a Pyrex all-glass still, fitted with baffle plates, and collected in sterile 2 l. Pyrex flasks. The flasks were then plugged with cotton wool, the wool plugs covered with tin foil and the flasks stocked in this way until the next day. Solutions of bactericides were made up immediately before each experiment. By this method solutions of constant hydrogen-ion concentration

were obtained throughout the work. It is important that this should be so, for small changes in *p*H have been found to modify the activity of bactericides, an effect noted by many observers (Chick, 1908*a*; Norton & Hsu, 1916; Friedenthal, 1919; Bigelow & Esty, 1920; Bittenbender, Digering & Tetrault, 1939; Goedrich, 1938; Joachimoglu, 1923; Vermast, 1921; Waterman & Kuiper, 1924; Kuroda, 1926; and Keysser & Orstein, 1926).

The bactericides

Phenol of A.R. standard and para-chlor-meta-cresol in fine white crystals, kindly supplied by Messrs Monsanto, were used throughout the work. In this paper the experiments recorded were all made with phenol 0·5%, or para-chlor-meta-cresol 0·05%. For every experiment the solutions were made up in 1 l. quantities to avoid any error associated with the weighing of small quantities. It was necessary to use hot water to make the solutions of para-chlor-meta-cresol, as the rate of solution in cold water is very slow, but closed containers were used in the preparation of these solutions as recommended by Rapps (1933).

Technique of a complete experiment

Throughout this work the two bactericides at 20° C. were inoculated with organisms washed off from the same 24 hr. culture. Temperature was controlled in a thermostatic bath at $\pm 0{\cdot}1°$ C. Each experiment consisted of following the death-rate of the organisms in the two solutions, which were treated in exactly the same way. The sample used to determine the first count (which is indicated throughout at time = 0) was in every case removed within 30 sec. of the inoculation of the bactericide. Thereafter at regular intervals samples were removed, diluted and seeded into molten agar medium at 42° C. The tubes were immediately rolled under the tap, inverted and incubated at 37° C. for 3 days.

The technique of the roll-tube dropping pipette method has been given in greater detail elsewhere (Wilson, 1922; Withell, 1938).

(*b*) *The results from single experiments*

(i) *Bactericidal action of phenol* 0·5% *on* Micrococcus *spp.*

A series of fifteen experiments was performed using phenol 0·5% and the aerial *Micrococcus*. Although each experiment was carried out with the greatest possible care, considerable variation was observed in the shape of the time-survivor curves. At the beginning of the series the results seemed to show that these organisms died in accordance with 'some rule analogous to the Mass Law, so that if the disinfectant is present in large excess, disinfectant rate at any moment is proportional to the concentration of bacteria' (Chick, 1910). This is illustrated in Fig. 2, where two experiments are shown which agree closely with the exponential hypothesis (Chick, 1908*a*, 1910; Madsen & Nyman, 1907; Paul, 1909; Cohen, 1922; etc., see Table 1). Little difference

was noticed when the concentration of bacteria was varied. Thus the initial concentration of organisms in Exp. 224 was approximately 100,000 per ml., while in Exp. 230 the initial concentration was approximately 20,000,000 per ml. (see Fig. 2).

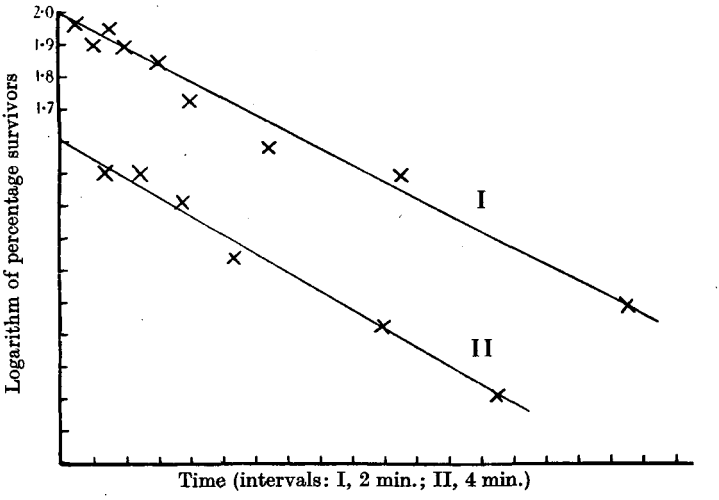


Fig. 2. Death-rate of a *Micrococcus* in phenol 0·5%. Temp. 20° C. I, Exp. 224; II, Exp. 230. All counts given in Appendix. The point signifying 100% viable on the zero time axis, has been shifted down in II to permit the representation of more than one experiment on the graph.

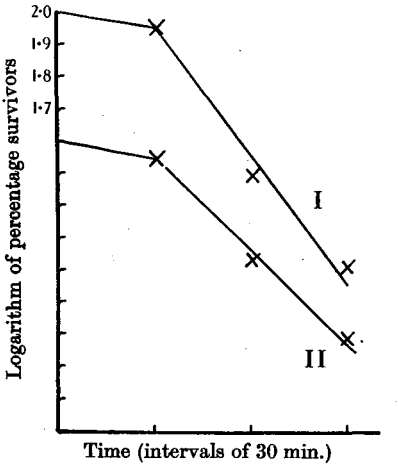


Fig. 3. Death-rate of a *Micrococcus* in phenol 0·5%. Temp. 20° C. I, Exp. 241; II, Exp. 242. All counts given in Appendix. The point signifying 100% viable on the zero time axis has been shifted down in II, to permit the representation of more than one experiment on the graph.

As the work progressed it was noticed that this type of curve was not the only type observed. For example, a definite lag phase sometimes occurred before the reaction settled down and proceeded approximately in an exponential fashion. Fig. 3 shows the type of curve obtained.

(ii) *Bactericidal action of para-chlor-meta-cresol* 0·05% *on* Micrococcus *spp.*

Thirteen separate and comparative experiments were carried out using para-chlor-meta-cresol 0·05% and the aerial *Micrococcus*. In general the same type of variation occurred as with phenol 0·5%. Figs. 4 and 5 show the variation of the time-survivor curves. (The counts are given in Appendix II.)

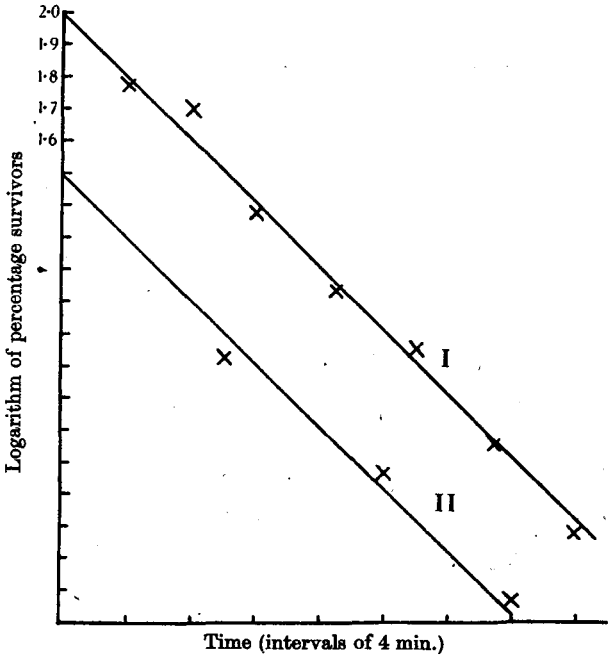


Fig. 4. Death-rate of a *Micrococcus* in para-chlor-meta-cresol, 0·05% solution. Temp. 20° C. I, Exp. 224; II, Exp. 229. All counts given in Appendix.

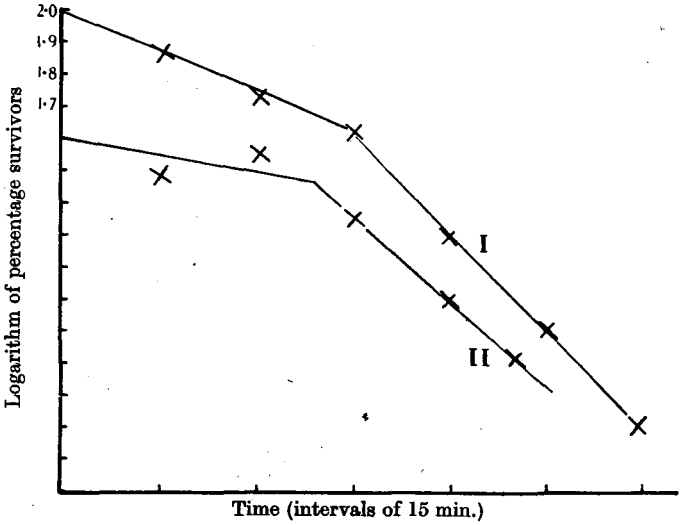


Fig. 5. Death-rate of a *Micrococcus* in para-chlor-meta-cresol 0·05% solution. Temp. 20° C. I, Exp. 241; II, Exp. 242. All counts given in Appendix.

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

(iii) *Bactericidal action of phenol* 0·5% *and para-chlor-meta-cresol* 0·05% *on* Bact. coli

The two bactericides are considered together in this part of the work because time-survivor curves of similar type were observed in both cases. Figs. 6 and 7 show the general shape of curve obtained. These curves did not vary in type but did vary in detail. Curves of this type have been interpreted

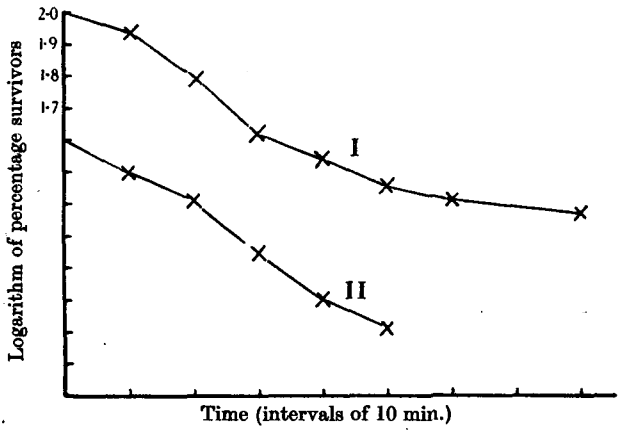


Fig. 6. Death-rate of *Bact. coli* in phenol solution, 0·5%. Temp. 20° C. I, Exp. 267; II, Exp. 268. All counts given in Appendix.

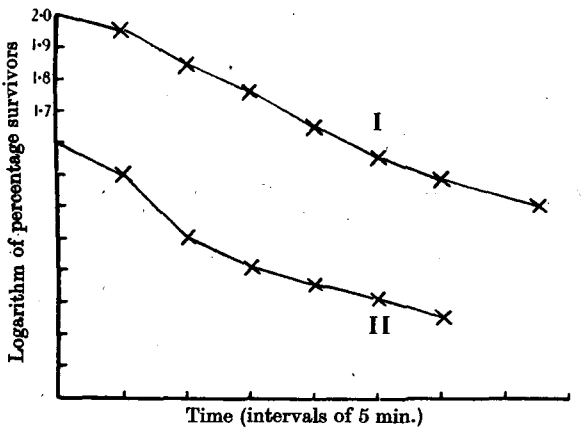


Fig. 7. Death-rate of *Bact. coli* in para-chlor-meta-cresol solution, 0·05%. Temp. 20° C. I, Exp. 267; II, Exp. 268. All counts given in Appendix.

(*a*) by drawing a straight line through the points of the lag period and another through the remainder of the points, or (*b*) by fitting a curve to the points when in general a sigmoid curve of very gradual slope is obtained.

(*c*) *Discussion*

It is well known that a variation of the type of time-survivor curve does occur when cultures of different ages are inoculated into the same strength bactericide (see Table 1). Similarly when bactericides of different strength

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

are employed a progressive variation of the type of curve from that of sigmoid to that of an exponential type is observed (Smith-Henderson, 1921; Clark, 1933; Withell, 1938; etc.; see Table 1). In this paper another type of variation has been noted. The different types obtained cannot be explained as being derived from one type of curve by the errors of the experiment, for the variation is much greater than can be accounted for, by errors of counting (see Withell (1938) for errors of dilution and the sampling error).

Various observers (Rahn, 1930; Chick, 1908*a*, 1910; Phelps, 1911; Falk & Winslow, 1926; see Table 1) have laid great emphasis on the shape of the time-survivor curves, paying attention to whether it has a lag or induction period, or whether it proceeds smoothly in an exponential fashion. Many theories have been built up on the basis of an inflexible type of time-survivor curve. For example, Phelps (1911) says: 'The rate of dying, whether under the influence of heat, cold, or chemical poison, is unfailingly found to follow the logarithmic curve of the velocity law, if the temperature be constant.' This statement is hardly substantiated by the data recorded in Table 1. Some have gone so far as to calculate how many molecules of bactericide react with one organism from the shape of the time-survivor curve (Rahn, 1929, 1930). In the series of experiments quoted here a variation of curve has been noted which, according to Rahn, would mean that at one time one type of reaction was proceeding between organism and bactericide and at another time a fundamentally different reaction was in progress.

It seems then that even in experiments which are carried out in a manner as nearly identical as possible, variation of type of time-survivor curve does occur. The diagrams, drawn from the counts in Appendix II, have all been constructed by plotting logarithm of percentage survivors against time. This has been done to show irregularities from the exponential curve. If diagrams are made from the same counts, by plotting percentage survivors against time, they are not very informative as, in all cases, the bulk of the organisms die in the first few units of time. In the next part an attempt is made to calculate the mean response of the organisms to the bactericide.

(*d*) *Summary*

A number of types of response to two phenolic bactericides have been observed when experiments are carried out in as nearly an identical a manner as possible using a *Micrococcus* and *Bact. coli*. They include time-survivor curves (*a*) giving a straight line when the logarithm of survivors is plotted against time, and (*b*) with a lag phase before the straight line when logarithm of survivors is plotted against time.

III. MEAN TIME-SURVIVOR CURVES

(a) *Method of calculation*

In Part II of this work it has been shown that some variation occurs in the response of organisms to phenolic bactericides in comparative experiments. The variations include at least two types of time-survivor curve which had previously been distinguished (Table 1, p. 125).

Enough evidence was collected at first to prove that the time-survivor curve was (1) of exponential type, i.e. that the number of organisms dying during any period was always a definite proportion of those alive at the start of that period. As more experiments were made it was obvious that this was not the invariable response. Other time-survivor curves were obtained as often as the exponential curves and included those which could be interpreted (2) as a lag phase followed by an exponential curve.

If the successive points (determined by the counts) on any such curve are joined by straight lines, then the reaction rate for any particular stage can be calculated. When this is done by using the formula

$$k = \frac{1}{t_1 - t_2} \log_{10} \frac{N}{n},$$

where k is the reaction rate, $t_1 - t_2$ is the time interval over which k is calculated, N is the number of viable organisms at the start of that interval, and n is the number of viable organisms at the end of that interval. The k that is calculated shows the slope of the time-survivor curve between successive points of the curve.

It is important that N and n should be the numbers of viable organisms at the beginning and end of each separate period. In some of the published work the same initial value of N is used for calculating k during each successive period $t_1 - t_2$, $t_2 - t_3$, etc.

If this is done the value of k for each separate period is biased by including the reaction velocity for the previous part of the curve. If the logarithms of the counts lie directly on a straight line this is immaterial, since the value of k is the same throughout. But if the counts do not lie on a straight line, then any method of calculation adopting the initial number of viable organisms as N throughout the calculations, will obscure any variation in the value of k during successive stages of the reaction.

The following is an example (Table 2) of how the calculation has been made and how the method of calculation can be altered to give more information about the way k (i.e. the slope of the curve) varies with time (Table 3).

It will be seen that the first set of figures are more nearly equal than the second set, and indeed this must be so, for they are all derived from a common point of origin. Further, the first set of figures give very little information about the real course of the reaction, they record the slope of the line which

Table 2. *Disinfection of* Bact. coli commune *with hot water* (*Chick*, 1910)

Temp. 49° C.

Time in min.	Survivors	k
0·75 = 0	331	
1·50	317	$k_1 = \frac{1}{0{\cdot}75} \log_{10} \frac{331}{317} = 0{\cdot}025$
2·50	275	$k_2 = \frac{1}{1{\cdot}75} \log_{10} \frac{331}{275} = 0{\cdot}046$
4·00	249·5	$k_3 = \frac{1}{3{\cdot}25} \log_{10} \frac{331}{249{\cdot}5} = 0{\cdot}038$
6·00	175	$k_4 = \frac{1}{5{\cdot}25} \log_{10} \frac{331}{175} = 0{\cdot}053$
9·00	165	$k_5 = \frac{1}{8{\cdot}25} \log_{10} \frac{331}{165} = 0{\cdot}037$
14·00	71	$k_6 = \frac{1}{13{\cdot}25} \log_{10} \frac{331}{71} = 0{\cdot}050$
20·00	39·5	$k_7 = \frac{1}{19{\cdot}25} \log_{10} \frac{331}{39{\cdot}5} = 0{\cdot}048$

Table 3. *Data exactly as in Table* 2 *but* N *in the formula* $k = \frac{1}{t_1 - t_2} \log_{10} \frac{N}{n}$ *is always the number of survivors at the beginning of the interval over which* k *is calculated*

k	k
$k_1 = \frac{1}{0{\cdot}75} \log_{10} \frac{331}{317} = 0{\cdot}025$	$k_5 = \frac{1}{3} \log_{10} \frac{175}{165} = 0{\cdot}009$
$k_2 = \frac{1}{1{\cdot}0} \log_{10} \frac{317}{275} = 0{\cdot}062$	$k_6 = \frac{1}{5} \log_{10} \frac{165}{71} = 0{\cdot}077$
$k_3 = \frac{1}{1{\cdot}5} \log_{10} \frac{275}{249{\cdot}5} = 0{\cdot}028$	$k_7 = \frac{1}{6} \log_{10} \frac{71}{39{\cdot}5} = 0{\cdot}042$
$k_4 = \frac{1}{2} \log_{10} \frac{249{\cdot}5}{175} = 0{\cdot}077$	

joins each count with the initial count. If there is any variation in the slope this can be seen more easily in the second method of calculation, for within the limits of the experimental error (which can be better appreciated in this way!) k is calculated in stages over the reaction.

In this paper the second method of calculation has been used to analyse the sets of time-survivor curves. This analysis was undertaken because variations were observed in individual curves and because the experimental error was such that different interpretations could be applied to the same set of figures in each curve. All sets of figures were derived from identical experiments using the same organism of the same age and the same strength bactericide. The general aim was to see if useful information could be gained from the average response of the organisms. To determine this mean response each of the time-survivor curves obtained was divided into a number of sections, each of which corresponded to the death of a given proportion of

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

organisms. Fig. 8 is an example of one of the curves obtained and has been divided to correspond with 5, 10, 20, 30%, etc., deaths. That is, for each experiment the mean counts were plotted against time, the counts joined by straight lines and the continuous line divided into portions corresponding to certain percentage deaths. From these divisions the times corresponding to the various percentage deaths can be easily deduced. If this time is inserted in the formula

$$k = \frac{1}{t} \log_{10} \frac{N}{n},$$

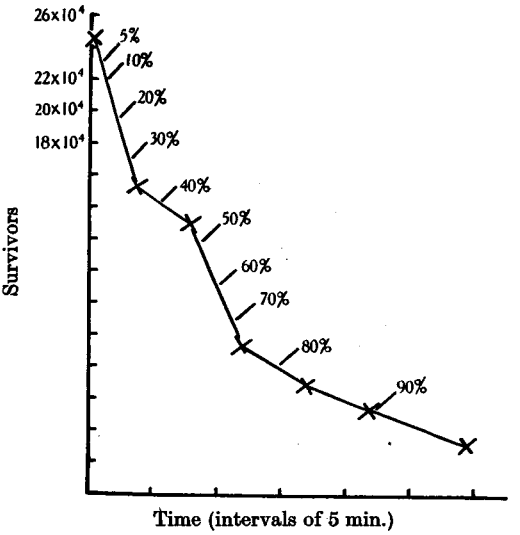


Fig. 8. Death-rate of a *Micrococcus* in para-chlor-meta-cresol solution, 0·05%. Temp. 20° C. The curve is divided to correspond with successive and regular percentage deaths. From such curves the mean curves were constructed (see text).

then k can be determined, or in other words the slope of the curve can be found for each section. Thus, for the period 0–5% deaths the formula is

$$k_1 = \frac{1}{t_1} \log_{10} \frac{100}{95},$$

and for 5–10% deaths

$$k_2 = \frac{1}{t_2 - t_1} \log_{10} \frac{95}{90},$$

and so on. When this has been done for each experiment the mean slope of the curve as the reaction progresses can be calculated. From these figures a hypothetical curve can be constructed which reflects the mean response of the organisms to the bactericide. Tables 4–7 show the figures obtained. A slide rule was used for all the calculations in this paper, and all logarithms are to the base 10.

Table 4. *Variation of k with the course of reaction*

Phenol 0·5 % on *Micrococcus*. Temp. 20° C.

Exp. no.	Deaths % 0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
224	0·02240	0·03200	0·01850	0·04650	0·02420	0·02870	0·03230	0·04550	0·05870
229	0·01490	0·01650	0·02650	0·02320	0·02780	0·03720	0·02570	0·02940	0·04400
231	0·01280	0·01380	0·01700	0·02320	0·01670	0·01670	0·01170	0·01250	0·01850
230	0·01490	0·01840	0·02550	0·00750	0·01560	0·03500	0·02760	0·01170	0·01360
233	0·00930	0·00828	0·00685	0·01580	0·01210	0·01144	—	—	—
234	0·01980	0·01840	0·02050	0·03230	0·03820	0·04520	0·01550	0·02170	—
236	0·00898	0·00828	0·01022	0·00968	0·01220	0·01218	0·00775	0·01920	—
237	0·00746	0·00690	0·00302	0·01453	0·01334	0·00990	—	—	—
238	0·00830	0·00750	0·01022	0·00775	0·00194	—	—	—	—
241	0·00101	0·00133	0·00905	0·01242	0·01700	0·01810	0·02498	0·01560	—
242	0·00179	0·00184	0·00425	0·00725	0·00888	0·01022	0·01450	0·01660	—
244	0 00106	0·00188	0·01540	0·00767	—	—	—	—	—
245	0·00320	0·00285	0·00326	0·01162	0·00860	0·00357	0·00450	0·00403	—
247	0·00496	0·00386	0·00556	0·00725	0·00265	0·00718	0·01211	0·00962	0·00587
249	0·00448	0·00487	0·00535	0·00695	0·00490	0·00545	0·00373	0·00366	0·00490

Table 5. *Variation of k with the course of reaction*

Para-chlor-meta-cresol 0·05 % and *Micrococcus*. Temp. 20° C.

Exp. no.	Deaths % 0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
224	0·03730	0·03220	0·05110	0·04650	0·03340	0·02640	0·06420	0·10000	0·06020	0·04020	0·04620
229	0·02240	0·02750	0·03400	0·04650	0·05320	0·05220	0·06430	0·12490	0·03920	0·06020	0·04280
231	0·01790	0·02070	0·02920	0·03320	0·04450	0·03960	0·06400	0·07150	0·10040	0·02400	0·01280
233	0·01870	0·01800	0·02230	0·02450	0·02550	0·01550	0·02150	0·02300	—	—	—
236	0·01280	0·01380	0·01700	0·01940	0·01208	0·01058	0·01290	0·01190	0·01760	—	—
237	0·01280	0·01500	0·01860	0·02320	0·01334	0·00528	0·00969	—	—	—	—
238	0·00746	0·00920	0·01460	0·02320	0·02223	0·01131	0·00807	0·01561	—	—	—
241	0·00347	0·00622	0·01180	0·00830	0·00910	0·00920	0·00808	0·01040	0·02201	0·02100	0·01481
242	0·00899	0·00253	0·00845	0·01180	0·00898	0·01320	0·01490	0·01318	0·01530	—	—
244	0·00670	0·00540	0·00340	0·01970	0·01810	0·01310	—	—	—	—	—
245	0·00485	0·00485	0·00600	0·00612	0·00635	0·00752	0·00580	0·00635	0·04430	0·01200	—
247	0·00899	0·00902	0·01460	0·01452	0·01668	0·01980	0·02423	0·02498	0·01360	0·01870	0·01072
249	0·00662	0·00517	0·00569	0·00485	0·00606	0·00528	0·00202	0·00960	0·00705	0·01003	0·00550

Table 6. *Variation of k with the course of the reaction*

Phenol 0·5 % and *Bact. coli*. Temp. 20° C.

Exp. no.	Deaths % 0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
262	0·0159	0·0400	0·0568	0·0225	0·0511	0·0620	0·0680	0·0960	0·0800	0·0910	0·0652
267	0·0590	0·0640	0·0682	0·0775	0·0760	0·1270	0·1102	0·0835	0·0945	0·1720	0·1200
268	0·0496	0·0435	0·0511	0·0581	0·0667	0·0792	0·0775	0·1000	0·1580	0·2670	0·1090
269	0·0160	0·0172	0·0183	0·0182	0·0218	0·0254	0·0184	0·0152	0·0587	—	—

Table 7. *Variation of k with the course of the reaction*

Para-chlor-meta-cresol 0·05 % and *Bact. coli*. Temp. 20° C.

Exp. no.	Deaths % 0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
262	0·0224	0·0218	0·0426	0·0726	0·0277	0·0342	—	—	—	—	—
267	0·0715	0·0385	0·0485	0·0425	0·0585	0·0580	0·0705	0·1000	0·1180	0·0800	0·0960
268	0·0448	0·0640	0·0585	0·0775	0·0885	0·0792	0·1282	0·1000	0·1175	0·2400	0·0858
269	0·0186	0·0320	0·0126	0·0342	0·0202	0·0360	0·0283	0·0248	0·0322	—	—

(*b*) *Results*

The figures from Tables 4–7 have been analysed, and the mean figures are given in Tables 8–11.

(i) *Reaction between phenol* 0·5% *and* Micrococcus *spp.*

The figures in Table 8 have been calculated from the reaction rates of Table 4. From these figures time-survivor curves can be constructed by substituting for k in the following formula, where every variable is known except t:

$$k = \frac{1}{t} \log_{10} \frac{N}{n},$$

Table 8. *Reaction between phenol* 0·5% *and* Micrococcus *spp.*
Mean value of k

No. of exps. used for mean values	Deaths % 0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
15	0·00842	0·00978	0·01194	0·01613	—	—	—	—	—
14	0·00895	0·01034	0·01149	0·01603	0·01448	—	—	—	—
13	0·00898	0·01057	0·01158	0·01666	0·01555	0·01853	—	—	—
11	0·00910	0·01111	0·01279	0·01693	0·01607	0·01996	0·01639	0·01715	—
6	0·01241	0·01491	0·01556	0·01883	0·01868	0·02171	0·01884	0·01859	0·02426

For example, the first figure quoted in Table 8 above is the rate while 5·0% of the population are killed. Hence

$$0{\cdot}00842 = \frac{1}{t} \log_{10} \frac{100}{95},$$

$$t = \frac{0{\cdot}0224}{0{\cdot}00842} = 2{\cdot}65 \text{ min.}$$

When t has been calculated in each case then time-survivor curves can be constructed by plotting the percentage deaths or their logarithms against time. In Fig. 9 this has been done for the most complete set of figures (from 0 to 80% deaths) in Table 8, and the resulting time-survivor curve is shown in Fig. 9, and shows that the mean survivor curve is a straight line when the logarithm of the survivors is plotted against time. From Table 8 the rate varies from 0·01241 to 0·02426, yet this variation can hardly be appreciated from the shape of the curve in Fig. 9, for a straight line is the obvious way to fit the counts and times.

For all practical purposes then the logarithm of survivors plotted against time gives very little deviation from a straight line and confirms the observations of many workers that this is a common mode of response of organisms to bactericides. That this is the mean response calculated from six experiments may perhaps lend more weight to this view.

(ii) *Reaction between para-chlor-meta-cresol* 0·05% *and* Micrococcus *spp.*

The figures in Table 9 below have been calculated from the individual reaction rates in Table 5. From the last set of figures (mean of six experiments) in Table 9 a time-survivor curve has been constructed by calculating t for each rate in the same way as for phenol. The time-survivor curve is

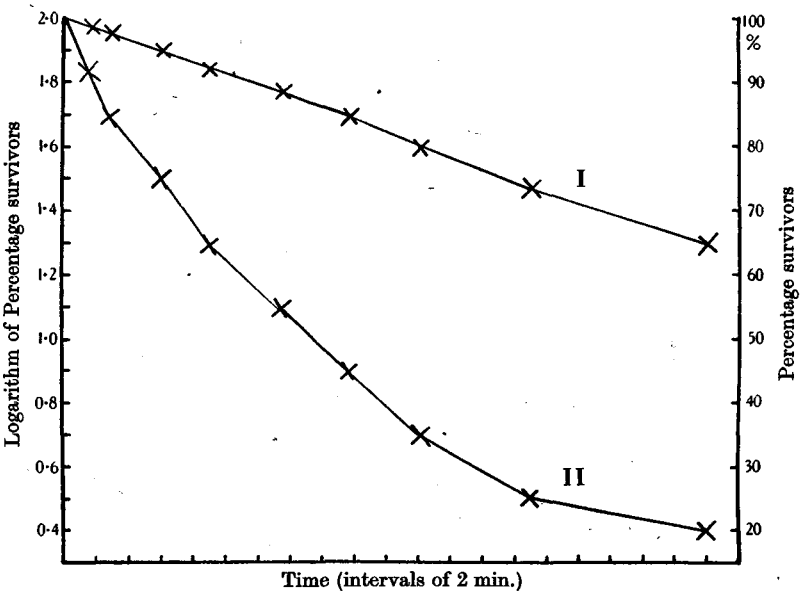


Fig. 9. Mean time-survivor curves for the *Micrococcus* in phenol 0·5% solution. Temp. 20° C. I, logarithm of percentage survivors against time; II, percentage survivors against time. The graph was constructed from the figures in Table 8 (0–80% deaths).

shown in Fig. 10. For para-chlor-meta-cresol and the *Micrococcus* the logarithm of the survivors plotted against time does not give a straight line. There is an initial period where the rate is slow, then a period where the rate gradually increases, and then a final slowing off.

Table 9. *Reaction between para-chlor-meta-cresol* 0·05% *and* Micrococcus *spp.*

No. of exps. used for mean values	Deaths %										
	0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
13	0·01315	0·01306	0·01816	0·02106	0·02039	0·01838	—	—	—	—	—
12	0·01369	0·01369	0·01939	0·02117	0·02058	0·01882	0·02447	—	—	—	—
11	0·01377	0·01358	0·01947	0·02099	0·02123	0·02005	0·02636	0·03740	—	—	—
9	0·01067	0·01358	0·01976	0·02016	0·02058	0·02042	0·02905	0·04142	0·03552	—	—
7	0·01061	0·01513	0·02177	0·02143	0·02417	0·02286	0·03317	0·04967	0·04096	0·02501	—
6	0·01611	0·01683	0·02442	0·02565	0·02616	0·02541	0·03781	0·05689	0·04041	0·03236	0·02214

(iii) *Reaction between phenol* 0·5% *and* Bact. coli

The mean figures in Table 10 below have been calculated from the individual reaction rates in Table 6. From the last set of figures (mean of three

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

experiments) in this table a time-survivor curve has been constructed by calculating t for each rate. The mean curve has not been illustrated as it is very similar to the curve in Fig. 6, and shows an initial lag period.

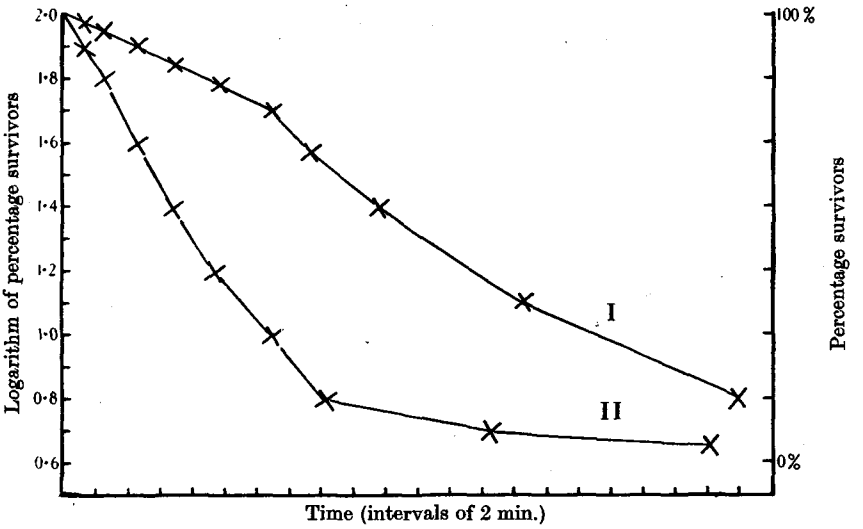


Fig. 10. Mean time-survivor curve for the *Micrococcus* in para-chlor-meta-cresol 0·05% solution. Temp. 20° C. I, logarithm of percentage survivors against time; II, percentage survivors against time. The graph was constructed from the figures in Table 9.

Table 10. *Reaction between phenol* 0·5% *and* Bact. coli.
Mean values of k

No. of exps. used for mean values	Deaths %										
	0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
4	0·0351	0·0412	0·0489	0·0441	0·0539	0·0734	0·0685	0·0737	0·0987	—	—
3	0·0415	0·0492	0·0587	0·0527	0·0646	0·0894	0·0852	0·0932	0·1108	0·1766	0·0981

(iv) *Reaction between para-chlor-meta-cresol* 0·05% *and* Bact. coli

The mean figures in Table 11 below have been calculated from the individual reaction rates in Table 7. From the last set of figures (mean of two experiments) in Table 11, a time-survivor curve has been constructed by calculating t for each mean rate. The mean curve has not been drawn, as it is very similar to the curve in Fig. 7, and shows a lag period.

Table 11. *Reaction between para-chlor-meta-cresol* 0·05% *and* Bact. coli.
Mean value of k

No. of exps. used for mean values	Deaths %										
	0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–95
4	0·0393	0·0441	0·0406	0·0567	0·0488	0·0519	—	—	—	—	—
3	0·0446	0·0512	0·0399	0·0514	0·0557	0·0577	0·0757	0·0747	0·0892	—	—
2	0·0633	0·0563	0·0535	0·0600	0·0735	0·0686	0·0994	0·1000	0·1178	0·1600	0·0909

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

(*c*) *Summary*

The mean time-survivor curves of bacteria immersed in two phenolic disinfectants show the same variation of type as the individual curves, that is, (*a*) an exponential rate, (*b*) a lag phase followed by an exponential rate. The significance of all these curves and of others is discussed in the next part of the paper.

IV. The significance of time-survivor curves

(*a*) *Theories of time-survivor curve variation*

The shape of time-survivor curves has been attributed by different observers to the essential similarity in resistances of the individuals in a suspension and also to the fundamental differences in resistances of the organisms. The first assumption is the keystone of the theory that the shape of curve is determined by the fundamental reaction between organism and reagent ('mechanistic hypothesis'). When the logarithm of the survivors is plotted against time, a straight line is often obtained, and the death-rate is then said to be similar to monomolecular chemical reactions, and death due to one event. The theory demands two assumptions: (i) that the organisms which show this type of curve are essentially uniform in resistance, and (ii) that the death of the organisms is due to one event. Both these assumptions are at variance with the observed facts regarding relationship of cells and reagents. Indeed, as Clark (1933) points out, 'the monomolecular theory, or quantum theory of cell destruction, has therefore very little solid evidence in its support and its application leads to conclusions that are so absurd that they are difficult to discuss'.

The obvious difficulties in the way of an acceptance of such a theory are that variation is a fundamental law of living matter, and to postulate a uniform cell population is to assume a condition that has never been demonstrated. All the evidence points to the diffusion of reagents through a cell wall, or to the adsorption of the reagent over the surface of the cell wall. The figures that have been given show that the number of molecules required for some response by the cell are so enormous as to make the idea of one molecule being sufficient patently absurd. For example, many observers (Arrhenius, Henri, Liebermann and Fennyvessey) have shown that haemolysis of erythrocytes follows a 'monomolecular' course, yet some of the figures calculated as the *minimum* number of molecules to cause haemolysis are enormous. Ponder (1930) and Clark (1933) give the following *minimum figures per erythrocyte*:

Saponin	10^8 molecules per cell
Sodium oleate	2×10^8 molecules per cell
Acids or alkalis	10^9 molecules per cell

In this work about 3×10^{20} molecules of phenol were added to 10^7 or less organisms, allowing 10^{13} molecules per cell. The volume of the phenol molecule is about $1/10^9$ that of a coccus. In the face of facts like these it is difficult to discuss further the monomolecular theory, for all evidence points to the fixing of millions of molecules of drug by each cell. This has been commented on by Reichenbach (1911) as follows: 'The molecules are so small compared to the bacteria and their number is so much greater, that doubtless even in very dilute solutions every bacterium is surrounded by a similar number of molecules and therefore is exposed equally to the action of the disinfectant. And still less can one say that the bacteria arrive one after another to the condition necessary for destruction by temperature. Therefore if the bacteria are of equal resistance one cannot understand why one should die quicker than another.' This objection to the theory that assumes equal resistance of the organisms has been answered by Rahn (1930) using arguments based on Yule's calculations (1910). Rahn still holds the view that the organisms are of equal resistances.

The monomolecular theory attempts to describe in very simple physico-chemical methods, a complicated process involving the death of the organism. It has been devised to explain the facts that exponential rates are frequently observed when bacteria are killed. When other types of time-survivor curves are obtained then some of those who rely on the monomolecular theory for exponential time-survivor curves fall back on differences of resistance (Chick, 1930, p. 187). Others (Rahn, 1930) assume that the reaction becomes bi- and termolecular or multimolecular. The latter assumptions are subject to the same disadvantages as the monomolecular theory incurs. Any other theory which takes its place must account for exponential time-survivor curves, together with those of sigmoid type.

The obvious alternative to the monomolecular theory is to say that the length of time an organism can survive in a bactericide is proportional to its resistance. This is an assumption, but an assumption so simple that it makes no reference to any of the complicated reactions both physical and chemical which may occur between the organism and reagent. Indeed, we may reasonably define resistance as the time an organism can remain viable in a solution. If we do use this assumption or definition, how are the resistances distributed? When the sigmoid time-survivor curves found in bacteriological data are transformed to frequency diagrams, then slightly skew curves are obtained (Chick, 1930, p. 183). When exponential curves are treated in the same way then extremely skew curves of resistance distribution are found (Chick, 1930; Rahn, 1930). The fact that skew distributions are obtained is one of the major arguments of those who hold the monomolecular theory. For example, Chick (1930, p. 186) says: 'an explanation on the grounds of differing inherent resistances must assume that these resistances are distributed among the individual bacteria in a manner which is an exception to other studied instances of biological variation'. Rahn (1930) says: 'It is not very easy however to

fit the laws of chance to the actual facts of the order of death, as shall be demonstrated by one of the experiments of Madsen & Nyman (1907) and of Hewlett (1909).' He then shows that when curves showing the distribution of resistances are constructed from Madsen & Nyman's data, which give an exponential time-survivor curve, these curves are sharply skewed to the left (i.e. with the majority of the organisms dying in the first time units). Hewlett's figures for the death of mustard seeds in mercuric chloride solution give an approximately normal distribution of survivor times. Rahn's comment is that this difference is difficult to explain. Later in the paper, referring to what are described as 'vitalist and mechanistic theories' on the subject of time-survivor curves, he says: 'When two groups of research workers hold such opposed views it is fairly safe to assume that some essentially new principle is involved, which none of the two parties realised.' I believe that this is so, but I do not think that Rahn's new principle is the one at issue here. He emphasizes the relative sizes of molecules and organisms, and finds by mathematical methods that if the reaction be of some higher order (say involving six molecules of bactericide) that time-survivor curves of sigmoid type occur. For those cases where the bacteria die more rapidly than a monomolecular rate indicates, he suggests, clumping of the organisms. His main assumption is that the organisms are all alike in resistance, and this I believe is fundamentally wrong even though he derives sigmoid curves from such a population by assuming multimolecular reactions.

I believe that the main principle which has been neglected by both 'vitalists and mechanists' alike is the general law of distribution of resistances. Both groups have assumed that when resistances are measured by survivor times then the distribution of these resistances should be approximately normal, i.e. that the general rule for any measured biological attribute is for it to be distributed in an approximately normal manner. This is *not always* the case. Galton in 1879 pointed out that 'the assumption which lies at the basis of the well-known law of Frequency of Error (commonly expressed by the formula $y = e^{-h^2x^2}$) is incorrect in many groups of vital and social phenomena, although that law has been applied to them by statisticians with partial success and corresponding convenience. Next I will point out the correct hypothesis upon which a law of Error suitable to these cases ought to be calculated.... The assumption to which I refer, is that errors in excess or in deficiency of the truth are equally probable, or conversely that if two fallible measurements have been made of the same object their arithmetic mean is more likely to be the true measurement than any other quantity that can be named. This assumption cannot be justified in vital phenomena'. He proceeds to give examples where this rule does not apply, and concludes, 'in other words the true mean is geometric'. This statement he applies to 'vital and social statistics'. He gives examples where the geometric mean is obviously a more correct estimate of the true mean of a set of observations. The logical outcome of Galton's remarks is that we should think in terms of powers of

integers and not the integers themselves when concerned with vital and social statistics. Gaddum's work (1933) makes it clear that the logarithms of the individual sensitivities of a number of experimental animals to a great variety of drugs and hormones are normally distributed. The individual sensitivities themselves are not all normally distributed. Hemmingsen (1934) shows in a striking manner that the logarithm of the length of many animals is always approximately normally distributed, while the distribution of the lengths themselves was often very sharply skewed to the left, and never normally distributed. His lengths were obtained by direct measurements of adult

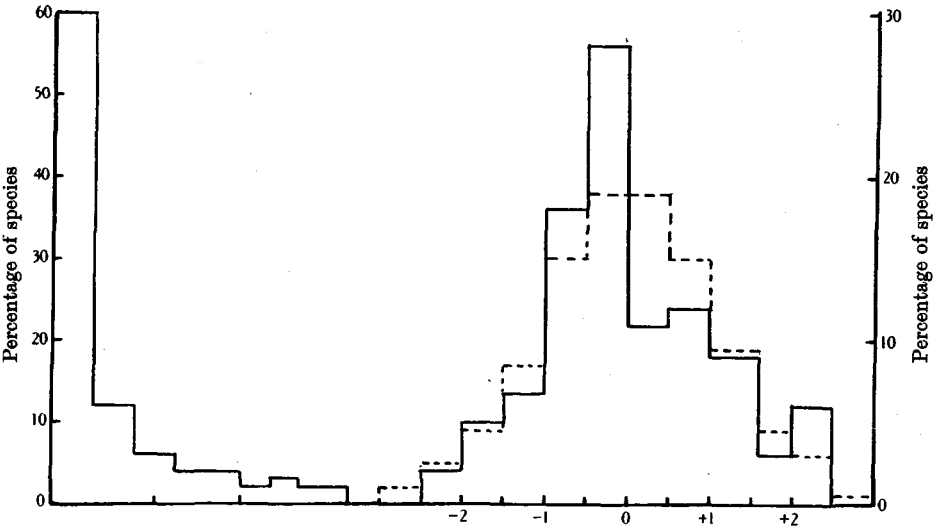


Fig. 11. The left-hand figure shows that the distributions of species with different length-mantle gives a curve sharply skewed to the left. When these lengths are expressed as logarithms, then the distribution of species with different logarithmic lengths is approximately normal. (From Hemmingsen (1933), part of Fig. 15.) Distribution of dorsal lengths of mantle of species of Cephalopods (*Decapoda Oigopsida*).

animals of different species but within the same genus from all the main systematic groups of the animal kingdom (i.e. interspecific distribution). The figures he obtained show, when plotted in a frequency diagram, a skewness always to the left. One of his figures is reproduced in Fig. 11.

Hemmingsen says: 'It will be seen that in general the logarithms show a closer approximation to the normal distribution, than the actual measures whose distribution in most cases, probably in all, is incompatible with the normal distribution. The approximation of the distribution of the logarithms to the normal distribution is particularly striking in those cases in which the distribution of actual measures is exceedingly skew.' The twenty-six figures in the appendix to his work are very clear evidence that this is the case.

With the background of Galton, Gaddum and Hemmingsen we can proceed to investigate the way in which the survival times of bacteria are distributed. It is the intention to see if the logarithms of the resistances of the organisms used in this work, and by other observers, are normally distributed. To do this we must take the resistance of the organism as proportional to its survivor time. Any reactions that take place between bactericide and organism are considered to be a manifestation of the resistance of that organism, and no assumptions are made regarding the nature of these reactions. It has been shown that if the distribution of the resistances is estimated on this assumption then skew distributions are often obtained, just as Hemmingsen obtained skew distributions with his linear interspecific measurements of animals. We can now show that the observed time-survivor curves of different observers all give approximately normal distributions of survivor times when the frequency diagrams are constructed from the *logarithms* of the survivor times. I have applied this method to many types of disinfection and, in nearly every case, the distribution of the logarithm of survival times is more nearly normal than the distribution of the survival times themselves. If the distribution of survival times is normal, this will bring the interpretation of bacteriological time-survivor curves in line with other pharmacological data where the logarithm of a biological attribute is often found more nearly normally distributed than the attribute itself. It must be emphasized that we are not assuming that the logarithm of the resistances are approximately normally distributed—we shall demonstrate that within the experimental error this is so.

In this connexion it is well to anticipate those who will say that the use of a logarithm instead of a natural number is a mathematical trick, used to explain the results. The reverse is the case. It has been found *experimentally* that the logarithms of many biological measurements are more normally distributed than the measurements themselves, and these practical observations accord with the general theory. There are also good theoretical reasons why this should be so. To calculate these resistances in bacteriology we have to define the resistance of the organism as the time it can remain viable in a solution. That is the difference between the direct observations of Hemmingsen and the observations included in this paper. The remarks of Gaddum on this subject are quoted below; they need no comment, except, that to apply them to the problem under review here we must substitute the logarithm of time for the logarithm of the dose, i.e. the different times after which an organism ceases to be viable are equivalent to different doses that are required to kill a series of animals.

'If the percentage mortality is plotted against the logarithm of the dose, the curves are always approximately symmetrical whether the variation is large or small. The shape of the curves indicates that if the resistance of an animal is measured by the dose which is just necessary to kill it, the logarithm of the resistance is normally distributed. The S-shaped curves are in fact

integrals of approximately normal frequency distributions.... The standard deviation of the distribution is easily calculated from the curve and the slope of the curve is usually expressed in terms of λ, the standard deviation of normal distribution which most nearly fits the results when the mortality is plotted against logarithms of the dose to the base 10. It has been thought that the use of the logarithm of the dose instead of the dose itself, represents a subtle mathematical device for obscuring the nature of the curve. This is not so. Both in practice and in theory the logarithm of any biological measurement is more likely to be normally distributed.' I would refer the reader in particular to Gaddum's report on 'Methods of Biological Assay depending on

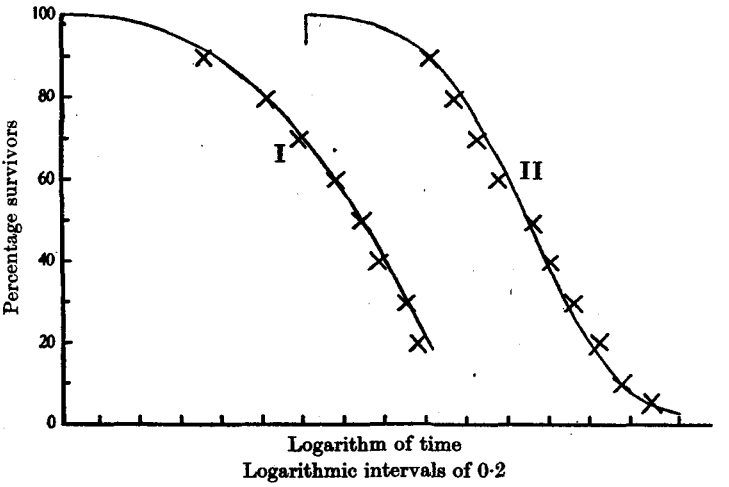


Fig. 12. Mean curves calculated in Part II drawn to a logarithmic time scale. I, reaction between a *Micrococcus* and phenol 0·5%. (This curve gives an exponential curve when plotted against time; see Fig. 9.) II, reaction between a *Micrococcus* and para-chlor-meta-cresol 0·05%. (This curve gives a lag phase and then an exponential rate when plotted against time; see Fig. 10.)

a Quantal Response' (1933), for a list of experiments where the evidence of normal distribution of the logarithm of the dose is clearly set out. Those who, like myself, do not profess to be mathematicians, might benefit from the story of the maggot and the elephant! (Gaddum, 1940, p. 360).

(*b*) *The evidence for the normal distribution of the logarithm of survivor times in bacteriology*

There are a number of ways in which the approximately normal distribution of the logarithms of survival times can be demonstrated.

(i) If percentage survivors are plotted against logarithm of time then sigmoid curves should result if the above statement is true. These sigmoid curves are the integrals of a normal curve of frequency distribution of logarithmic survival times. The mean curves calculated in Part III are drawn to a logarithmic time scale in Figs. 12 and 13. There is no doubt that each of

these curves is of sigmoid type, and it must be remembered that the first of these curves (Fig. 12, I) gives a straight line when the logarithm of the survivors is plotted against time (see Fig. 9). The second of these curves (Fig. 12, II) shows a lag phase followed by an exponential curve when plotted in

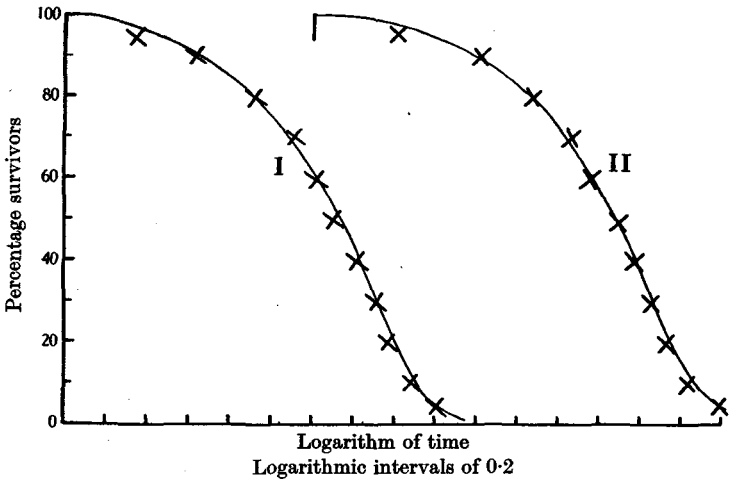


Fig. 13. Mean curves calculated in Part II drawn to a logarithmic time scale. I, reaction between *Bact. coli* and phenol 0·5%. II, reaction between *Bact. coli* and para-chlor-meta-cresol 0·05%. Both these curves when plotted against time give an approximately exponential curve, and when plotted as logarithm of survivors against time, show evidence of a lag period.

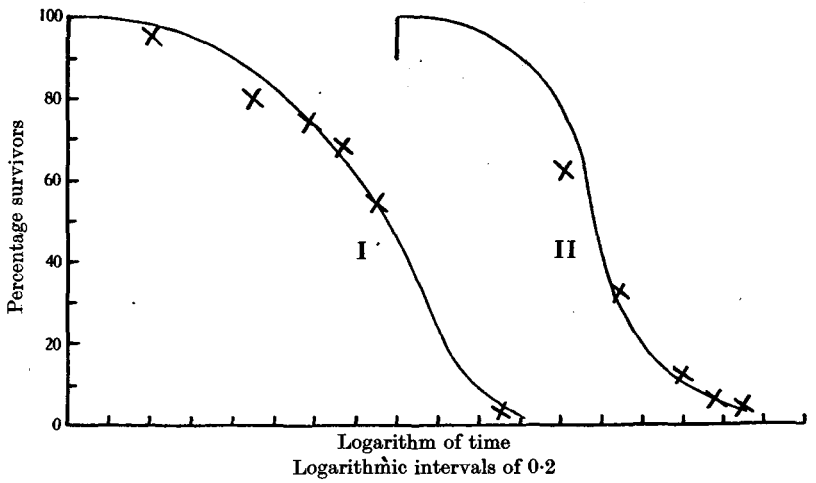


Fig. 14. Disinfection of anthrax spores by 5% phenol. Data from Chick (1908): I, from Table I, p. 97; II, from Table II, p. 99. Both these curves would give exponential ('monomolecular rate') curves when plotted against time.

similar manner (see Fig. 10). I and II in Fig. 13 also show a lag phase when logarithms of survivors are plotted against time. All these show a general resemblance when a logarithmic time scale is used which suggests a common origin of the shape of these curves.

Other observers' figures also give very good agreement with this general type. In Figs. 14 and 15 are figured data from:

(*a*) Anthrax spores and phenol (Chick, 1908*a*) (Fig. 14, I and II).

Both these sets of figures give good agreement with the monomolecular reaction rate, i.e. are exponential curves, and yet when plotted against the logarithm of time show general agreement with other curves in their sigmoid type.

(*b*) *Staphylococcus aureus* and phenol (Chick, 1910) (Fig. 15). This curve shows a lag phase and well-defined exponential rate thereafter, if the logarithms of survivors are plotted against time; but here again shows a sigmoid type when drawn to the logarithm of time.

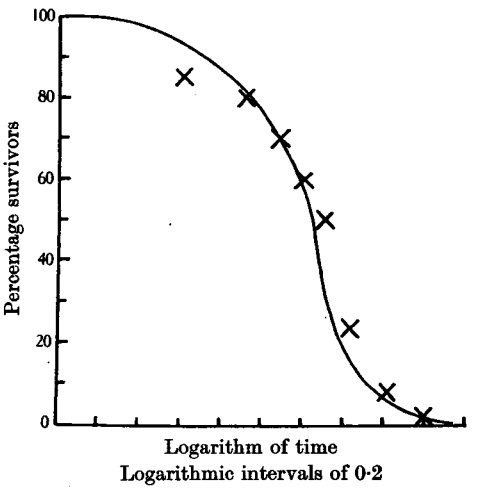


Fig. 15. Disinfection of *Staph. aureus* with phenol 0·6%. Data from Chick (1910), Table VII, p. 246. The same data shows a well-marked lag period and then gives good agreement with exponential rate, when logarithm of survivors is plotted against time.

Diagrams showing the percentage of organisms dying in equal logarithmic time units can be constructed from time-survival curves. Four of these diagrams, which show the distribution of logarithmic survival times are shown in Figs. 16–19, which are derived from:

(*a*) Anthrax spores and phenol (Chick, 1908*a*) (Fig. 16).

(*b*) Disinfection of a *Micrococcus* by phenol 0·5% (Exp. 247) (Fig. 17).

(*c*) *Staphylococcus* and phenol (Chick, 1910) (Fig. 18).

(*d*) Mustard seeds and mercuric chloride (Hewlett, 1909) (Fig. 19).

These four sets of data all give approximately normal distributions of resistances when the logarithms of survival times are used. They include data which was formerly thought to be fundamentally different. The data from mustard seeds is included to show that those experiments which give approximately normal distributions when plotted against time give a better agreement when the logarithm of the time is used. Further, all the other types

quoted agree more or less closely with this general rule, although they give extremely skew distributions if plotted against time. The skew distributions are shown to the left of each of the diagrams (Figs. 16–19). In all cases a more normal distribution of resistances is obtained by plotting the percentage of organisms against the logarithms of survival times.

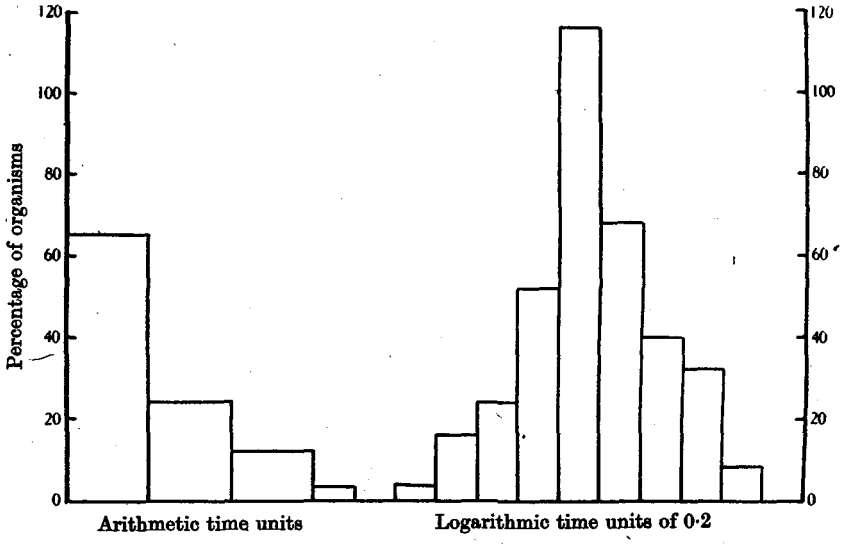


Fig. 16. Disinfection of anthrax spores by phenol 5% (data from Chick, 1908*a*, Table I, p. 99). Distribution of resistances: (*a*) left-hand figure, from survivor times, skew distribution; (*b*) right-hand figure, from logarithm of survivor times, approximately normal distribution.

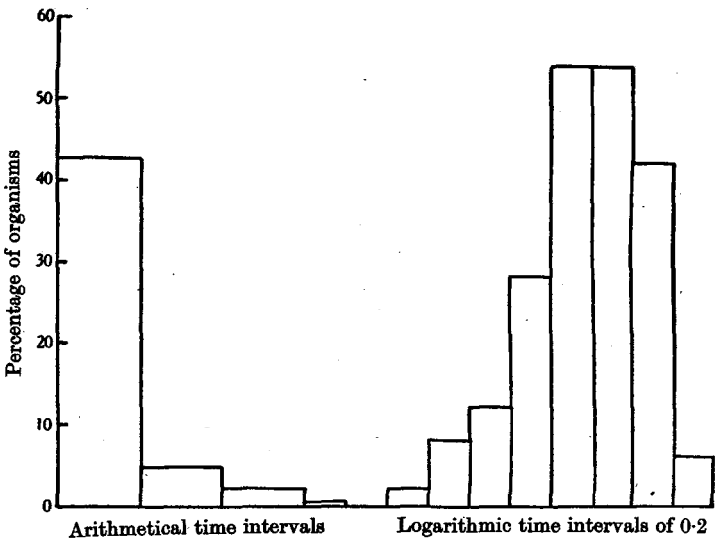


Fig. 17. Disinfection of the *Micrococcus* by phenol 0·5% (Exp. 247). Distribution of resistances: (*a*) left-hand figure, from arithmetical survivor times, skew distribution; (*b*) right-hand figure, from logarithmic survivor times, approximately normal distribution.

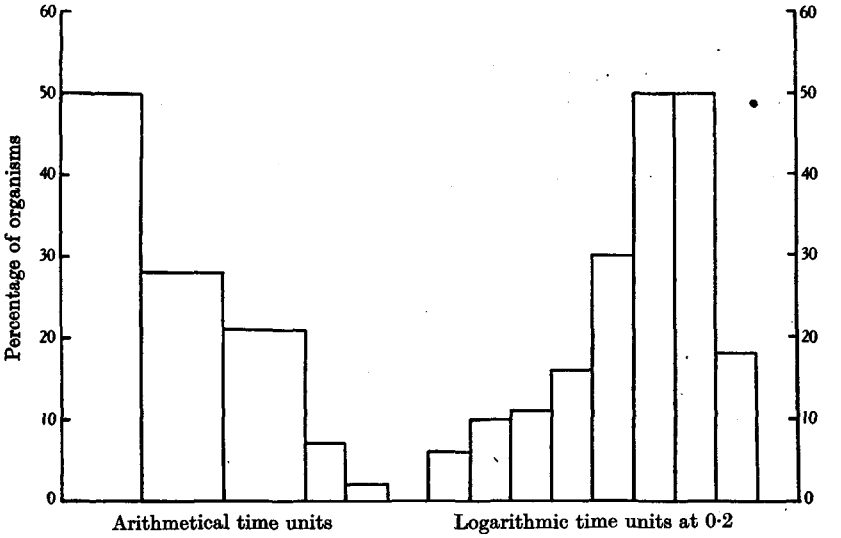


Fig. 18. Disinfection of *Staph. aureus* by phenol 0·6% (data from Chick, 1910, Table VII, p. 246). Distribution of resistances: (*a*) left-hand figure, calculated from arithmetical survivor times, skew distribution; (*b*) right-hand figure, calculated from logarithmic survivor times, approximately normal distribution.

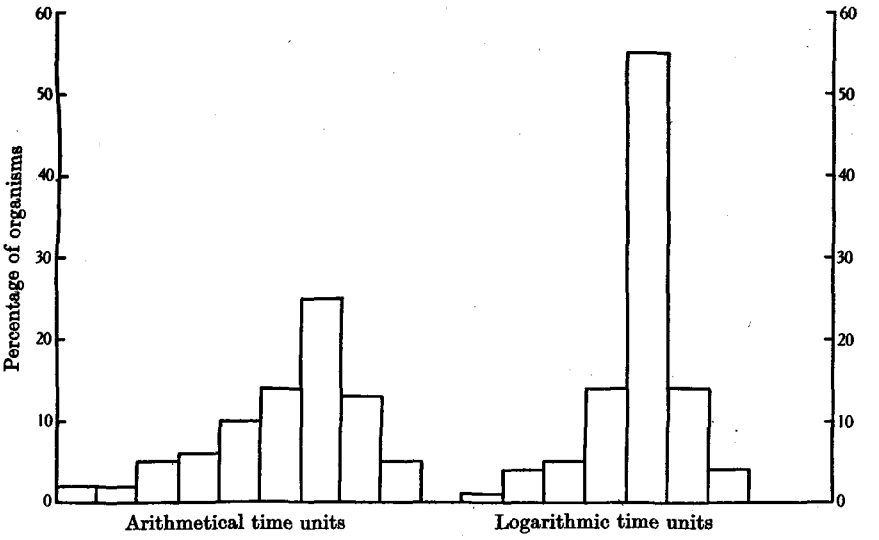


Fig. 19. Destruction of mustard seeds with mercuric chloride (Hewlett, 1909). The figure shows that if survivor times are approximately normally distributed, then the logarithm of survivor times is similarly distributed.

(ii) Another way to show the truth of the argument is to plot percentage deaths against the logarithm of the times, calculated as percentages of the logarithmic time for 50% deaths. Fig. 20 shows two curves drawn through the points obtained.

(I) From data which gives a sigmoid time-survivor curve when percentage survivors are plotted against time (Smith-Henderson (1921)—*Botrytis* spores and phenol 0·5%).

(II) From data which gives an exponential curve when time survivor curves are similarly constructed (anthrax spores and phenol 5% (Chick, 1908*a*)).

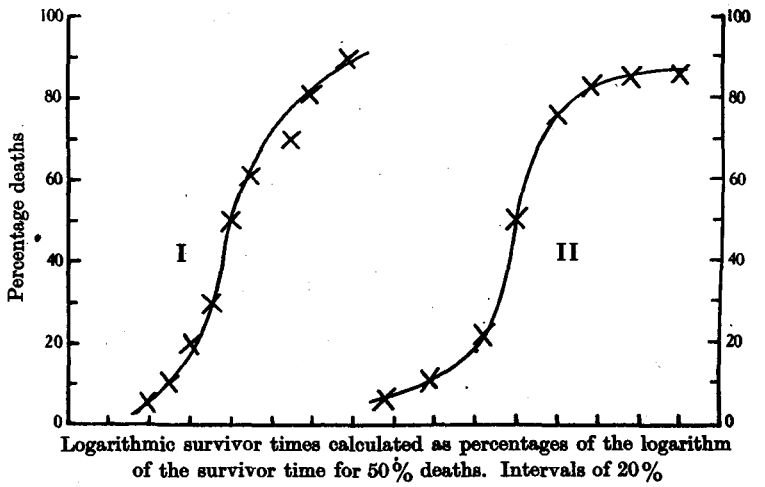


Fig. 20. Distribution of logarithmic survivor times. I, data which gives a sigmoid curve when plotted against time (Smith-Henderson (1921), *Botrytis* spores and phenol 0·6%). II, data which gives an exponential curve when plotted against time (Chick, 1908*a*, anthrax spores and phenol 5·0%). The curves appear fundamentally different when plotted against time, but show agreement in type when plotted on logarithmic scale.

Fig. 21 shows:

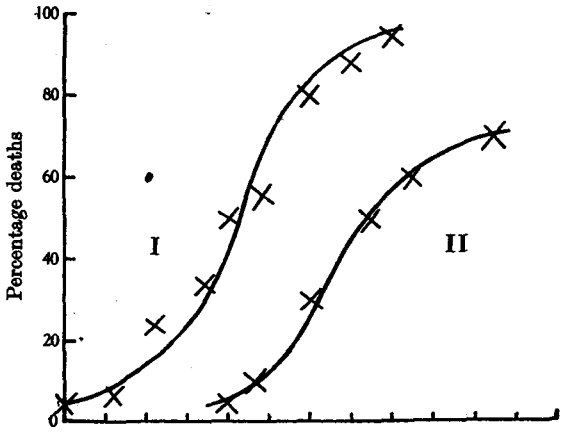
(I) A curve obtained from data which gives a lag phase followed by a straight line when logarithm of survivors is plotted against time (*Bact. coli* and phenol 0·5% quoted in Part I of this work).

(II) From similar data for *Staph. aureus* and phenol 0·6% (Chick, 1910, p. 246).

These curves, which have been considered fundamentally different, give similar results when plotted in this way and are capable of similar explanation as will be pointed out later.

(iii) There is a more accurate method of demonstrating the distribution of survival times (resistances), and that is to make use of the normal equivalent deviation (N.E.D.) of Gaddum (1933) or the 'probit' of Bliss (1938). If a response to any stimulus depends on a distribution of resistance which is normal, then, if the percentage response is plotted against the logarithm of the stimulus, sigmoid curves result. By the use of the N.E.D. and probit

these sigmoid curves can be turned into straight lines. When this is done the results are more easily interpreted, more information can be conveniently derived from them and the observations of different observers can be compared more easily. The N.E.D. is percentage response (e.g. percentage death) translated into terms of standard deviation and with a standard deviation as



Logarithmic survivor times, calculated as a percentage of the logarithm of the survivor time for 50% deaths. Intervals of 20%

Fig. 21. Distribution of logarithmic survivor times. I, data which gives a lag phase followed by approximately exponential series (*Bact. coli* and phenol 0·5%, mean curve, see Part II and Fig. 6). II, similar data for *Staph. aureus* and phenol 0·6% (Chick, 1910, p. 246).

unit. Suppose two points are marked on the logarithm of time abscissa to a normal curve, each corresponding to plus or minus one standard deviation from the mean. If perpendiculars are drawn from these points to cut the normal curve they will enclose between them two-thirds of the total area of the curve. This is shown in Fig. 22. The area *ACE* would then be approximately 16% of the total area of the curve. If this curve represents distri-

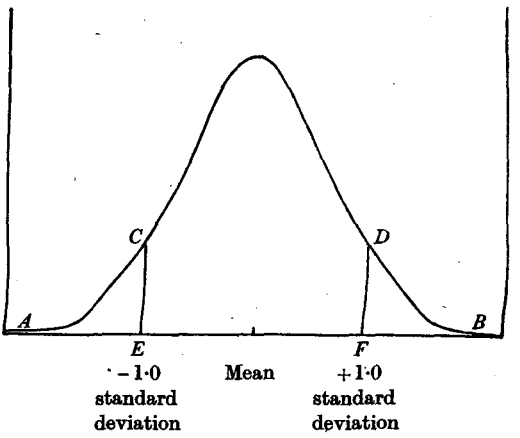


Fig. 22. Calculation of normal equivalent deviation from percentage response (see text).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

bution of resistances then the number of organisms with resistances equal to or less than any point on the curve (say C) is proportional to the area to the left of a perpendicular to the abscissa at E. In this case the area ACE represents 16% of the total resistances. If mortality is directly proportional to resistances, we can translate percentage mortality to areas of the curve proportional to resistances. A percentage death of 16 would therefore be equal to 16% of the total area of the curve and be represented on the abscissa by $-1{\cdot}0$ standard deviations. Similarly, 84% deaths are represented by $+1{\cdot}0$ standard deviations. 50% deaths would be translated to 0·0 in the N.E.D. terminology. The translation of the percentiles into terms of N.E.D. is easily effected by tables (Bliss, 1938) and by a figure in Gaddum's work (1933). If N.E.D. is plotted against logarithm of time straight lines will result if the distribution is normal. If the distribution is not normal, there will be a variation from the straight line and the abnormality can be estimated by the shape of the line. It must be mentioned that to show normal distribution it is most important for the line to be approximately straight round the centre part of the line, about N.E.D. of 0·0. Deviations in the extremes of the lines are not so important as they refer to very small percentages. There is another point—the translation of percentiles into N.E.D., by means of Gaddum's figure and Bliss's tables, depends on the assumption that the logarithm of the survivor times are normally distributed. Gaddum (1933) has shown that the logarithms of the individual effective doses for many animals are approximately normally distributed, and that slight deviations from normality will not effect the calculations significantly. The device is used for its convenience, and as a further demonstration of the fact that the logarithm of survivor times is approximately normally distributed.

The probit of Bliss (1938) is derived from the N.E.D. by adding 5·0 to the figures obtained when percentiles are changed to N.E.D., and serves to render all the figures positive.

Figs. 23–26 show for individual experiments in this series the way in which probit is related to the logarithm of the time. It will be seen that the line is very nearly straight around probit 5·0. At small values of probit the line bends in some cases, and the significance of this will be discussed later. The mean curves calculated in this work (p. 135) are drawn on a probit-logarithm of time graph in Fig. 27.

I have prepared a number of diagrams, based on the observations of other workers, to illustrate and emphasize the skew distributions that are obtained when frequency diagrams are constructed from survivor times and the more normal distribution of the logarithm of the survivor times. These will be found in Appendix I and comprise Figs. 28–46.

Each diagram has been similarly constructed and is divided into three parts. The right-hand diagram shows probit plotted against time (upper curve) and probit plotted against logarithm of time (lower curve). When such diagrams yield curves, a deviation from normality is indicated, and this is reflected

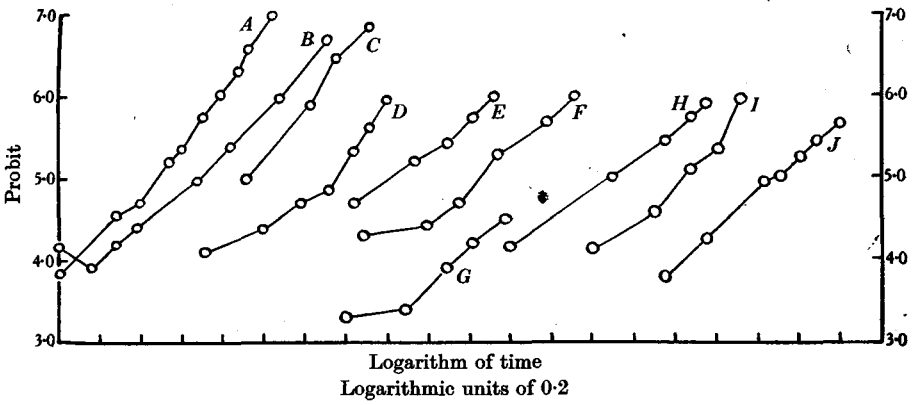


Fig. 23. Probit plotted against logarithm of time. Disinfection of a *Micrococcus* by phenol 0·5%. *A*, Exp. 247; *B*, Exp. 224; *C*, Exp. 229; *D*, Exp. 249; *E*, Exp. 231; *F*, Exp. 230; *G*, Exp. 233; *H*, Exp. 234; *I*, Exp. 236; *J*, Exp. 245. All counts given in Appendix. Each curve has a different zero on abscissa.

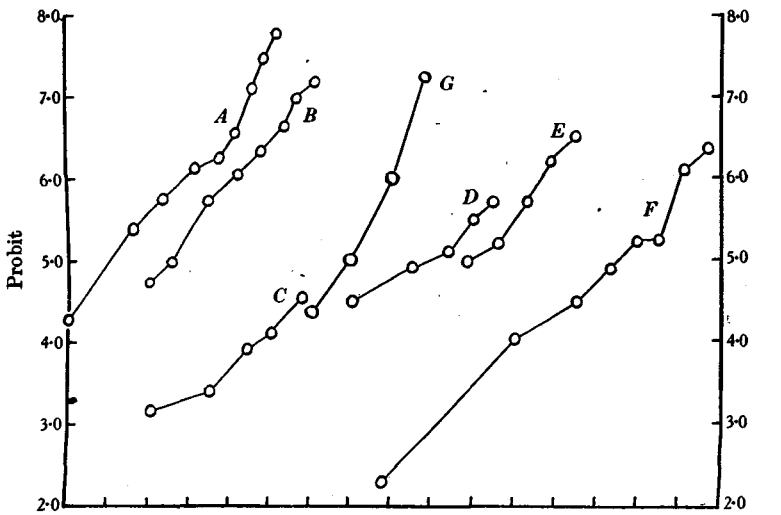


Fig. 24. Probit plotted against logarithm of time. Disinfection of the *Micrococcus* by para-chlor-meta-cresol 0·05%. *A*, Exp. 247; *B*, Exp. 224; *C*, Exp. 233; *D*, Exp. 236; *E*, Exp. 236; *F*, Exp. 241; *G*, Myer's data for *Bacillus* 25 (see p. 126). All counts given in Appendix. Each curve has a different zero in abscissa.

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

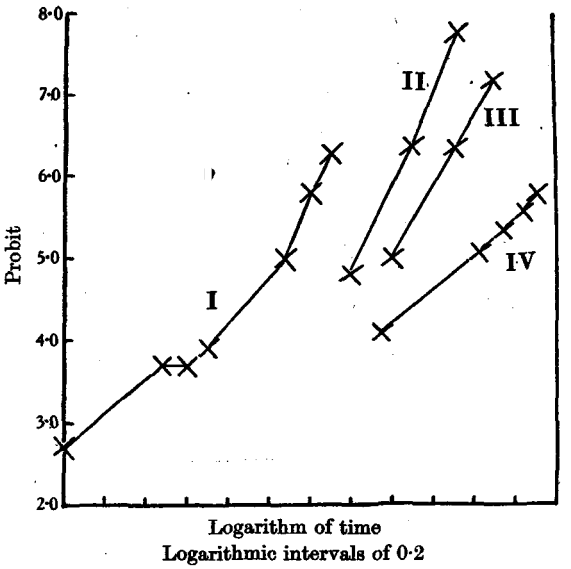


Fig. 25. Probit plotted against logarithm of time. Disinfection of *Bact. coli* by phenol 0·5%. I, Exp. 262; II, Exp. 267; III, Exp. 268; IV, Exp. 269. All counts given in Appendix. Each curve has a different zero on abscissa.

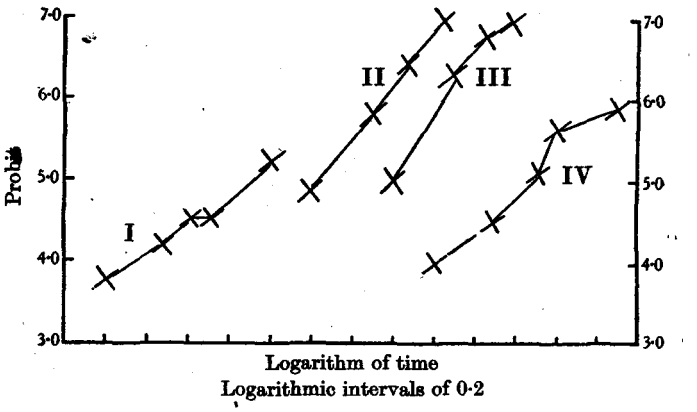


Fig. 26. Probit plotted against logarithm of time. Disinfection of *Bact. coli* by para-chlor-meta-cresol 0·05%. I, Exp. 262; II, Exp. 267; III, Exp. 268; IV, Exp. 269. All counts given in Appendix. Each curve has a different zero on abscissa.

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

in the frequency diagrams which have been constructed from these lines. The distribution of logarithmic survivor times is illustrated in the central diagram and the distribution of survivor times in the left-hand diagram.

It was my endeavour to select a representative fraction of the bacteriological literature, but the work of selection has been difficult for two reasons. First, some observers have given no counts, which means that although they have drawn graphs, I could not accurately derive from their observations the information I required. Secondly, the course of the reaction is sometimes followed over a small range. I have not regularly used counts of this nature because it is not easy to obtain a clear judgement of the shape of the probit-time curve when the information extends over a small range. If Appendix I is consulted it will be seen that all the examples quoted give a better agreement of survivor-time distribution with the normal curve when logarithms

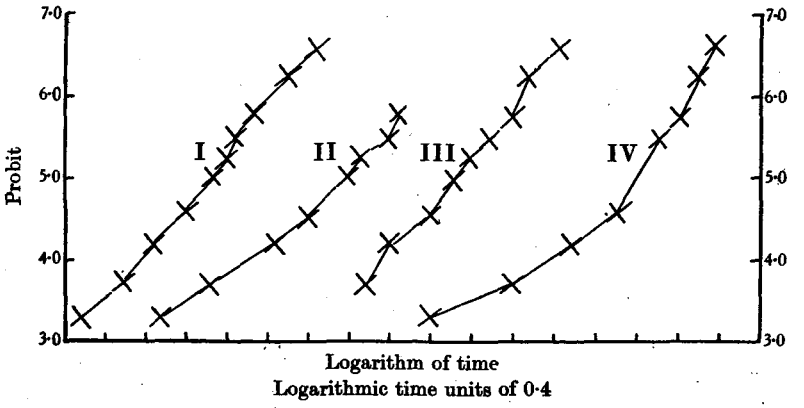


Fig. 27. Probit plotted against logarithm of time. Mean curves calculated in Part II. I, disinfection of a *Micrococcus* with para-chlor-meta-cresol 0·05%. II, disinfection of a *Micrococcus* with phenol 0·5%. III, disinfection of *Bact. coli* with phenol 0·5%. IV, disinfection of *Bact. coli* with para-chlor-meta-cresol 0·05%. Each curve has a different zero on the abscissa.

are used. In some cases this is very evident. If Table 1 (pp. 125 f.) is referred to at the same time, the shape of the curves which these observers reported when percentage survivors are plotted against time is given in the last column. All the various types of survivor curve that have been reported in bacteriology are illustrated in Appendix I, and every case gives a better approximation to normality when logarithms are used. In the appendix Fig. 45 shows the data of Hewlett (1909) for the death of mustard seeds in mercuric chloride solution, while Fig. 46 shows the distribution of survival times for the confused flour beetle (Oothuisen, 1935). These two diagrams show that when the survivor times are distributed normally, the logarithmic survivor times are also normally distributed. The probit-time curve and probit-logarithm of time curve in Figs. 45 and 46 both show deviations from a straight line, but the deviation concerns a small proportion of the organisms, which is reflected in the approximately normal distribution diagrams. These diagrams have, in each case, been constructed from the smoothed curve of the probit diagrams.

For each of the curves where the data is sufficient, the standard deviation of the logarithms of the survivor times (λ) has been calculated. This has been done by halving the difference between the logarithm of the time when 84% of the organisms are viable and that when 16% are viable. The reciprocal

Table 12. *Values of λ and b for experiments in this work*

Exp.	Bactericide	Organism	λ	$b=1/\lambda$	Shape of time-survivor curve on arithmetical time scale
224	Phenol 0·5%	*Micrococcus*	0·46	2·17	Exponential
229	,,	,,	0·52	1·92	,,
231	,,	,,	0·54	1·84	,,
230	,,	,,	0·56	1·78	,,
233	,,	,,	0·48	2·08	,,
234	,,	,,	0·64	1·56	,,
248	,,	,,	0·40	2·50	,,
247	,,	,,	0·44	2·26	,,
241	,,	,,	0·26	3·85	Lag phase then exponential
242	,,	,,	0·30	3·33	,, ,, ,,
244	,,	,,	0·22	4·55	,, ,, ,,
224	Para-chlor-meta-cresol 0·05%	,,	0·51	1·98	Exponential
229	,,	,,	0·45	2·22	,,
231	,,	,,	0·46	2·17	,,
233	,,	,,	0·43	2·32	,,
241	,,	,,	0·52	1·96	,,
245	,,	,,	0·46	2·17	,,
247	,,	,,	0·50	2·00	,,
242	,,	,,	0·36	2·78	Lag phase then exponential
269	Phenol 0·5%	*Bact. coli*	0·50	2·00	Exponential
262	,,	,,	0·32	3·11	Lag phase then exponential
267	,,	,,	0·30	3·33	,, ,, ,,
268	,,	,,	0·34	2·93	,, ,, ,,
267	Para-chlor-meta-cresol 0·05%	,,	0·32	3·11	,, ,, ,,

Table 13. *Values of λ and b from other observers' counts*

Reference	Poison	Organism	λ	$b=1/\lambda$	No. of figure in Appendix I
Chick (1908*a*, p. 108)	Mercuric chloride	*Bact. paratyphosum*	0·55	1·82	28
Lee & Gilbert (1918, Table IV)	Phenol	*Bact. typhosum*	0·38	2·63	29
Lee & Gilbert (1918)	Mercuric chloride	Anthrax spores	0·46	2·17	30
Chick (1910, p. 258)	Hot water	*Bact. coli*	0·48	2·08	31
Chick (1910, p. 260)	Hot water	*Bact. paratyphosum*	0·38	2·63	32
Chick (1908*a*, p. 97)	Phenol	Anthrax spores	0·55	1·82	33
Withell (this paper, Part II)	Phenol	*Micrococcus*	0·50	2·00	34
Lea, Haines & Coulson (1937)	Alpha particles	*B. mesentericus*	0·60	1·67	35
Brooks (1918)	Specific haemolytic serum	Red cells	0·48	2·08	36
Smith-Henderson (1921)	Phenol	*Botrytis* spores	0·30	3·33	37
Falk & Winslow (1926)	0·145 *M* calcium chloride	*Bact. coli*	0·46	2·17	38
Wyckoff (1932)	Ultra-violet light	*Colon bacilli*	0·40	2·50	39
Wyckoff & Rivers (1930)	Cathode rays	*Staph. aureus*	0·36	2·77	40
Wyckoff (1930)	X-rays	*Bact. coli*	0·52	1·97	41
Wyckoff (1930)	X-rays	*Bact. aertrycke*	0·44	2·27	42
Gates (1929*a*)	Ultra-violet light	*Staph. aureus*	0·45	2·23	43
Withell (this paper, Part II)	Para-chlor-meta-cresol	*Micrococcus*	0·43	2·32	44
Hewlett (1909)	Mercuric chloride	Mustard seeds	0·20	5·00	45
Oothuisen (1935)	Moist heat	Confused flour beetle	0·15	6·67	46

of λ ($=b$) is the most convenient estimation of the slope of the characteristic curve. The figures are given in Tables 12 and 13 above. Table 12 shows λ and b for my own experiments and Table 13 λ and b for all the experiments illustrated by diagrams in Appendix I. These figures will be referred to in the discussion which follows. Sufficient has been said now to show that the

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

logarithms of the survivor times are often more normally distributed than the survivor times themselves. The significance of this fact is discussed in the next section.

(*c*) *Discussion*

Theories advocated by different bacteriologists to explain the shape of time-survivor curves have recently been reviewed by Chick (1930), Rahn (1930) and Clark (1933). All the reviewers divide the theories into two groups. First, those which assume that the organisms are of equal resistance. This theory has lately been elaborated (Chick, 1930) to include a rhythmical internal change in the organization of the bacterial contents so that at one time only a portion of the organisms are liable to attack by the disinfectant molecules: the organisms are still supposed to be equal in resistance. It has been shown that this latter assumption has never been demonstrated in practice and is not in accord with available evidence. Though the second group of workers base their theories on a distribution of resistances, they have been unable to explain the distribution of resistances from an exponential type curve without assuming other facts. In Part IV (*b*) it is shown that if the resistances are considered logarithmically then many types of bacteriological time-survivor curves show a nearly normal distribution of resistances. In other words those cases which give an approximate normal distribution of survival times, and those which give a skew distribution of survival times, both give a nearly normal distribution of the *logarithms* of survival times. It has been shown that this is not confined to bacteriology, and besides the examples quoted in Part IV we can add that Gaddum (1940, p. 361) has pointed out that Alvarez's figures for distribution of blood pressure do not give a normal distribution of these blood pressures, but do so if the distribution of the logarithm of the blood pressure is considered.

If the resistances are distributed as we have indicated then all the types give very similar curves when plotted on a logarithmic time scale, whereas on a time scale they appear fundamentally different. When the slope of the normal curve is steep, i.e. when λ is small, then there will be few organisms of little resistance and few of great resistance—the bulk of the resistances will be more closely centred round the logarithmic mean. If this type of curve is integrated to give a time-survivor curve plotted against arithmetical units, then, after a lag phase the bulk of the organisms will die in a few units of time. When the percentage survivors are plotted against time, in arithmetical units, the lag phase may not be observed if the strength of the bactericide kills the organisms of little resistance in a very short time. Smith-Henderson (1921) has shown with *Botrytis* spores and Withell (1938) with *Bact. coli* that as the strength of bactericide is increased (in both cases from 0·5 to 0·75% phenol), a progression from a sigmoid curve to an exponential curve is obtained. That is why lag phase disappears. It probably only disappears because measurements of bacterial population cannot be performed at intervals of fractions of a minute or second. When a logarithmic time scale is used we are made aware of this fact.

When the slope of the characteristic curve of resistances is less steep (i.e. when λ is large), the resistances are more widely distributed and a bigger percentage of the organisms will have resistances farther from the logarithmic mean. When such a curve is integrated to give a time-survivor curve then the rate will increase more gradually. A sigmoid curve may be shown when percentage survivors are plotted against time, if the strength of the bactericide permits a count to be made early enough. When an arithmetic time scale is used, the apparent differences in the type of time-survivor curve depend on λ (or b) and the strength of the bactericide.

The different types of time-survivor curve which were found in Part II can all be explained by this type of argument involving resistance distribution on a logarithmic time basis. We do know that the slope of the curve (b) does vary for the sensitivities of animals. For example, Gaddum says: 'the results..., amply confirm Trevan's conclusion that different drugs may produce curves of very different slope. The slope of the curve is to a certain extent characteristic of the drug used, but variations in the details of the technique of the experiment have been found to produce definite variations in the order of the slope. Among the variables which may be controlled in order to increase the homogeneity of the animals are their genetic composition, weight, age, sex, diet and environmental temperature. The influence of some of these factors has been studied, but our knowledge of their relative importance is far from complete'. A glance at the figures in Tables 12 and 13 for the values of λ of different organisms suspended in different bactericides will show that they also vary, and it is this variation which is the root of the apparent differences when a time scale of natural numbers is used. For example, if the figures of λ for a single organism are considered (*Micrococcus*—phenol 0·5%, Table 12), the curve when plotted against an arithmetical time scale appears to be exponential when λ varies from 0·40 to 0·64, for this particular strength bactericide.

A lag phase appears to be shown when λ falls to values from 0·22 to 0·30. It is obvious that the differences observed when an arithmetical time scale is used are only apparent differences, and that all these curves show a fundamental resemblance when plotted on a logarithmic time scale.

The reciprocal of λ is directly proportional to the slope of the curve. Gaddum calls this function $b\ (=1/\lambda)$. λ is then a measure of the variability of the animals or organisms. In the case of bacteria when λ is large the resistance of organisms differs from the mean by a greater amount than when λ is small (see Tables 12 and 13).

The resistance distribution is always nearer normal when logarithms are used. Such deviations as have been observed from normal relate to relatively small proportions of organisms, which do not materially affect the normal distribution. This can be seen in the diagrams illustrating distributions of survivor times in Appendix I. It is unlikely that all suspensions of bacteria will have their logarithmic survivor times normally distributed because each

suspension will contain organisms of different ages, and since the resistance of an organism has been found to vary with its age, the normal distribution may be obscured. Somewhat similar 'abnormalities' have been found when animals of mixed genetic composition and weight are used. I have some evidence that in a few cases both probit-logarithm of time lines and probit-time lines are curved. In the cases I have examined, such deviations from the straight line do not affect greatly the normal distribution, and I hope to make a more thorough search of the literature to discover more examples of such deviations.

Smith-Henderson (1921, 1923) has mentioned logarithms in connexion with survival times but did not show their approximately normal distribution. His theory was advanced to account for the skew type of distribution which has now been shown to be common in biological measurements. He maintains that the extremely skew curves found from exponential rates are due to the plotting of resistances in terms of survivor times, a practice which he regards as unjustifiable. If R is the resistance and T is the time taken to kill, then if

$$\frac{R}{\log T} = k,$$

these skew curves are not obtained. This expression, he suggested, is not necessarily the correct expression, but does show that if the alteration in the rate of reaction between cells and poison is of the above nature then it is possible to obtain a 'logarithmic' (i.e. exponential) survivor curve from a frequency distribution which is approximately normal. Although he used this relationship he did not show that the logarithm of survivor times was normally distributed, and his formula related to the time taken for the poison to penetrate the cell wall or perform some other function leading to the death of the cell. It is well, however, to remember that Smith-Henderson pointed out that resistance distribution was the main factor in determining the shape of time survivor curves. His use of the formula

$$\frac{R}{\log T} = k$$

implies that the resistance of an organism is proportional to the logarithm of the survival time. As far as I am aware there is no theoretical or practical evidence that this is true.

The shape of observed time-survivor curves in bacteriology, whether spores or vegetative organisms are concerned, are of three general types:

(1) The exponential type, i.e. death-rate constant when an arithmetical time scale is used.

(2) The type with a lag phase, then a quicker, exponential rate. Here, death-rate increases.

(3) The type where the death-rate falls off throughout the reaction. This type is rarely seen, and one set of figures is shown in Fig. 28 drawn on a probit-log time graph (Chick, 1908).

All these types of curve have been shown to yield approximately normal distribution of the logarithm of survivor times and are all capable of similar explanation, viz. that the survivor time is proportional to resistance and that the logarithm of the survival time is normally distributed. Variations observed in the shape of time-survivor curve on an arithmetical time scale can be explained by variations in the logarithmic standard deviations and by the use of bactericides of different strengths.

(*d*) *Summary and conclusions*

This work has been concerned with the response of bacteria to various disinfectants. Variations in the response of organisms are most clearly seen by constructing time-survivor curves from the counts showing the death-rate. The literature has been reviewed, and in Table 1 the work of other observers is tabulated. The types of response obtained by these workers can be classified according to the shape of the time-survivor curve recorded.

There are three general types of response:

(*a*) those which give sigmoid time-survivor curves,

(*b*) those which give exponential time-survivor curves, and

(*c*) those which give a lag phase followed by an exponential curve.

The problem was to find a rational explanation for the occurrence of all these types of curve and also to explain why the same system of organism and reagent will give varying time-survivor curves when the experiment is repeated a number of times.

In Part II a number of experiments are recorded using a *Micrococcus* and *Bact. coli*. Two bactericides, phenol 0·5% and para-chlor-meta-cresol 0·05%, were used in each case. A constant response to these bactericides was not observed, although the experiments were performed in as nearly an identical manner as possible. The *Micrococcus*, when immersed in each bactericide, gave, at first, counts which agreed very well with an exponential rate. Later experiments showed a more or less well-marked lag phase. *Bact. coli* showed with each bactericide a lag phase, but the extent of the lag varied.

In Part III each set of time-survivor curves has been analysed and the mean curve constructed, by calculating the mean slope of the time-survivor curve, in stages over the course of the reaction. These mean curves showed the same variations as the individual time-survivor curves, and still left unsolved the problem of the explanation of this variation in response.

Present theories which attempt to explain this variation in response are reviewed in Part IV. The 'mechanistic' theory assumes that the organisms are all of equal resistance. When time-survivor curves are exponential it is assumed that death is due to one event, and when other time-survivor curves approaching sigmoid types are obtained, it is assumed that death is due to more than one event. These assumptions have been shown to be not in accord with the available evidence.

Vitalist theories assert the variation of time-survivor curves as due to a

variation in the way the resistances are distributed. Workers who hold this view have assumed that when resistances are measured by survival times, the survival times should be normally distributed. The apparent difficulty in this theory is that the survival times are nearly always distributed in an extremely skew manner—most of the organisms dying in the first few units of time.

I have shown that all types of time-survivor curve yield an approximately normal distribution if the survival times are plotted as logarithms instead of absolute numbers. This is true whether the distribution of survivor times is normal or skew. In Part IV all the experiments recorded in Parts II and III, and in Appendix I, the work of other observers are shown to yield a much better approximation to the normal curve when a logarithmic time scale is used.

The use of a logarithmic time scale agrees with pharmacological data, and more especially with the work of Gaddum, who has shown that the variation in animal response to a stimulus is usually more normally distributed when logarithms are used.

The shape of the characteristic curve for any suspension of organisms in a particular experiment can be calculated from the logarithmic standard deviation (λ) of the survivor times. I have tabulated values of λ for my own series of experiments and for those of other workers, when the counts permitted the calculation of such values. From these values for a series of experiments with the same organism and bactericide, it has been shown that the 'fundamental' difference in shape of time-survivor curves obtained when arithmetic units are used is only apparent. All the shapes of curve found in the literature are due to variations in the way the resistances of the organisms are distributed. This distribution is measured by the standard deviation of the logarithm of the survivor times. When λ varies then:

(*a*) When an arithmetical time scale is used, fundamentally different equations can be fitted to the time mortality data, in different cases.

(*b*) When a logarithmic time scale is used, a variation of λ is shown by a variation in the steepness of the time-survivor curve.

Apart from the way λ varies, the only other factor which is of importance in determining the shape of the curve is the strength of the bactericide. Occasionally the bactericide acts so quickly that no counts can be made early in the reaction. Such an experiment yields little information about the course of the reaction.

The main conclusion of this work is that the different rates of destruction of bacteria under the influence of a bactericide is determined essentially by differences in the manner in which the resistances of the organisms are distributed.

It is with great pleasure that I record my thanks to Prof. G. S. Wilson and Prof. J. H. Gaddum. I have received invaluable assistance from them both. I wish also to mention H. Hadley, Esq., P. Dormer, Esq., and Miss E. Grigg for technical and preparation work cheerfully undertaken.

V. Appendix I, comprising Figs. 28–46

Each diagram is constructed on a common plan. The right-hand portion shows probit-logarithm of time curve (lower curve) and probit-time curve (upper curve). The central diagram shows the distribution of logarithmic survivor times and the right-hand distribution diagram shows survivor times. λ and b for each diagram are shown in Table 13.

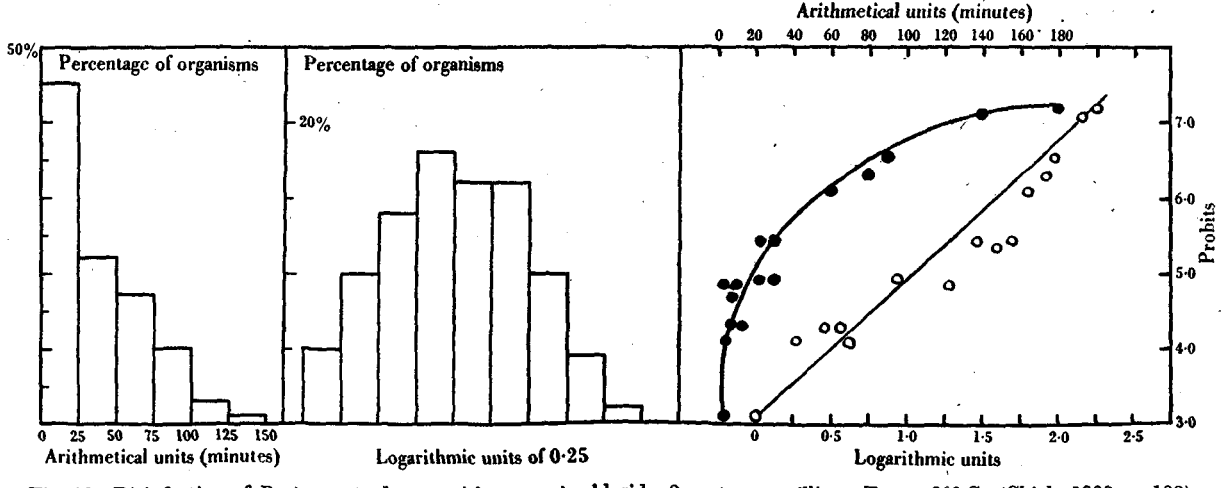


Fig. 28. Disinfection of *Bact. paratyphosum* with mercuric chloride, 2 parts per million. Temp. 20° C. (Chick, 1908, p. 108).

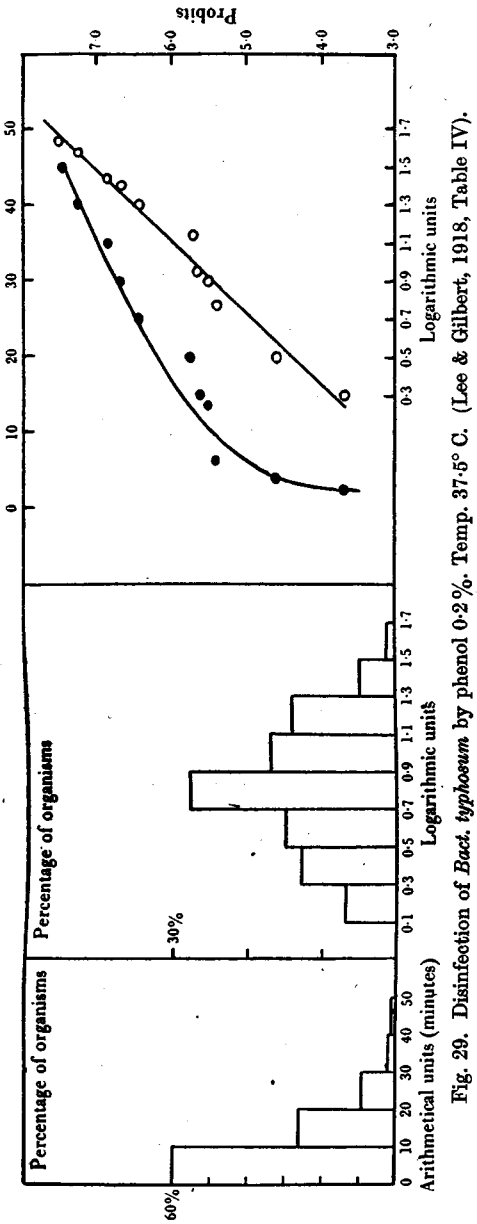


Fig. 29. Disinfection of *Bact. typhosum* by phenol 0·2%. Temp. 37·5° C. (Lee & Gilbert, 1918, Table IV).

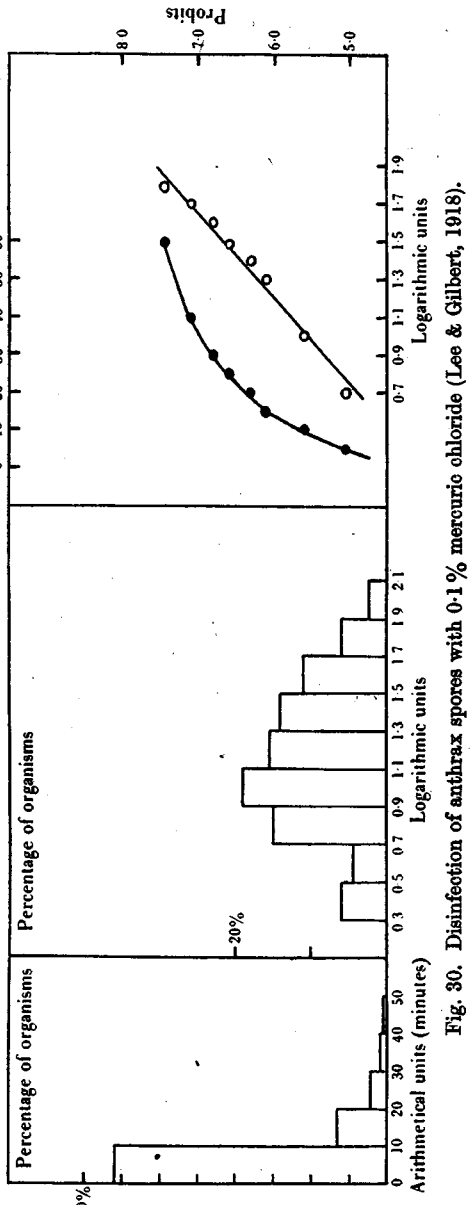


Fig. 30. Disinfection of anthrax spores with 0·1% mercuric chloride (Lee & Gilbert, 1918).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

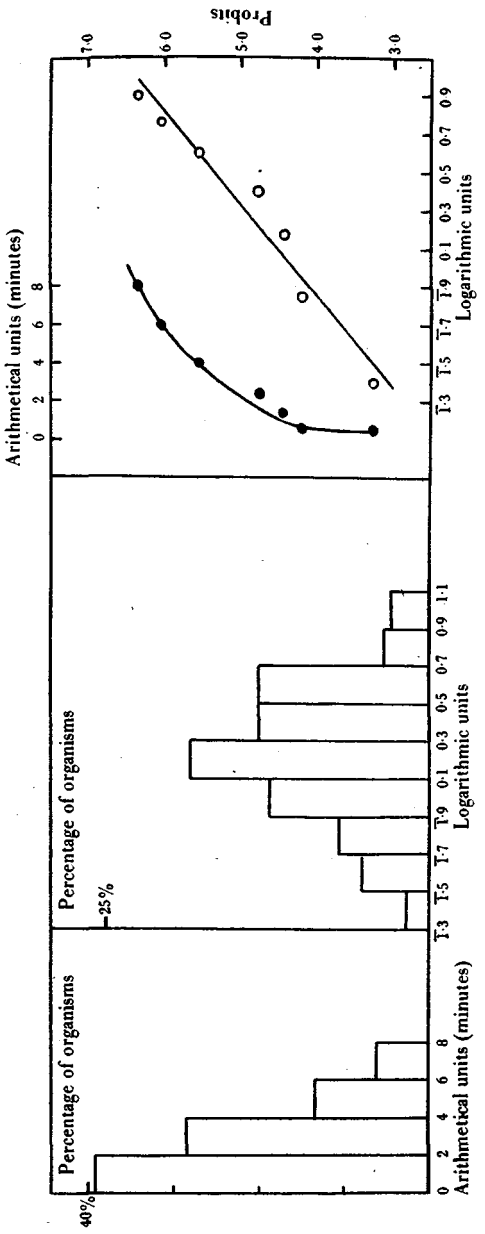


Fig. 31. Disinfection of *Bact. coli* by hot water. Temp. 52·5° C. (Chick, 1910, p. 258.)

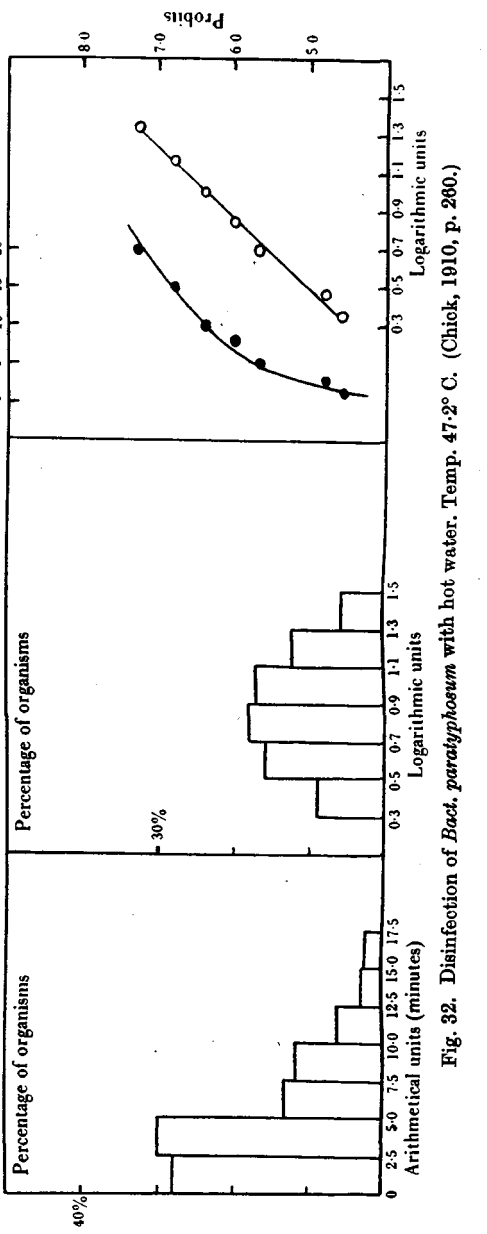


Fig. 32. Disinfection of *Bact. paratyphosum* with hot water. Temp. 47·2° C. (Chick, 1910, p. 260.)

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

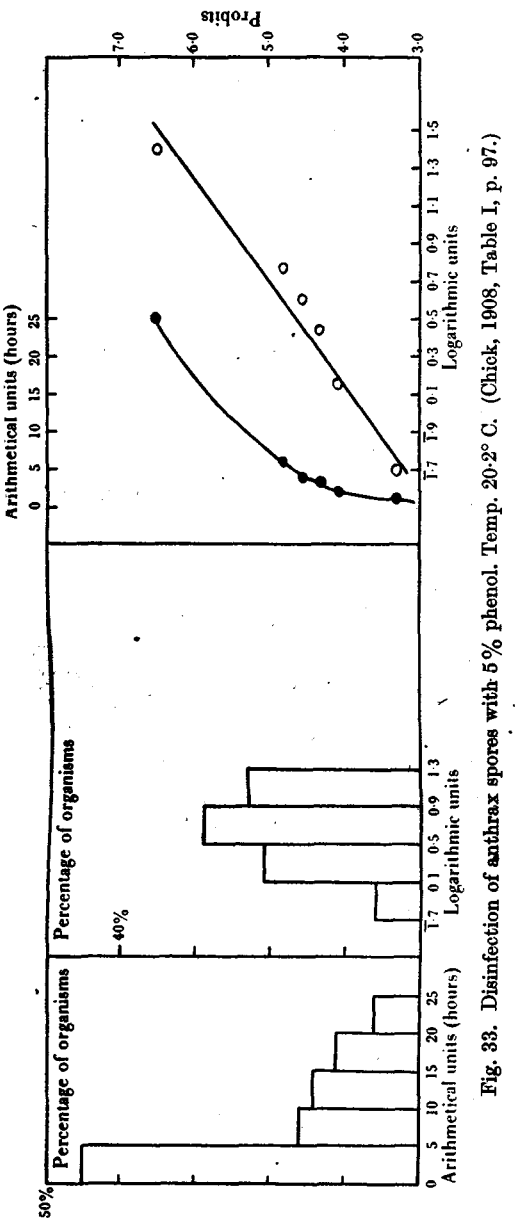


Fig. 33. Disinfection of anthrax spores with 5% phenol. Temp. 20·2° C. (Chick, 1908, Table I, p. 97.)

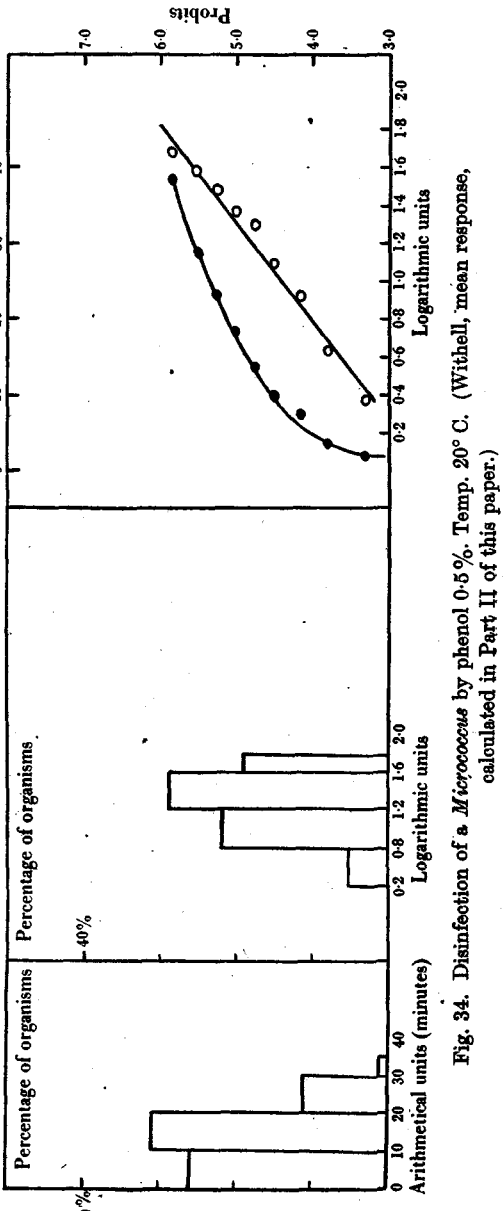


Fig. 34. Disinfection of a *Micrococcus* by phenol 0·5%. Temp. 20° C. (Withell, mean response, calculated in Part II of this paper.)

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

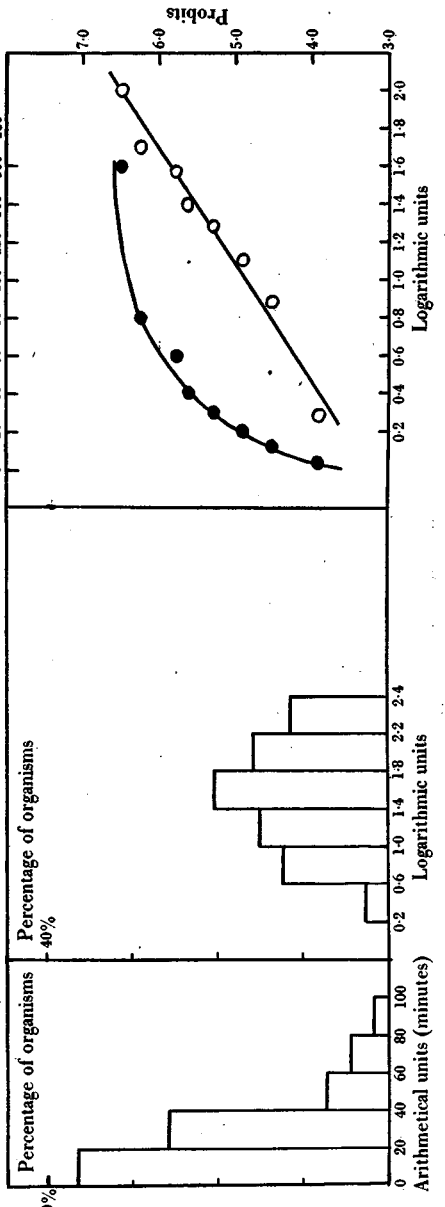


Fig. 35. Destruction of *B. mesentericus* spores with alpha particles (Lea *et al.* 1937).

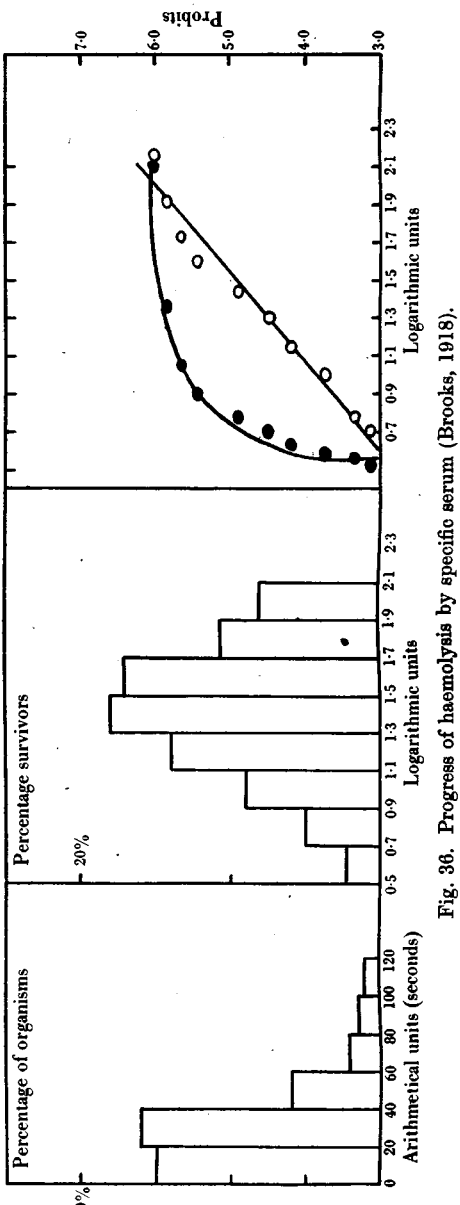


Fig. 36. Progress of haemolysis by specific serum (Brooks, 1918).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

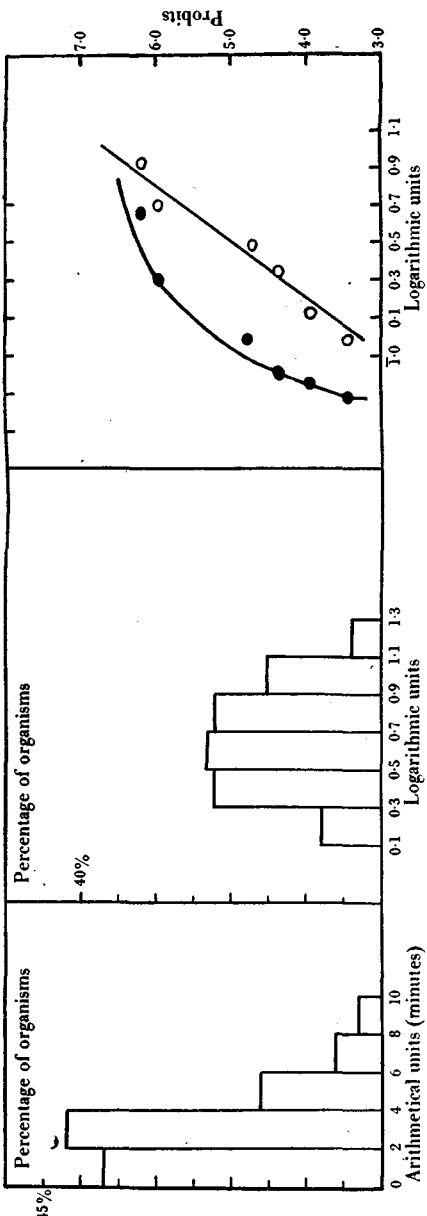


Fig. 37. Disinfection of *Botrytis* spores by 0·7% phenol (Smith-Henderson, 1921).

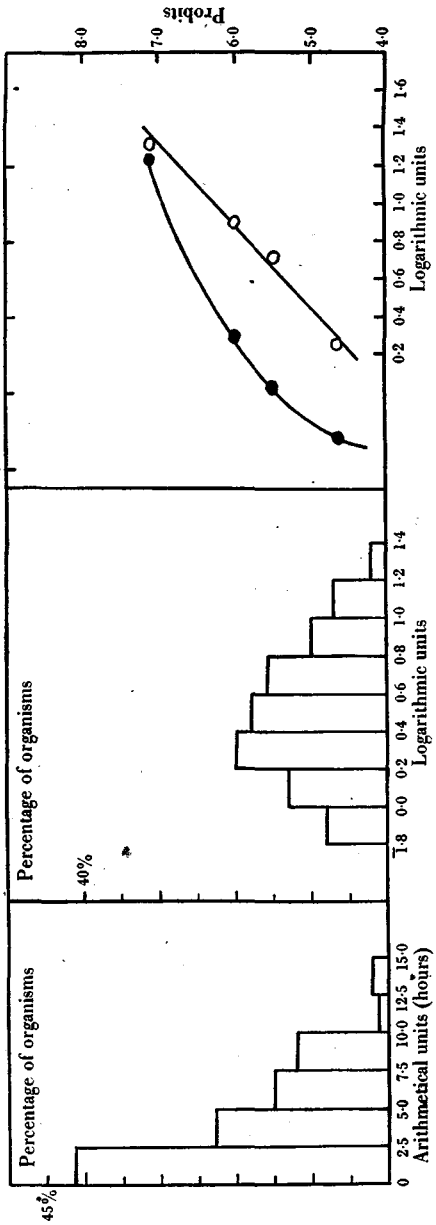


Fig. 38. Viability of *Bact. coli* in 0·145 *M* calcium chloride (Falk & Winslow, 1926).

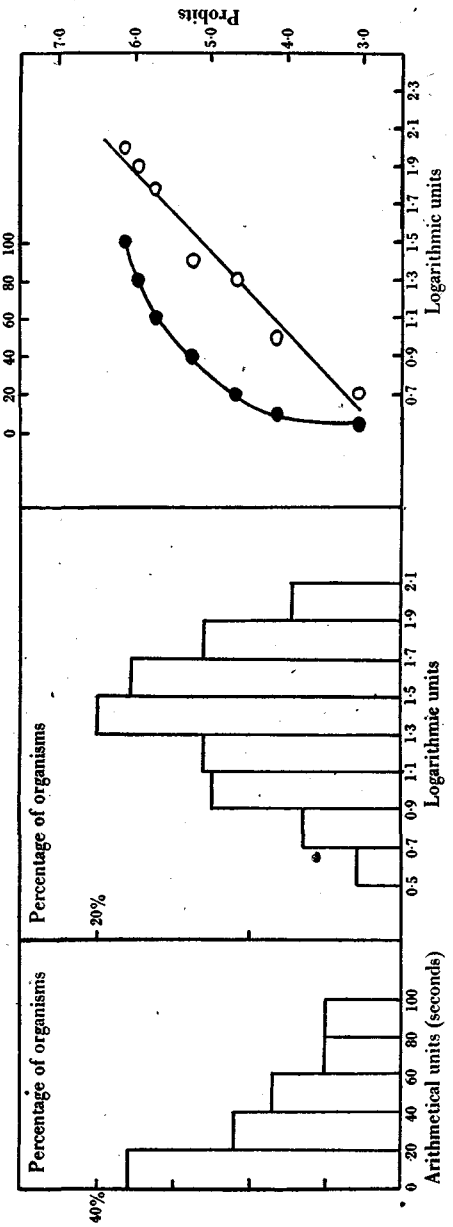


Fig. 39. Destruction of colon bacilli by ultra-violet light (Wyckoff, 1932).

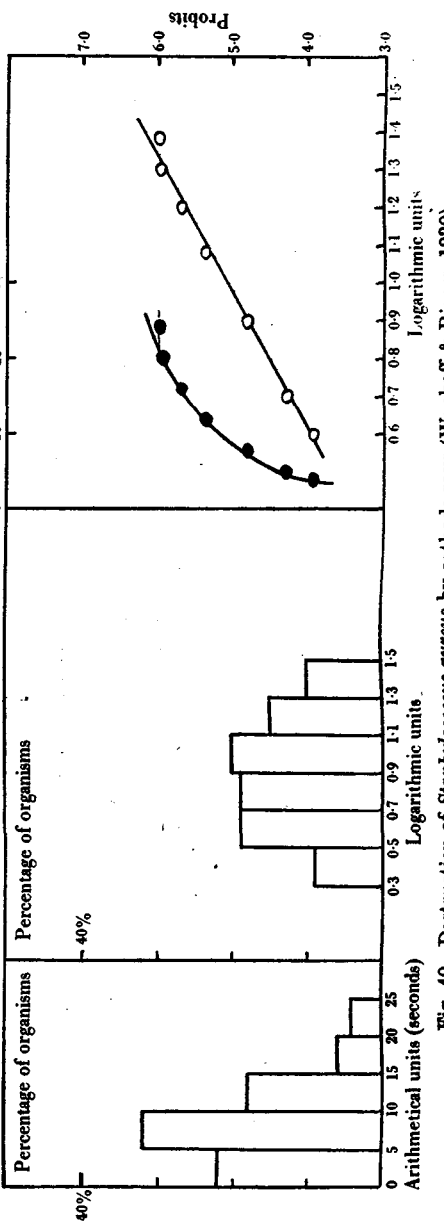


Fig. 40. Destruction of *Staphylococcus aureus* by cathode rays (Wyckoff & Rivers, 1930).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

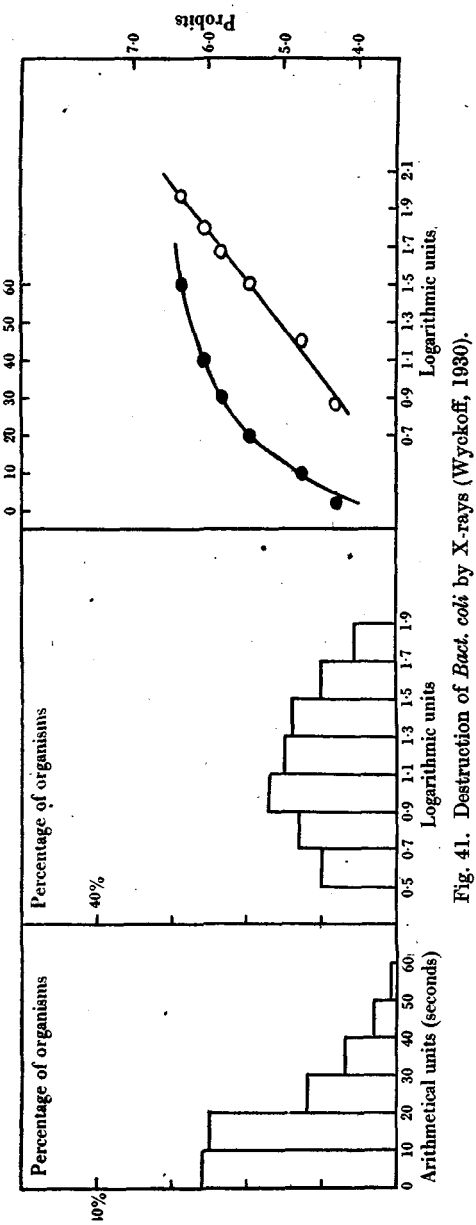


Fig. 41. Destruction of *Bact. coli* by X-rays (Wyckoff, 1930).

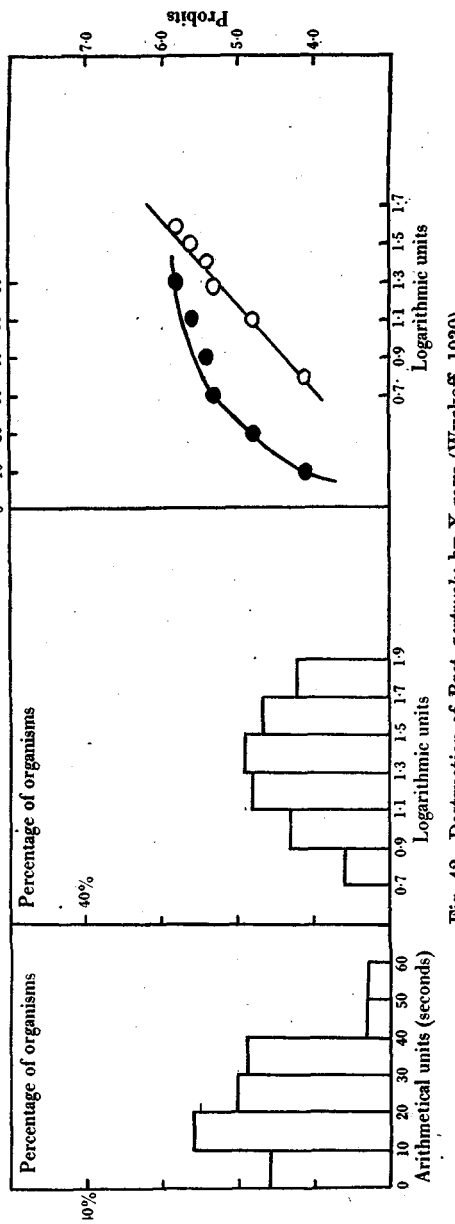


Fig. 42. Destruction of *Bact. aertryche* by X-rays (Wyckoff, 1930).

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

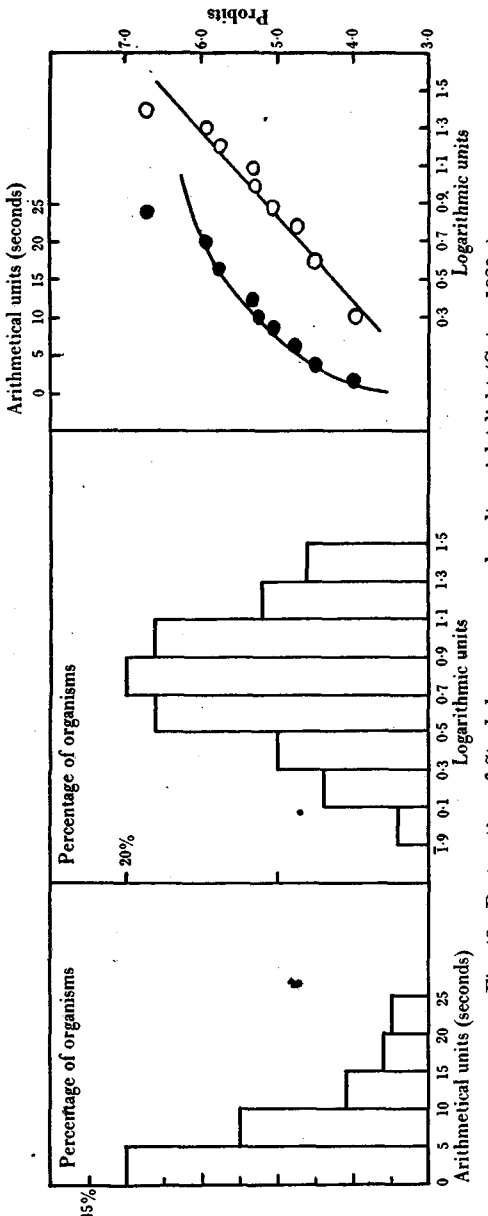


Fig. 43. Destruction of *Staphylococcus aureus* by ultra-violet light (Gates, 1929*a*).

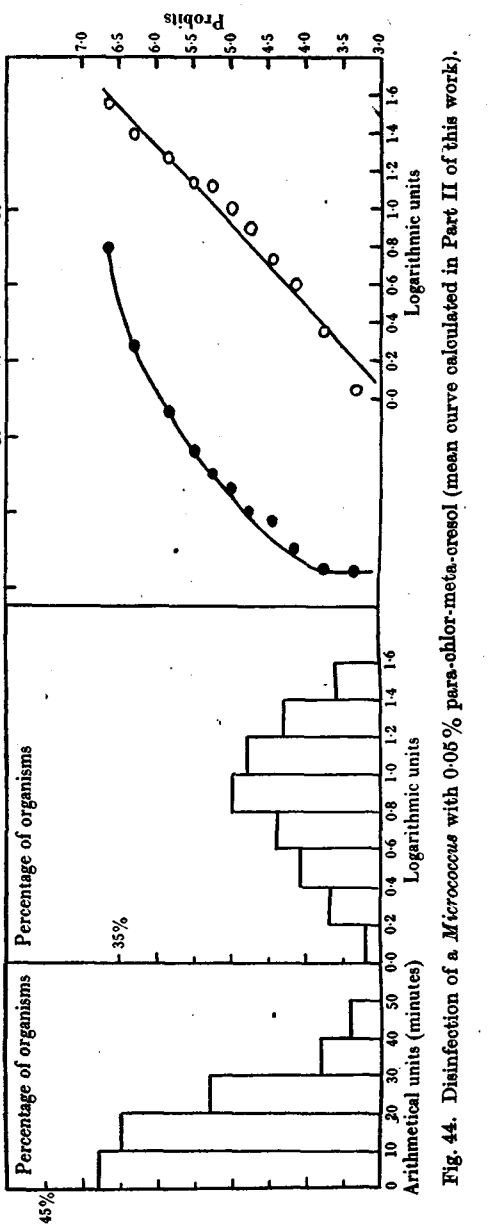


Fig. 44. Disinfection of a *Micrococcus* with 0·05% para-chlor-meta-cresol (mean curve calculated in Part II of this work).

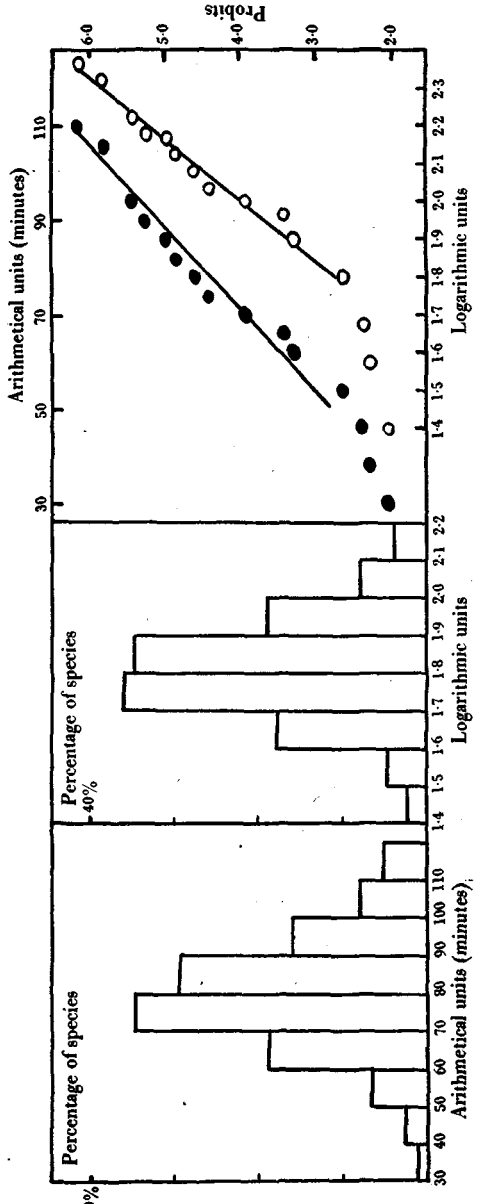
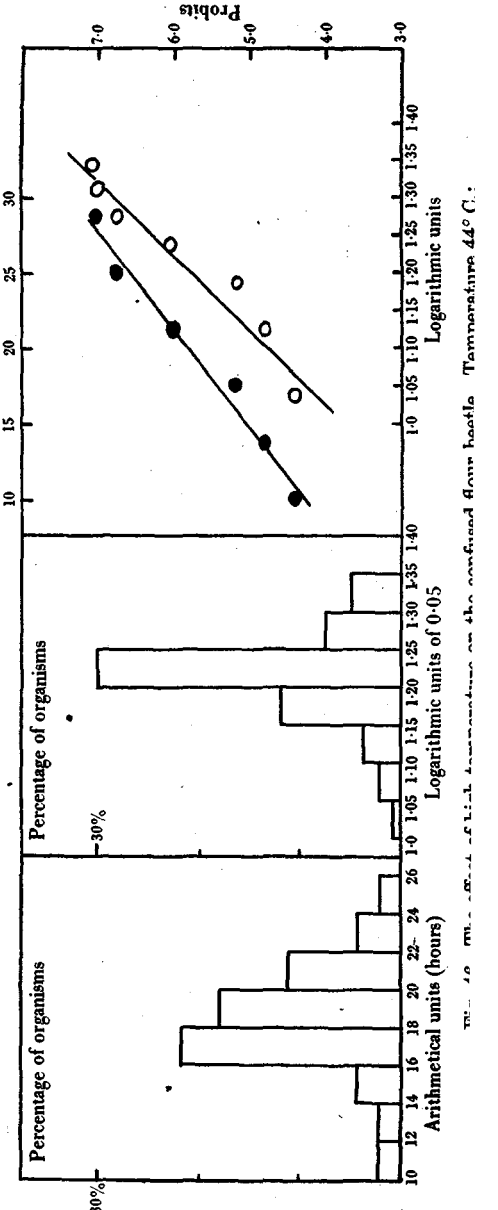


Fig. 45. Death of mustard seeds in 0·2% mercuric chloride (Hewlett, 1909).



VI. Appendix II

(*a*) Reactions between phenol 0·5% and *Micrococcus.*

(*b*) Reactions between para-chlor-meta-cresol 0·05% and *Micrococcus.*

(*c*) Reactions between phenol 0·5% and *Bact. coli.*

(*d*) Reactions between para-chlor-meta-cresol 0·05% and *Bact. coli.*

(*e*) Experiments showing the bactericidal effect of diluents autoclaved *with rubber washers.*

(*a*) *Reactions between phenol* 0·5% *and* Micrococcus *spp.*

Temp. 20° C.

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
224	0.0	88	85	64	83	90	82	3·0973	102,600
	1.0	85	70	70	79	85	78	3·0973	97,590
	2.0	56	72	69	56	73	65	3·0973	81,320
	3.0	64	63	80	87	66	72	3·0973	90,070
	4.0	59	65	68	104	74	64	3·0973	80,070
	5.0	54	54	48	67	60	57	3·0973	71,320
	10.0	40	41	41	51	44	43	3·0973	53,800
	15.0	32	31	23	41	24	30	3·0973	37,540
	25.0	19	14	18	23	28	20	2·7965	12,510
	41.0	4	10	3	1	17	7	2·7965	4,381
229	0.0	138	149	152	134	—	143	3·0973	178,800
	12.0	48	89	97	91	58	77	3·0973	85,860
	24.5	23	16	33	30	—	25	3·0973	31,270
	30.0	26	25	23	22	13	21·8	2·7890	13,360
	41.0	11	15	16	14	24	16	2·5051	5,119
	45.0	29	38	26	36	43	34·4	2·2043	5,507
231	0.0	183	147	142	162	129	153	5·1079	19,620,000
	10.0	81	105	156	88	61	98	5·1079	12,560,000
	20.0	197	243	294	209	368	262	4·5136	8,549,000
	40.0	251	259	309	317	—	284	4·2204	4,718,000
	50.0	294	360	398	—	—	350	3·9275	2,962,000
230	0.0	141	136	153	125	117	134	5·1291	18,040,000
	5.0	101	106	86	109	110	102	5·1291	13,730,000
	10.0	94	100	106	110	93	101	5·1291	13,560,000
	15.0	95	116	92	69	69	86	5·1291	11,580,000
	21.0	67	67	71	28	42	55	5·1291	7,404,000
	40.0	133	133	130	138	—	134·5	4·5248	4,492,000
	54.0	158	149	167	174	161	162	4·2398	2,814,000
233	0.0	295	356	345	298	384	336	3·0973	420,000
	5.0	310	234	328	312	332	303	3·0973	378,000
	10.0	297	325	290	345	309	293	3·0973	366,600
	15.0	267	282	151	258	262	244	3·0973	305,300
	20.0	449	447	442	419	440	439	2·7789	263,800
	30.0	588	719	619	750	669	669	2·4857	204,200
234	0.0	52	70	75	56	76	65·8	3·0973	82,310
	5.0	49	44	62	52	50	51·4	3·0973	64,310
	10.0	37	37	16	33	44	33·4	3·0973	41,780
	19.0	35	51	51	39	55	46·2	2·7900	28,480
	24.0	29	30	26	35	48	33·6	2·7900	20,710
	30.0	61	60	60	69	35	57·0	2·5051	18,240
236	0.0	213	209	203	220	222	213·4	3·0973	267,000
	10.0	169	140	219	163	165	171·2	3·0973	214,200
	20.0	125	137	137	123	145	133·4	3·0973	166,900
	30.0	126	95	95	103	79	99·6	3·0973	124,700
	40.0	88	81	83	100	87	87·8	3·0973	109,800
	50.0	79	109	114	106	146	110·8	2·7900	68,140

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

(*a*) *Reactions between phenol* 0·5% *and* Micrococcus *spp.* (continued)

Temp. 20° C.

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
237	0.0	171	162	163	173	177	169	3·0973	211,500
	10.0	83	91	173	174	174	139	3·0973	173,900
	20.0	163	163	143	156	107	146·2	3·0973	186,900
	30.0	183	214	171	241	234	208·6	2·7900	128,600
	40.0	194	198	169	154	121	171·2	2·7900	105,500
	50.0	319	370	307	300	297	316	2·5051	101,000
238	0.0	128	116	112	114	93	112·6	5·1291	15,180,000
	15.0	62	93	96	91	77	80·4	5·1291	10,810,000
	30.0	90	53	84	66	77	74	5·1291	9,961,000
	45.0	149	163	170	155	128	153	4·8218	10,150,000
	60.0	255	259	192	—	—	235	4·5369	8,091,000
241	0.0	181	208	212	192	195	197·6	3·0973	247,400
	50.0	170	206	224	162	164	185·2	3·0973	231,700
	60·0	116	154	112	138	—	130	2·7900	80,150
	75.0	76	88	72	71	65	74·0	2·7900	45,620
242	0.0	229	256	255	255	—	249	3·0973	311,600
	30.0	230	217	216	197	—	215	3·0973	268,900
	60.0	148	91	104	122	—	116·3	3·0973	145,500
	75.0	131	137	144	116	—	132	2·7900	81,390
244	0.0	145	147	154	123	—	142	3·0761	166,800
	10.0	129	142	145	131	—	138	3·0761	164,000
	20.0	129	145	130	146	—	138	3·0761	164,000
	30.0	114	114	94	110	—	108	2·9881	105,100
	40.0	104	95	101	—	—	100	3·0761	119,100
245	0.0	314	262	260	275	252	271	3·0973	337,800
	15.0	220	258	246	220	249	238·6	3·0973	299,000
	30.0	226	208	209	224	212	216	3·0973	270,300
	45.0	176	181	133	115	—	151	3·0973	188,900
	60.0	141	138	135	135	159	141·6	3·0973	176,400
	75.0	122	100	100	152	144	123·6	3·0973	154,600
	90.0	147	154	96	103	17	103·4	3·0973	129,300
	105·0	98	76	80	116	110	98	3·0973	122,600
	120.0	219	225	150	163	30	157·4	2·7900	97,050
247	0.0	233	264	230	264	248	248	3·0973	312,400
	15.0	224	222	212	211	—	217	3·0973	271,500
	30.0	193	204	184	147	98	165	3·0973	206,500
	45.0	181	167	138	165	163	163	3·0973	204,400
	60.0	151	140	127	132	—	137·5	3·0973	172,000
	75.0	104	84	107	73	97	93	3·0973	116,300
	90.0	64	70	67	61	63	65	3·0973	81,320
	105.0	54	67	60	53	62	59	3·0973	73,810
	135.0	31	38	34	28	24	31	3·0973	38,790
	150.0	19	23	17	17	20	19	3·0973	23,780
	165.0	13	27	19	22	16	19·4	2·7900	11,960
	190.0	10	13	4	8	10	9·0	2·7900	5,526
	250.0	3	7	11	8	2	6·2	2·5051	1,981
249	0.0	295	269	236	297	—	274	5·1291	36,890,000
	30.0	201	186	159	186	—	183	5·1291	24,630,000
	60.0	140	141	102	—	—	128	5·1291	17,230,000
	90.0	117	116	119	122	107	116	5·1291	15,630,000
	120.0	103	102	110	108	88	102	5·1291	13,730,000
	153.0	52	57	49	51	—	52	5·1291	7,000,000
	180.0	29	33	30	31	28	30	5·1291	4,038,000
	210.0	18	11	19	15	—	15	5·1291	2,154,000

(*b*) *Reactions between para-chlor-meta-cresol* 0·05% *and* Micrococcus *spp.*

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
224	0.0	204	255	178	214	227	216	3·0761	257,400
	4.0	187	171	126	88	107	136	3·0761	162,000
	8.0	139	101	119	114	111	117	3·0761	139,400
	12.0	65	46	42	72	46	54	3·0761	64,190
	17.0	36	39	38	23	22	32	3·0761	38,130
	22.0	13	23	22	22	24	21	3·0761	25,020
	27.0	32	23	14	19	19	20·4	2·7789	12,860
	32.0	86	46	21	34	29	43·2	2·1849	6,613
	37.0	34	32	32	58	12	33·6	2·1849	5,131
229	0.0	145	161	86	90	110	118	3·0761	140,600
	10.0	21	27	45	43	26	32	3·0761	38,130
	20.0	13	9	12	7	—	10·25	3·0761	12,210
	28.0	6	8	7	3	6	6·0	2·4857	1,836
	40.0	2	2	11	1	8	4·8	2·1849	734·7
231	0.0	100	125	114	130	114	127	5·1291	17,020,000
	15.0	18	27	22	31	5	20·6	5·1291	2,775,000
	30.0	4	7	9	8	23	10·2	5·1291	1,373,000
	40.0	27	11	37	13	26	23	4·5248	770,000
	50.0	16	37	7	45	31	27	4·2398	469,000
	60.0	17	59	35	42	63	43	3·9496	380,600
	70.0	35	52	23	27	37	35	3·9496	309,700
233	0.0	305	318	324	323	305	315	3·0761	375,300
	5.25	196	237	221	223	245	244	3·0761	290,700
	11.0	153	187	177	156	186	172	3·0761	204,900
	17.0	128	158	147	175	135	149	3·0761	177,500
	22.0	203	253	198	200	146	200	2·7789	120,100
	30.0	349	353	292	287	227	301·6	2·4857	92,300
236	0.0	212	221	193	230	244	220	3·0761	262,100
	10.0	156	156	144	128	146	146	3·0761	174,000
	20.0	119	120	128	113	96	115·2	3·0761	137,200
	30.33	76	111	88	105	92	94·2	3·0761	112,300
	40.0	167	122	137	163	80	133·8	2·7789	87,380
	50.0	195	149	188	211	270	202·6	2·4857	62,000
237	0.0	151	174	153	138	181	159·4	3·0761	189,800
	10.0	72	134	58	118	—	98·0	3·0761	116,800
	20.0	89	93	89	113	78	92·4	3·0761	110,100
	30.0	172	123	169	142	166	154·4	2·7789	92,790
	40.0	46	113	159	136	138	118·4	2·7789	71,170
	50.0	278	239	246	212	—	244	2·4857	74,660
238	0.0	139	128	133	141	141	136·5	5·1079	17,500,000
	15.0	65	86	59	88	52	70·0	5·1079	8,974,000
	30.0	79	57	54	66	80	67·2	5·1079	8,616,000
	45.0	85	79	80	78	61	76·6	4·8107	4,953,000
	60.0	132	201	153	142	—	157	4·5175	5,169,000
241	0.0	231	238	269	222	246	241·2	3·0761	287,000
	15.0	190	175	183	175	—	180·8	3·0761	215,400
	30.0	141	133	113	129	—	129·0	3·0761	153,700
	45.0	120	99	88	106	90	101·8	3·0761	121,300
	61.0	118	92	98	96	97	100·2	2·7789	60,230
	75.0	70	66	76	68	64	68·8	2·6323	29,500
	98.0	58	53	43	40	42	47·2	2·4857	14,440
242	0.0	254	255	241	250	—	250	3·0761	297,900
	15.0	175	187	187	—	—	183	3·0761	218,100
	30.0	212	234	239	245	—	232·5	3·0761	277,600
	45.0	143	138	131	160	—	143	3·0761	170,400
	60.0	85	95	83	95	72	86	3·0761	102,400
	75.0	140	108	109	99	134	108	2·7789	64,910
244	0.0	222	207	268	216	—	223	3·0973	279,000
	10.0	190	185	201	199	—	194	3·0973	242,800
	20.0	185	187	219	213	188	198	3·0973	247,700
	30.0	125	137	157	118	113	130	3·0973	162,700
	40.0	106	97	84	—	—	96	3·0973	120,100

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

(*b*) *Reactions between para-chlor-meta-cresol* 0·05 % *and* Micrococcus *spp.* (continued)

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
245	0.0	291	258	251	279	219	255·4	3·0761	304,300
	15.0	215	198	201	235	—	212·0	3·0761	252,500
	30.0	216	146	185	155	—	175·5	3·0761	209,000
	45.0	142	124	130	142	142	136·0	3·0761	162,000
	60.0	104	108	82	—	—	98·0	3·0761	116,800
	75.0	115	104	103	114	—	109·0	3·0761	129,900
	90.0	86	101	53	76	42	71·6	3·0761	85,330
	120.0	165	120	98	52	47	96·4	2·7789	57,940
247	0.0	259	244	293	259	223	255	3·0761	303,800
	15.0	164	161	156	—	—	160	3·0761	191,100
	30.0	94	88	83	90	—	88·8	3·0761	83,990
	60.0	27	15	22	47	—	28	3·0761	26,510
	75.0	20	21	18	22	—	20·25	3·0761	24,070
	90.0	7	10	9	5	5	9·0	3·0761	10,730
	105.0	3	1	5	6	3	3·6	3·0761	4,289
	120.0	2	1	1	0	3	1·2	3·0761	1,430
	135.0	0	0	2	1	0	0·4	3·0761	488
249	0.0	267	303	306	254	283	286	5·1079	36,670,000
	30.0	183	189	173	180	—	181	5·1079	23,200,000
	60.0	143	123	109	124	133	126	5·1079	16,150,000
	90.0	143	104	116	86	86	107	5·1079	17,270,000
	120.0	81	61	82	—	—	74	5·1079	9,486,000
	157.0	48	45	28	26	—	39	5·1079	5,000,000
	180.0	59	73	82	70	—	71	4·5136	2,317,000
	210.0	16	27	9	24	15	16	5·1079	2,651,000

(*c*) *Reactions between phenol* 0·5 % *and* Bact. coli

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
262	0.0	125	140	155	136	128	136·6	5·1291	17,160,000
	1.0	133	127	118	123	127	125·6	5·1291	16,900,000
	2.0	117	129	96	121	108	114·2	5·1291	15,380,000
	3.0	97	98	107	96	100	99·6	5·1291	13,410,000
	4.0	90	102	108	108	99	101·2	5·1291	13,610,000
	5.0	103	94	91	82	95	93·0	5·1291	12,520,000
	11.0	147	143	167	145	108	142·0	4·5369	4,889,000
	16.0	169	100	150	116	165	140·0	3·9469	1,239,000
	20.0	120	113	108	—	—	114·0	3·6458	504,300
267	0.0	273	299	280	301	285	287·6	3·0973	359,800
	5.0	100	127	125	91	80	104·6	3·0973	130,900
	10.0	13	22	23	25	33	23·2	3·0973	29,160
	15.0	3	4	3	4	2	3·2	3·0973	4,001
	20.0	1	0	1	0	—	0·5	3·0973	625·[illegible]
	25.0	1	1	0	0	0	0·4	2·7900	246·[illegible]
	30.0	0	1	0	0	—	0·25	2·7900	154·[illegible]
	40.0	0	1	0	0	0	0·20	2·5051	63·[illegible]
268	0.0	221	269	250	266	—	201·2	3·0973	251,700
	5.0	62	124	112	124	74	99·2	3·0973	124,100
	10.0	11	13	17	18	17	15·6	3·0973	19,510
	15.0	4	2	6	2	1	3·0	3·0973	3,753
	20.0	2	0	1	1	1	1·0	3·0973	1,251
269	0.0	273	272	317	320	—	295·5	3·0973	369,700
	5.5	231	225	240	217	270	236·6	3·0973	296,000
	16.0	293	271	229	316	—	277·0	2·7900	170,800
	22.0	190	232	247	222	—	222·8	2·7900	137,400
	27.0	339	395	453	—	—	396·0	2·5051	126,700
	32.0	267	322	—	—	—	294·5	2·3373	64,010

(*d*) *Reactions between para-chlor-meta-cresol* 0·05% *and* Bact. coli

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
262	0.0	159	173	156	156	178	164·4	5·1079	21,080,000
	1.0	106	116	99	109	140	114	5·1079	14,602,000
	2.0	148	153	164	131	—	149	5·1079	19,100,000
	3.0	145	126	102	117	118	141·8	5·1079	18,160,000
	4.0	100	120	116	83	—	104·8	5·1079	13,440,000
	5.0	107	104	102	112	110	107	5·1079	13,690,000
	10.0	247	289	301	276	—	278·3	4·5175	9,158,000
267	0.0	252	288	287	315	290	286·4	3·0761	341,300
	5.0	154	169	162	166	148	159·8	3·0761	190,400
	10.0	43	39	66	59	68	55·0	3·0761	65,540
	15.0	16	24	26	14	19	19·8	3·0761	23,590
	20.0	6	4	6	4	7	5·2	3·0761	6,197
	25.0	2	5	5	2	—	3·5	2·7789	2,104
	30.0	0	7	0	0	0	1·4	2·7789	841·4
	40.0	0	0	2	—	—	0·66	2·4857	201·8
268	0.0	134	147	156	236	—	168·3	3·0761	200,400
	5.0	66	51	39	39	51	49·2	3·0761	58,620
	10.0	4	4	5	7	7	5·2	3·0761	6,195
	15.0	1	3	2	2	1	1·8	3·0761	2,145
	20.0	2	1	0	0	2	1·0	3·0761	1,191
	25.0	1	1	2	2	—	1·2	2·7789	721·3
	30.0	1	1	1	0	0	0·6	2·7789	286·5
269	0.0	227	197	254	267	276	244·2	3·0761	291,000
	5.0	201	242	179	208	183	202·6	3·0761	241,400
	10.0	178	182	183	155	150	169·6	3·0761	202,100
	15.0	229	228	208	—	—	221·6	2·7789	133,200
	26.0	230	229	209	226	—	223·5	2·4857	68,390
	31.0	224	234	232	—	—	230·0	2·3170	47,720

(*e*) *Experiments showing bactericidal effect of Ringer solution, after sterilization, on* Micrococcus *spp.*

(i) Ringer sterilized by filtering bulk (5 l.) through Doulton filter candle and filled into sterile bottles

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
213 A	0.0	222	248	290	218	221	240	0·733	1349
	20.0	266	315	290	365	227	273	0·733	1476
	40.0	304	279	281	264	284	282	0·733	1524
	65.0	221	279	255	279	281	263	0·733	1422
	86.0	256	244	236	242	252	246	0·733	1330
	106.0	248	248	195	200	232	224	0·733	1211
213 B	0.0	905	1011	867	938	955	935	0·733	5058
	30.0	967	908	847	1062	893	935	0·733	5058
215	0.0	207	271	244	233	203	232	0·733	1256
	60.0	249	235	247	251	196	236	0·733	1276
	120.0	212	266	267	280	228	251	0·733	1358
	180.0	289	235	258	250	236	254	0·733	1374
	240.0	250	261	250	256	238	251	0·733	1358
217	0.0	221	179	186	217	189	198	0·733	1081
	240.0	203	215	184	186	194	196	0·733	1059
	300.0	154	222	188	178	237	195	0·733	1054
	360.0	108	93	122	250	157	146	0·733	789

https://doi.org/10.1017/S0022172400035361 Published online by Cambridge University Press

(ii) Ringer sterilized by steaming (i) 30 min., (ii) 60 min., in screw-cap bottles sealed by a rubber washer

Exp.	Time	Counts					Mean count	Log factor	No. of organisms
215	0.0	206	208	200	174	165	191	0·733	1033
	60.0	84	98	54	93	66	79	0·733	427
	120.0	9	24	4	2	4	9·4	0·733	508
	180.0	24	17	30	39	33	29	0·733	157
217	0.0	216	214	191	212	241	215	0·733	1161
	60.0	26	33	31	—	—	30·0	0·733	162
	120.0	10	3	3	8	6	6·0	0·733	324
	180.0	1	5	4	4	4	3·6	0·733	195
	240.0	12	14	57	6	5	19·0	0·733	102
	300.0	3	0	7	8	2	4·0	0·733	22
	360.0	4	11	14	9	4	10·5	0·733	57

(iii) Ringer sterilized by autoclaving (i) at 10 lb. excess pressure for 20 min., (ii) at 10 lb. excess pressure for 30 min., in screw-cap bottles fitted with rubber washers

	Exp.	Time	Counts					Mean count	Log factor	No. of organisms
(i)	215	0.0	235	216	198	165	184	200	0·733	1081
		60.0	165	162	141	161	180	162	0·733	877
		120.0	102	118	86	151	93	110	0·733	594
		180.0	82	86	97	90	94	90	0·733	486
(ii)	217	0.0	218	262	233	218	242	233	0·733	1259
		120.0	50	47	37	86	50	54	0·733	292
		180.0	25	9	24	20	18	19	0·733	103
		240.0	23	22	18	20	33	23	0·733	125
		300.0	27	20	19	42	43	28	0·733	151
		360.0	29	16	13	19	24	20	0·733	108

REFERENCES

ARRHENIUS, S. (1915). *Quantitative Laws in Biological Chemistry.* 164 pp. London.

ARRHENIUS, S. & MADSEN, TH. (1903). Anwendung der physikalischen Chemie auf das Studium der Toxine und Antitoxine. *Z. phys. Chem.* **44**, 7.

AXELROD, AEBERSOLD & SPEAR (unpublished, Jan. 1941). Cited in Lea, Haines & Bretscher (1941).

BALLNER (1902). Experimentelle Studien über die Desinfectionskraft gesättigter Wasserdampfe bei verschiedenen Siedetemperaturen. *S.B. Akad. Wiss. Wien*, **111**, Abt. 3, 97.

BAKER, S. L. & NANAVUTTY, S. H. (1929). A quantitative study of the effect of ultra-violet rays on the bacteriophage. *Brit. J. Exp. Path.* **10**, 45.

BIGELOW, W. D. & ESTY, J. R. (1920). The thermal death point in relation to time of typical thermophilic organisms. *J. Infect. Dis.* **27**, 602.

BITTENBENDER, W. A., DIGERING, E. F. & TETRAULT, P. A. (1939). Bactericidal properties of commercial antiseptics. *Industr. Engng Chem.* **31**, 742.

BLISS, C. I. (1937). The calculation of the time mortality curve. *Ann. Appl. Biol.* **24**, 815.

BLISS, C. I. (1938). The determination of the dosage mortality curve from small numbers. *Quart. J. Pharm.* **11**, 192.

BOYCOTT, A. E. (1920). The killing of tadpoles by heat. *J. Path. Bact.* **29**, 237.

BROOKS, S. C. (1918). A theory of the mechanism of disinfection, hemolysis and similar processes. *J. Gen. Physiol.* **1**, 61.

BURBURY, Y. M. (1928). *Third Progress Report, Foot and Mouth Disease Research Committee*, p. 88.

CHICK, H. (1908 *a*). An investigation of the laws of disinfection. *J. Hyg., Camb.*, **8**, 92.

CHICK, H. (1908 *b*). The death rate of bacteria under the action of disinfectants. *78th Rep. Brit. Ass. Adv. Sci.* p. 901.

CHICK, H. (1910). The process of disinfection by chemical agencies and hot water. *J. Hyg., Camb.*, **10**, 237.

CHICK, H. (1912). The factors conditioning the velocity of disinfection. Reprinted from *Original Communications, 8th International Congress of Applied Chemistry*, **26**, 167.

CHICK, H. (1913). The bactericidal properties of blood serum. *J. Hyg., Camb.*, **12**, 414.

CHICK, H. (1930). *The Theory of Disinfection. A System of Bacteriology in Relation to Medicine*, **1**, 179. Med. Res. Coun.

CHICK, H. & MARTIN, C. J. (1908). The principles involved in the standardization of disinfectants. *J. Hyg., Camb.*, **8**, 655.

CLARK, A. J. (1933). *The Mode of Action of Drugs on Cells.* 296 pp. London: Edward Arnold and Co.

CLARK & GAGE (1903). *34th Annual Report of the State Board of Health, Massachusetts.* Quoted by Chick (1910, p. 280).

COBLENTZ, W. W. & FULTON, H. R. (1924). A radioactive investigation of the germicidal action of ultra-violet radiation. *Sci. Pap. U.S. Bur. Stand.* **19**, no. 495, 641.

COHEN, B. (1922). Disinfection studies. The effects of temperature and hydrogen-ion concentration upon the viability of *Bact. coli* and *Bact. typhosus* in water. *J. Bact.* **7**, 183.

DARWIN, N. & BLACKMANN (1909). The death rate of cells of higher plants in fatal conditions. *Rep. 78th Meeting Brit. Ass. Adv. Sci.* p. 902.

DAVIS, H. (1940). The effect on bacteria of substances in use for the preparation of solutions for parenteral administration. *Quart. J. Pharm.* **8**, 32.

DIENES, L. (1911). Zeigen die Blutkörperchen einer Blutkörperchenaufschwemmung bei der Haemolyse messbare individuelle Verschiedenheiten? *Biochem. Z.* **33**, 268.

DREYER, G. & HANSSEN, O. (1907). Sur la loi de la vitesse d'hémolyse des hématics sous l'action de la lumière, de la chaleur, et de quelques corps hémolitiques. *C.R. Acad. Sci., Paris*, **145**, 371.

EIJKMANN, C. (1908). Die Ueberlebungscurve bei Abtötung der Bakterien durch Hitze. *Biochem. Z.* **11**, 12.

EIJKMANN, C. (1909). Investigations on the subject of disinfection. *Verh. K. Akad. Amst.* **2T**, 11.

EIJKMANN, C. (1912). *Folia Microbiol., Delft*, **1**, 1.

FALK, I. S. (1923). *Abstr. Bact.* **7**, 66.

FALK, I. S. & WINSLOW, C. E. A. (1926). A contribution to the dynamics of toxicity and the theory of disinfection. *J. Bact.* **11**, 1.

FRIEDENTHAL, H. (1919). Absolute und relative Desinfectionskraft von Elementen und chemischen Verbindungen. *Biochem. Z.* **94**, 47.

FULMER, E. I. & BUCHANAN, R. E. (1923). Studies on toxicity. *J. Gen. Physiol.* **6**, 77.

GADDUM, J. H. (1933). Reports on biological standards. III. Methods of biological assay depending on a quantal response. *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 183.

GADDUM, J. H. (1937). Discussion on the chemical and physical basis of pharmacological action. *Proc. Roy. Soc.* B, **121**, 598.

GADDUM, J. H. (1940). *Pharmacology.* 407 pp. Oxford University Medical Press.

GALTON, F. (1879). The geometric mean in vital and social statistics. *Proc. Roy. Soc.* **29**, 365.

GATES, F. L. (1929 *a*). A study of the bactericidal action of ultra-violet light. I. *J. Gen. Physiol.* **13**, 231.

GATES, F. L. (1929 *b*). A study of the bactericidal action of ultra-violet light. II. *J. Gen. Physiol.* **13**, 249.

GATES, F. L. (1930). A study of the bactericidal action of ultra-violet light. III. *J. Gen. Physiol.* **14**, 31.

GEPPERT, J. (1889). Zur Lehre von den Antisepticis. *Berl. klin. Wschr.* **26**, 789.

GLADSTONE, G. P. & FILDES, P. (1940). A simple culture medium for general use without meat extract or peptone. *Brit. J. Exp. Path.* **21**, 161.

GLYNN, E., POWELL, M., REES, A. A. & COX, G. L. (1913). Standardization of bacterial vaccines. *J. Path. Bact.* **18**, 379.

GOEDRICH, P. (1938). Increase of bactericidal action of germicides by variation of *p*H. *J. Amer. Pharm. Ass.* **27**, 1233.

GROVES, J. F. (1917). Temperature and life duration of seeds. *Bot. Gaz.* **63**, 169.

HABERMANN, S. & ELLSWORTH, L. D. (1940). Lethal and dissociative effects of X-rays on bacteria. *J. Bact.* **40**, 483.

HARVEY, H. W. (1909). The action of poisons on *Chlamydomonas* and other vegetable cells. *Ann. Bot., Lond.*, **23**, 181.

HELLER, G. (1941). A quantitative study of environmental factors involved in survival and death of bacteria in the desiccated state. *J. Bact.* **41**, 109.

HEMMINGSEN, A. M. (1933). The accuracy of insulin assay on white mice. *Quart. J. Pharm.* **6**, 39.

HEMMINGSEN, A. M. (1934). A statistical analysis of the differences in body size of related species. *Vidensk. Medd. naturh. Foren. Kbh.* **98**, 125.

HENRI, V. (1905). Étude de la loi de la vitesse d'hémolyse des hématics de poulet par le serum de chien. *C.R. Soc. Biol., Paris*, **58**, 37.

HEŘČIK, F. (1936). Action of ultra-violet light on spores and vegetative forms of *B. megatherium* sp. *J. Gen. Physiol.* **20**, 589.

HEWLETT, R. T. (1909). Milroy Lectures on Disinfectants. *Lancet*, **1**, 741, 815, 889.

HOLLAENDER, A. & CLAUS, W. D. (1936). The bactericidal effect of ultra-violet radiations on *E. coli* in liquid suspensions. *J. Gen. Physiol.* **19**, 753.

HOLLAENDER, A. & DUGGAR, B. M. (1936). The irradiation of plant viruses and of micro-organisms with monochromatic light. III. *Proc. Nat. Acad. Sci., Wash.*, **22**, 19.

HOLWECK, F. (1929). Production de rayons X monochromatiques de grande longueur d'onde. Action quantique sur les microbes. *C.R. Acad. Sci., Paris*, **188**, 197.

IKÉDA, K. (1897). Die chemische Grundlage der Lehre von der Giftwirkung und Desinfektion. *Z. Hyg. InfektKr.* **25**, 95.

IRWIN, J. O. (1937). Statistical methods applied to biological assays. *J. Roy. Statist. Soc.* Suppl. **4**, 1–48.

JENNISON, M. W. (1937). Relations between plate counts and direct microscopic counts of *Escherichia coli* during the logarithmic growth period. *J. Bact.* **33**, 461.

JENNISON, M. W. & WADSWORTH, G. P. (1940). Evaluation of the errors involved in estimating bacterial numbers by the plating method. *J. Bact.* **39**, 389.

JOACHIMOGLU, G. (1923). Über den Einfluss der Wasserstoffionenkonzentration auf die antiseptische Wirkung des Sublimats. *Biochem. Z.* **134**, 489.

KEYSSER & ORSTEIN (1926). Das Optimum der Wasserstoffionenkonzentration (*p*H) als wichtigster Desinfektorischer Faktor bei örtlichen und allgemeinen Infektionen und seine Bedeutung für die Behandlung eitriger Bauchfellentzündungen. *Klin. Wschr.* **5**, 404.

KNAYSI, G. (1935). A microscopical method of distinguishing dead from living bacterial cells. *J. Bact.* **30**, 193.

KRÖNIG, B. & PAUL, T. (1897). Die chemische Grundlage der Lehre von der Giftwirkung und Desinfektion. *Z. Hyg. InfektKr.* **25**, 1.

KURODA, T. (1926). Über den Einfluss der Wasserstoffionenkonzentration auf die antiseptische Wirkung des Sublimats. *Biochem. Z.* **169**, 28.

LACASSAGNE, A. (1929). Action de rayons X de grande longueur d'onde, sur les microbes. *C.R. Acad. Sci., Paris*, **188**, 200.

LEA, D. E. & HAINES, R. B. (1940). The bactericidal action of ultra-violet light. *J. Hyg., Camb.*, **40**, 162.

LEA, D. E., HAINES, R. B. & BRETSCHER, E. (1941). The bactericidal action of X-rays, neutrons, and radioactive radiations. *J. Hyg., Camb.*, **41**, 1.

LEA, D. E., HAINES, R. B. & COULSON, C. A. (1937). The mechanism of the bactericidal action of radioactive substances. *Proc. Roy. Soc.* B, **120**, 47.

LEE, R. E. & GILBERT, C. A. (1918). Application of the mass law to the process of disinfection, being a contribution to the 'mechanistic theory', as opposed to the 'vitalistic theory'. *J. Phys. Chem.* **22**, 348.

LIEBERMANN, L. v. & FENNYVESSEY, B. v. (1912). Über den zeitlichen Verlauf der Hämolyse. *Z. Immunforsch.* **12**, 417.

LLOYD, L. (1920). The habits of the glasshouse tomato moth, *Hadena* (*Polia*) *oleracea*, and its control. *Ann. Appl. Biol.* **7**, 66.

LOEB, J. & NORTHROP, J. H. (1917). On the influence of food and temperature on the duration of life. *J. Biol. Chem.* **32**, 103.

MADSEN, T. & NYMAN, M. (1907). Zur Theorie der Desinfektion. I. *Z. Hyg. InfektKr.* **57**, 388.

MIONI, G. (1905). Influence de la quantité des globules et de la durée de la réaction sur les résultats de l'hémolyse. *C.R. Soc. Biol., Paris*, **42**, 485.

MIONI, G. (1905). *Ann. Inst. Pasteur*, **19**, 84.

MYERS (1929). Cited in Rahn (1930 *a*).

NORTON, J. F. & HSU, P. H. (1916). The physical chemistry of disinfection. *J. Infect. Dis.* **18**, 180.

OOTHUISEN, M. J. (1935). The effect of high temperature on the Confused Flour Beetle. *Tech. Bull. Minn. Agric. Exp. Sta.* no. 107.

PAUL, T. (1909). Der chemische Reactionsverlauf beim Absterben trockener Bakterien bei niederen Temperaturen. *Biochem. Z.* **18**, 1.

PAUL, T., BIRSTEIN, G. & REUSS, A. (1910). Beitrag zur Kinetik des Absterbens der Bakterien in Sauerstoff verschiedener Konzentration und bei verschiedenen Temperaturen. *Biochem. Z.* **25**, 367.

PAUL & PRALL (1907). Die Wertbestimmung von Desinfektionsmitteln mit Staphylococcen, die bei der Temperatur der flüssigen Luft aufbewahrt wurden. *Arb. GesundhAmt., Berl.*, **26**, 424.

PETERS, R. A. (1920). Variations in the resistance of protozoon organisms to toxic agents. *J. Physiol.* **54**, 260.

PHELPS, E. B. (1911). The application of certain laws of physical chemistry in the standardization of disinfection. *J. Infect. Dis.* **8**, 27.

PONDER, E. (1930). The form of the frequency distribution of red cell resistances to saponin. *Proc. Roy. Soc.* B, **106**, 543.

PORODKO, TH. M. (1926). *Ber. dtsch. bot. Ges.* **44**, 71.

PORODKO, TH. M. (1927). *Ber. dtsch. bot. Ges.* **45**, 4.

RAHN, O. (1929). The size of bacteria as a cause of the logarithmic order of death. *J. gen. Physiol.* **13**, 179.

RAHN, O. (1930). The non-logarithmic order of death of some bacteria. *J. Gen. Physiol.* **13**, 395.

RAHN, O. (1931). A chemical explanation of variability in the growth rate. *J. Gen. Physiol.* **15**, 257.

RAPPS, N. F. (1933). The bactericidal efficiency of chlorocresol and chlorozylenol. *J. Soc. Chem. Ind.* **52**, 16 June, 175 T.

REICHENBACH, H. (1911). Die Absterbeordnung der Bakterien und ihre Bedeutung für Theorie u. Praxis der Desinfektion. *Z. Hyg. InfektKr.* **69**, 171.

SALMONSEN, C. J. & DREYER, G. (1907). De la loi de l'effet hémolytique des rayons Becquerel. *C.R. Acad. Sci., Paris*, **144**, 999.

SATTLER, W. (1928).. Untersuchungen über die Absterbegeschwindigkeit, die Warme beschleunigung und den Resistenzwechselpunkt einiger für den Molkereibetrieb wichtiger Bakterien unter veschiedenen Bedingungen und ihr Verhalten bei den Pasteurisierungstemperaturen und bei raschen Temperaturwechsel. *Milchw. Forsch.* **7**, 100.

SMITH-HENDERSON, J. (1921). The killing of *Botrytis* spores by phenol. *Ann. Appl. Biol.* **8**, 27.

SMITH-HENDERSON, J. (1923). The effect of heat on *Botrytis* spores. *Ann. Appl. Biol.* **10**, 335.

TAMMAN, G. (1895). Zur Wirkung ungeformter Fermente. *Z. phys. Chem.* **18**, 426.

TREVAN, J. W. (1927). The error of determining toxicity. *Proc. Roy. Soc.* B, **101**, 483.

VERMAST, P. G. F. (1921). Beitrag zur Theorje d. Desinfektion im Lichte der Meyer-Overton'schen Lipoidtheorie. *Biochem. Z.* **125**, 107.

WATERMAN, H. I. & KUIPER, P. (1924). *Rec. trav. chim. Pays-Bas*, **43**, 232.

WATERMAN, H. I. & KUIPER, P. (1924). The antiseptic action of benzoic acid, salicylic acid, cinnamic acid and their salts. *Rec. trav. chim. Pays-Bas*, **43**, 323.

WATSON, H. E. (1908). A note on the variation of the rate of disinfection, with change in concentration of disinfectant. *J. Hyg., Camb.*, **8**, 536.

WILSON, G. S. (1922). The proportion of viable bacteria in young cultures, with special reference to the technique employed in counting. *J. Bact.* **1**, 405.

WITHELL, E. R. (1938). The evaluation of bactericidal action. *Quart. J. Pharm.* **9**, 736.

WOERZ, J. A. Cited in Rahn (1930 *a*).

WYCKOFF, R. W. G. (1930). The killing of certain bacteria by X-rays. *J. Exp. Med.* **50**, 435.

WYCKOFF, R. W. G. (1932). The killing of colon bacilli by ultra violet light. *J. Gen. Physiol.* **15**, 351.

WYCKOFF, R. W. G. & RIVERS, T. M. (1930). The effect of cathode rays on certain bacteria. *J. Exp. Med.* **51**, 921.

YULE, G. V. (1910). On the distribution of deaths with age when the causes of deaths act cumulatively, and similar frequency distributions. *J. Roy. Statist. Soc.* **73**, 26.

ZIEGLER, N. R. & HALVORSEN, H. O. (1935). Application of statistics to problems in bacteriology. IV. Experimental comparison of the dilution method, the plate count, and the direct count for the determination of bacterial populations. *J. Bact.* **29**, 609.

(*MS. received for publication* 1. I. 42.—Ed.)