COMPARATIVE STUDIES OF METHODS OF EVALUATING ANTIBACTERIAL SUBSTANCES

PART II. EVALUATION OF BACTERICIDAL ACTION. A COMPARISON OF AN EXTINCTION METHOD WITH A COUNTING METHOD

BY A. M. COOK and B. A. WILLS

From the Department of Pharmaceutics, School of Pharmacy, University of London

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INTRODUCTION

IN this paper the bactericidal action of phenol on *Bacterium coli* is examined by the method of Berry and Bean[1] and the results compared with those obtained by a counting technique.

THE LOGLOG ANALYSIS OF EXTINCTION DATA

Using the extinction data provided from this laboratory, it has been shown by Mather[2] that, using the loglog transformation of the proportion of negative samples at each contact time, the time when there is on the average one surviving organism per unit volume can be calculated. The method of analysis permits estimation of the sampling variances of the mean weighted loglog and the slope of the regression of loglog proportion of negative samples upon contact time. The standard error of an estimate of the killing time may be computed. The calculation of the regression line relating loglog to time follows the same course as probit analysis, but with the use of different weighting coefficients. The most informative observations are those where the proportion, p, lies between 0·2 and 0·3. The single mean survivor time is that at which $p = 0{\cdot}3679$, corresponding to loglog $= 0$.

Estimation of the mean single survivor time necessitates some modifications in the design of an experiment from that originally described. The reliability of an estimate will be improved by performance of as many replicate determinations as possible within an experiment and by reducing the intervals between contact times as far as possible subject to an adequate range being provided around the anticipated killing time. With aqueous solutions of phenols an adequate number of proportions of negative samples will not usually be found if the contact time intervals exceed 1/7 of the killing time. The most convenient structure of an experiment was usually found to consist of between 15 and 20 replicates at 6 contact times.

During the period of 5 consecutive days in which the percentages of survivors at various contact times were determined, as described in the following section, the single mean survivor time with 1·10 per cent. phenol was determined. The results of the experiment are shown in Table II and the values of the loglogs (y) are shown plotted against contact times in Figure 1, in which A represents the line best fitted by

inspection. By reading the value on the abscissa corresponding to $y = 0$ an estimate of 45·4 minutes was obtained for the mean single survivor time. A first approximation to the loglog relationships was made, following exactly the method described by Mather, from which an analysis of variance of the new relationship yielded the information expressed in Table I.

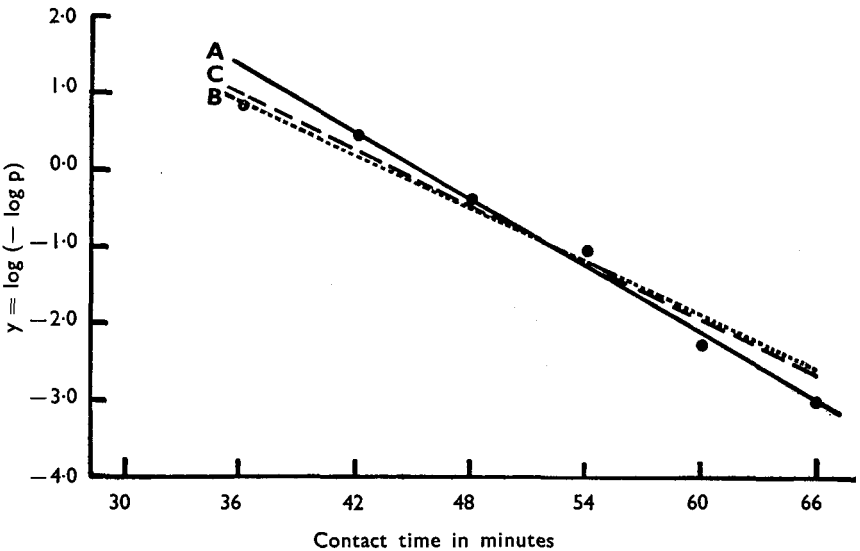

FIG. 1. The relationship between y (log[$-$log p]) and contact times and the calculation of the regression of y upon x: line best fitted by inspection, A; first approximation, B; and second approximation, C. Data for the exposure of *Bact. coli* to 1·10 per cent. phenol.

This variance ratio with the appropriate degrees of freedom corresponds to a probability level between 0·01 and 0·001, so that the regression is highly significant. The sampling variances of mean working loglog and slope were calculated as $\bar{y}_w = -0{\cdot}18189 \pm 0{\cdot}02657$ and $b = -0{\cdot}11442$

TABLE I

Source of variance	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Regression ..	27·62456	1	27·62456	$n_1 = 1$ 40·1547 $n_2 = 4$
Residual	2·75182	4	0·68795	
Total	30·37638	5		

$\pm$ 0·00047. The revised approximation to the loglog relationship was $Y = -0{\cdot}18189 - 0{\cdot}11442\ (X - 45{\cdot}6870)$, this giving the line B in

Figure 1. The standard error of an estimate was calculated from the expression:

$$S_{x1} = \left(\frac{1}{b^2}\left\{\frac{1}{\Sigma(nw)} + \frac{(x_1 - \bar{x})^2}{\Sigma[nw(x-\bar{x})^2]}\right\}\right)^{\frac{1}{2}},$$

and the new value of the mean single survivor time found to be 44·090 ± 1·457 minutes.

The cycle of computation was repeated in order to derive a second approximation to the relationship. The residual variance was thereby further reduced to 0·321, and the estimates of mean working loglog and slope recalculated as

$$\bar{y}_w = -0{\cdot}1092 \pm 0{\cdot}0232 \text{ and } b = -0{\cdot}1214 \pm 0{\cdot}00030$$

The second approximation to the relationship was

$$Y = -0{\cdot}1092 \pm 0{\cdot}12114\ (X - 45{\cdot}3434),$$

from which the line C in Figure 1 was obtained. The final estimate of the mean single survivor time was 44·444 ± 1·264 minutes, and this was used for the correlation with contact times required to give other percentages of survivors, which is described below. The second approximation has brought about small reductions in the residual variance and in the standard error of an estimate; however, as Mather found, the estimate based on the use of a line fitted by inspection yields a result lying within the standard errors of the estimates based on the corrected regressions.

Correlation between Extinction Data and Percentages of Survivors at Various Contact Times with Aqueous Solutions of Phenol

The use of the loglog analysis of extinction data and the development by Finney[3] of a loglog analysis of the data of dilution series suggested that a relation might exist between the mean single survivor time and the contact times required for varying percentages of survivors determined by dilution series. The establishment of such a correlation would signify that coefficients of disinfectant action based on end-point methods were of comparable reliability with those based on counts of bacterial survivors. Moreover, a comparison of these two methods was desirable, rather than a comparison of extinction results with direct colony counts, because any tendency of the organisms to clump in the presence of the solution of bactericide would be expected to lead to a similar discrepancy in both methods, thus making possible a more direct comparison. This consideration, coupled with the fact that the same form of analysis would be used for data of both methods, was thought to more than outweigh the loss of accuracy inherent in counts by dilution series as against direct colony counts.

Experimental

1. *Scheme.* The percentages of survivors were determined by adding 10 drops of a standardised suspension of a 24-hour growth of *Bact. coli*

to 5 ml. of a 1·10 per cent. phenol solution contained in a 60 ml. glass-stoppered bottle, which had previously been immersed in a water bath maintained at a temperature of 20° ± 0·05° C. The contents of the bottle were mixed by rotation and the bottle replaced in the water bath. After a fixed time exposure a sample of 10 drops of the reaction mixture was withdrawn and diluted by successive factors of 4, over a range exceeding that dilution at which one survivor could exist. 6 consecutive dilutions considered to lie around the end-point were selected and 5 drops of each inoculated into each of 10 aluminium-capped test-tubes containing 5 ml. of sterilised broth of the same composition and same batch preparation as that used in estimation of the single survivor time. The inoculated tubes were placed at once in a water bath maintained at 37° C. and afterwards incubated at the same temperature for 48 hours before reading the results. Further periods of incubation of up to 2 months resulted in no further decrease in the proportion of negative samples.

Each day experiments were carried out using exposures of varying times—3 or 4 different times each day. In order to express the results as percentages of survivors, an initial count was made by adding 10 drops of bacterial suspension to 5 ml. of water, diluting 10 drops of this with a single large volume, and diluting a sample of this by successive factors of 4. A correction, however, has to be applied for the larger volume of a drop of the reaction mixture as compared with the drop volume of the phenolic reaction mixture.

2. *Details*

(i) *The Dilutions.* In the beginning of the work these were made with sterilised distilled water. 25 drops of a previous dilution was added to 75 drops of water in a sterilised test-tube, the mixture shaken, and 25 drops of this dilution removed to the next tube. It was found that such dilutions took a considerable time to perform, and that although the percentages of survivors appeared to decrease logarithmically with time after exposures of up to 15 minutes, the decrease was very much more steep with longer exposures. It was considered that damaged organisms might not survive in water over the period between preparation of all dilutions and their inoculation into the broth. Dilutions were afterwards prepared using sterilised broth. The numbers of survivors at all time intervals were increased and a strong correlation found to exist between the logarithms of the percentages of survivors and contact times over a range of exposures from 5 to 25 minutes.

The first dilution was always obtained by addition of 10 drops of reaction mixture to 5 ml. of broth before commencing the dilutions by factors of 4. This was chosen in order that the phenol might be well diluted immediately on expiry of the period of exposure. In the case of the "blank" exposures which used water in place of the phenol solution, the first dilution was made by the addition of 10 drops to 350 ml. water, giving a 1 : 2000 dilution.

Owing to the variations in the drop volumes of solutions of phenol

diluted with broth, these dilutions further diluted with broth, and of water diluted with broth, the drop volumes of water, 1·10 per cent. phenol solution and the broth were determined by the method described by Withell[4], and the drop volumes of mixtures of these were determined. This enabled graphs to be plotted recording (*a*) decrease in drop volume as water was continuously diluted with broth; (*b*) decrease in drop volume as 1·10 per cent. solution of phenol was diluted with broth. In both cases only two corrections need be applied, for after 2 dilutions the discrepancies are smaller than the errors due to variation in drop volumes delivered by different dropping pipettes.

(ii) *The Dropping Pipettes.* Prepared from 5 mm. diameter glass tubing, one end being drawn out and ground to fit a "Record" hypodermic needle as described by Cook and Yousef[5]. The needles were ground and the tips polished to give a square and completely smooth end. The drop weights of water delivered from all the needles prepared for use were determined, taking 20 weighings from each needle. The results, when subjected to an analysis of variance, showed a significantly greater variation in the performance of different needles than in the performance of one needle. However, this difference is of little practical significance, since the difference between the highest and lowest needle means scarcely exceeded 2 per cent. of the weight delivered—comparing favourably with the performance of different delivery pipettes.

Results and Treatment

The treatment of the results of the dilution series closely followed that described by Finney. The proportions of media showing no growth at each dilution were observed and the corresponding value of the loglog found. A rough estimate of the bacterial density may be obtained by plotting the loglog against the natural logarithms of the dilution factors. The value of the dilution factor corresponding to a loglog of 0 is the dilution required to yield one survivor, and its value is thus the estimated number of organisms contained in a five drop sample of the reaction mixture.

Such estimations were found to be inaccurate owing to the fact that seldom did an experiment show more than 3 dilutions, sometimes only 2, with a proportion of negative samples. The remaining dilutions gave all positive or all negative samples. The method of calculation described by Finney was therefore used in all cases to obtain the final result. This consisted in estimating a series of working loglogs, Y, corresponding to the proportions of negative samples in each of 5 dilution series. The values of weighting coefficients, w, and working deviates, η, were found by reference to the tables published by Finney, and from these a correction factor, $\bar{\eta} = \frac{\Sigma nw\eta}{\Sigma nw}$, computed and subtracted from the values of Y to give a new series of corrected working loglogs. The process was repeated until the correction became sufficiently small to be ignored. In this case no further calculation was employed where the correction was

reduced to below 0·005, this usually requiring 2 or 3 cycles of computation. An example of the calculation is given below:—

EXPERIMENT PS/46

EXPOSURE TO 1·10 PER CENT. PHENOL FOR 15 MINUTES

Dilution Factor	p	Empirical Loglog	Y	nw	η	Y	nw	η
1/4	0	—	1·0	5·2204	−0·3679	1·1	4·7080	−0·3329
1/16	0·5	−0·366	−0·4	4·7057	−0·0386	−0·3	4·9999	0·0659
1/64	0·9	−2·250	−1·8	1·5201	0·3735	−1·7	1·6650	0·4401
1/256	1·0	—	−3·2	0·3994	1·0207	−3·1	0·4404	1·0229
1/1024	1·0	—	−4·6	0·1000	1·0050	−4·5	0·1105	1·0056
				11·9456			11·9238	

$$\Sigma nw = -1{\cdot}0263 \qquad \Sigma nw\eta = 0{\cdot}0566$$

$$\bar{\eta}\ (\text{1st cycle}) = -\frac{1{\cdot}0263}{11{\cdot}9456} = -0{\cdot}0859;\ \text{value of Y at 1/16 dilution} = -0{\cdot}3141$$

$$\bar{\eta}\ (\text{2nd cycle}) = +\frac{0{\cdot}0566}{11{\cdot}9238} = +0{\cdot}0047;\ \text{value of Y at 1/16 dilution} = -0{\cdot}3188$$

$$\text{Log}_e - 0{\cdot}3188 = 0{\cdot}7270$$

Hence density per sample volume = 11·632.

Initially 10 drops of reaction mixture were diluted with 5 ml. broth, corresponding to a further dilution factor of 37·23. The density per 10 drops of reaction mixture is therefore $11{\cdot}632 \times 37{\cdot}23 \times 2 = 8{\cdot}66 \times 10^2$. The density per 10 drops at zero time determined on the same day was found to be $12{\cdot}64 \times 10^6$, giving a percentage of survivors after 15 minutes exposure of 0·0063 per cent.

TABLE II

DEATH OF *Bact. coli* ON EXPOSURE TO 1·10 PER CENT. PHENOL

Series/Time (minutes)	36	42	48	54	60	66
1	+	+	+	−	−	−
2	+	+	+	+	−	−
3	+	−	−	−	−	−
4	+	+	+	+	+	+
5	+	−	−	−	−	−
6	−	+	+	−	−	−
7	+	+	+	+	−	−
8	+	+	+	−	−	−
9	+	+	+	+	−	−
10	+	+	−	−	+	−
11	−	−	+	−	−	−
12	+	+	−	+	−	−
13	+	+	+	−	−	−
14	+	+	−	−	−	−
15	+	+	−	−	−	−
16	+	−	−	−	−	−
17	+	+	+	+	−	−
18	+	+	−	−	−	−
19	+	+	−	−	−	−
20	+	+	−	−	−	−
Total of negative samples	2	4	10	14	18	19
p	0·1	0·2	0·5	0·7	0·9	0·95
$y = \log(-\log p)$	0·834	0·476	−0·366	−1·031	−2·250	−2·970

In all, 18 bacterial densities were estimated on 4 consecutive days: 4 of these consisted of the estimates of densities at zero time and the remainder were estimates of the survivors after varying periods of exposure. The results of these experiments are recorded in Table III.

In all, 10 estimations of the density of the original bacterial suspension had been made, these having been carried out on different days on freshly prepared and standardised suspensions. The results were a follows, expressed as densities per ml.:—

2.366×10^9, $2{\cdot}322 \times 10^9$, $1{\cdot}680 \times 10^9$, $1{\cdot}923 \times 10^9$, $2{\cdot}095 \times 10^9$, $2{\cdot}353 \times 10^9$, $2{\cdot}508 \times 10^9$, $2{\cdot}549 \times 10^9$, $2{\cdot}503 \times 10^9$, $1{\cdot}819 \times 10^9$.

Mean density $= 2{\cdot}211 \times 10^9 \pm 0{\cdot}3146 \times 10^9$.

This shows a reasonable degree of reproducibility both of standardisation of the suspension and of estimation of the densities.

Analysis of the Results

Figure 2 shows the logarithms of percentages of survivors plotted against the logarithms of the corresponding contact times with 1·10 per cent. phenol solution. On inspection, it was considered possible that there might be sufficient variability between the results obtained on different days that four significantly different relationships could be established. This variation was thought to be explained largely by variations in the densities of the original suspensions. Since only one single mean survivor time had been determined and since no initial count had been made on the suspension used for estimation of the single survivor time during the week occupied by these experiments, it was decided to express it as 4 percentages corresponding with the densities estimated on each of the other 4 days. These are shown as the 4 almost coincident points at the bottom of the graph. The problem then was to treat the results as 4 separate relationships, to ascertain whether the 4 regression lines differ significantly in slope, and if no such difference was indicated, to determine whether the 4 regression lines could be regarded as adequately represented by one coincident line (Tippett[6]).

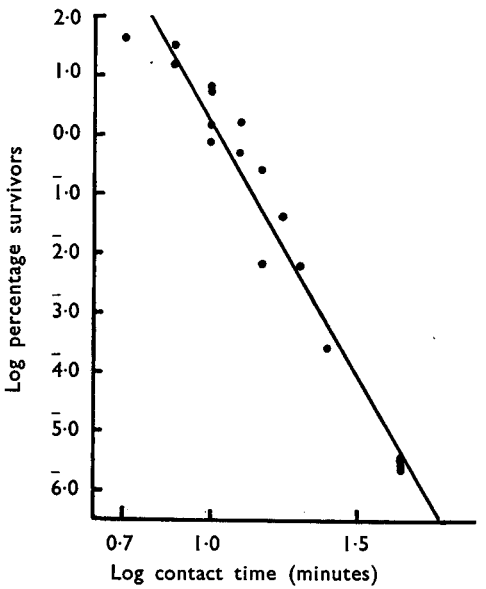

FIG. 2. The relationship between percentages of survivors and contact times after exposure to 1·10 per cent. phenol solution.

TABLE III

EXPOSURE OF *Bact. coli* TO 1·10 PER CENT. PHENOL SOLUTION OVER VARYING TIME INTERVALS

Experiment	Contact time (minutes)	Density per 10 drops of reaction mixture	Percentage survivors
PS/38	0	$13{\cdot}98 \times 10^6$	—
PS/39	10	$2{\cdot}036 \times 10^5$	1·455
PS/40	12·5	$6{\cdot}984 \times 10^4$	0·499
PS/41	20	$8{\cdot}70 \times 10^3$	0·00622
PS/42	25	33·27	0·000237
PS/43	0	$13{\cdot}742 \times 10^6$	—
PS/44	7·5	$2{\cdot}060 \times 10^6$	14·99
PS/45	10	$9{\cdot}754 \times 10^4$	0·710
PS/46	15	$8{\cdot}660 \times 10^3$	0·00630
PS/47	0	$9{\cdot}937 \times 10^6$	—
PS/48	7·5	$3{\cdot}176 \times 10^6$	31·96
PS/49	10	$6{\cdot}070 \times 10^5$	6·109
PS/50	12·5	$1{\cdot}602 \times 10^5$	1·613
PS/51	17·5	$4{\cdot}170 \times 10^3$	0·0419
PS/52	0	$10{\cdot}378 \times 10^6$	—
PS/53	5	$4{\cdot}641 \times 10^6$	44·72
PS/54	10	$5{\cdot}626 \times 10^5$	5·421
PS/55	15	$2{\cdot}712 \times 10^4$	0·261

First, the correlation coefficients and residual sums of squares were calculated for each relationship, coding the values of the ordinates by addition of 7 to the index of the logarithm.

(i) $\Sigma^2 x = 41{\cdot}520625$ $\quad \Sigma^2 y = 547{\cdot}861433$

$\Sigma x^2 = 8{\cdot}565273$ $\quad \Sigma y^2 = 132{\cdot}436315$

$\Sigma x \Sigma y = 150{\cdot}822907$ $\quad \Sigma xy = 27{\cdot}733522$

$\Sigma(x - \bar{x})^2 = 0{\cdot}261148$

$\Sigma(y - \bar{y})^2 = 22{\cdot}864028$

$\Sigma(x - \bar{x})(y - \bar{y}) = -2{\cdot}431059$

$r = -0{\cdot}9949$

Residual S. Sq. $= 0{\cdot}232999$

(ii) $\Sigma^2 x = 22{\cdot}079849$ $\quad \Sigma^2 y = 449{\cdot}870463$

$\Sigma x^2 = 5{\cdot}864064$ $\quad \Sigma y^2 = 138{\cdot}716550$

$\Sigma x \Sigma y = 99{\cdot}664798$ $\quad \Sigma xy = 21{\cdot}931006$

$\Sigma(x - \bar{x})^2 = 0{\cdot}344102$ $\quad \Sigma(y - \bar{y})^2 = 26{\cdot}273934$

$\Sigma(x - \bar{x})(y - \bar{y}) = -2{\cdot}985193$

$r = -0{\cdot}9928$

Residual S. Sq. $= 0{\cdot}376447$

(iii) $\Sigma^2 x = 34{\cdot}372189$ $\quad \Sigma^2 y = 939{\cdot}184058$

$\Sigma x^2 = 7{\cdot}229236$ $\quad \Sigma y^2 = 218{\cdot}836626$

$\Sigma x \Sigma y = 179{\cdot}671401$ $\quad \Sigma xy = 32{\cdot}636754$

$\Sigma(x - \bar{x})^2 = 0{\cdot}354797$

$\Sigma(y - \bar{y})^2 = 30{\cdot}999854$

$\Sigma(x - \bar{x})(y - \bar{y}) = -3{\cdot}297526$

$r = 0{\cdot}9943$

Residual S. Sq. $= 0{\cdot}352211$

(iv) $\Sigma^2 x = 20{\cdot}455991 \qquad \Sigma^2 y = 590{\cdot}879350$

$\Sigma x^2 = 5{\cdot}586893 \qquad \Sigma y^2 = 178{\cdot}090972$

$\Sigma x \Sigma y = 109{\cdot}940997 \qquad \Sigma xy = 23{\cdot}809900$

$\Sigma(x - \bar{x})^2 = 0{\cdot}472895 \qquad \Sigma(y - \bar{y})^2 = 30{\cdot}371134$

$\Sigma(x - \bar{x})\,(y - \bar{y}) = -3{\cdot}675349$

$r = -0{\cdot}9698$

Residual S. Sq. $= 1{\cdot}806251$

$$\text{Total residual variance} = \frac{0{\cdot}232999 + 0{\cdot}376447 + 0{\cdot}352211 + 1{\cdot}806251}{18 - 8}$$

$$= 0{\cdot}276791$$

Total residual S. Sq., assuming one regression coefficient, but different means of y for a given value of x

$$= \frac{\Sigma^2(x - \bar{x}_1)\,(y - \bar{y}_1)}{\Sigma(x - \bar{x}_1)^2} + \frac{\Sigma^2(x - \bar{x}_2)\,(y - \bar{y}_2)}{\Sigma(x - \bar{x}_2)^2} + \ldots .$$

$$\frac{[\Sigma(x - \bar{x}_1)\,(y - \bar{y}_1) + \Sigma(x - \bar{x}_2)\,(y - \bar{y}_2) + \ldots]^2}{\Sigma(x - \bar{x}_1)^2 + \Sigma(x - \bar{x}_2)^2 \ldots .}$$

$$= 107{\cdot}741002 - 107{\cdot}115618$$

$$= 0{\cdot}625384$$

There are $4 - 1 = 3$ degrees of freedom, so the estimate of this residual variance is 0·208461. This is smaller than the residual variance for the separate regression coefficients, so that the samples could be considered as being derived from a population having a regression of common slope. Next, the total of 18 results were pooled and the residual sum of squares calculated.

$\Sigma x^2 = 27{\cdot}245466 \qquad \Sigma^2 x = 463{\cdot}462534 \qquad \Sigma(x - \bar{x})^2 = 1{\cdot}497547$

$\Sigma y^2 = 668{\cdot}080463 \qquad \Sigma^2 y = 9914{\cdot}326390 \qquad \Sigma(y - \bar{y})^2 = 117{\cdot}284558$

$\Sigma xy = 106{\cdot}111182 \qquad \Sigma x \Sigma y = 2143{\cdot}57617$

$\Sigma(x - \bar{x})\,(y - \bar{y}) = -12{\cdot}976383$

$r = -0{\cdot}979136 \qquad b = -8{\cdot}665092$

Regression Equation: $Y = 5{\cdot}5317 - 8{\cdot}6651\,(X - 1{\cdot}196)$

Residual S.Sq. from common regression $= 4{\cdot}843001$ for $18 - 2$ $= 16$ degrees of freedom

Residual S.Sq. from separate regressions $= 2{\cdot}76791$ with 10 degrees of freedom

The difference between the two residual sums of squares is 2·07509, with $16 - 10 = 6$ degrees of freedom, giving a variance estimate of 0·3458. This may be tested against the residual variance for the separate regressions, the variance ratio being 1·249. This ratio, with the appropriate number of degrees of freedom, corresponds to a probability level of above 0·2. Hence the residual variance from the common regression is not significantly greater than that from the separate regressions, and the

4 separate relationships can be confidently regarded as being represented by one coincident regression line.

Discussion

A correlation between the percentages of survivors after varying periods of contact with an aqueous solution of phenol and the percentage of survivors corresponding with the mean single survivor time, as estimated from extinction data, has been demonstrated. This leads to the conclusion that the evaluation of bactericidal activity by an extinction method, provided the results are subjected to the loglog analysis, will yield as much information as can be derived from the use of counting methods in the region of virtual sterilisation. It is realised that estimations of bacterial densities by dilution series as employed in this investigation are not of comparable accuracy with direct colony counts; and it has been pointed out that such estimates were used in view of the equal tendencies to clumping of organisms in the two methods used.

Inspection of the log. per cent. survivors–log. contact time regression (Fig. 2) might lead to the conclusion that the deviations are due to the relationship being in fact sigmoid. If this were the case it might be expected that a more satifactory correlation would be evident on plotting the values of the percentages of survivors on a probit scale. Accordingly, the percentages of survivors were converted into probits.

In order to compare the significance of the two regressions, the data for each relationship were pooled, but the values of the percentages of survivors corresponding to the mean single survivor time were not included. In the case of the logarithmic survivor-time curve, the slope was calculated as -7.7908 and the correlation coefficient found to be -0.9287, with 12 degrees of freedom. The value of the contact time corresponding to the single survivor percentage was found to be 54·95 minutes, whereas the observed value was 44·44 minutes. With the probit per cent. survivors—log. contact time regression, the slope was calculated as -6.6319, the correlation coefficient as -0.9548, with 12 degrees of freedom, and the single survivor contact time as 30·69 minutes, as compared with the observed 44·44 minutes.

These results indicate that the probit relationship provides the more highly significant regression over the largest part of the curve, and the existence of the probit relationship is evidence of a log-normally distributed resistance to the bactericide amongst the test organisms, which has been demonstrated by many workers who have employed colony counting methods. However, the fact that the experimentally observed value of the single survivor time is smaller than the value calculated on the basis of the logarithmic regression appears to be reasonable on the grounds that sampling variations in obtaining a viable organism at the extreme of the curve would lead to an apparently shorter contact time than that expected; on the other hand, the probit regression permits calculation of a single survivor time smaller than that observed. In neither case can the discrepancy be regarded as serious since the limits of error of an estimate of a contact time at this extreme will be wide.

Evaluation of bactericidal activity by the extinction method described leads to a general economy in time and apparatus over counting methods. Moreover, the treatment of results is relatively simple and the results are rapidly read, so that it is conceivable that this method might well be applied to the routine testing of bactericidal activity, giving estimates of equal reproducibility to those of any other methods now in common use.

Summary

A correlation between the mean single survivor time and percentages of survivors at a series of shorter contact times as estimated by serial dilution counts has been established.

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