THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF ETHYLENE GLYCOL AND SOME OF ITS MONOALKYL ETHERS AGAINST *BACTERIUM COLI*

PART VIII

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So far, this work has shown that the course of disinfection of *Bact. coli* by ethylene glycol and its monoalkyl ethers follows that of an asymmetrical sigmoid curve; the actual appearance of the curve could be made to vary by alteration of the environmental conditions[1,2]. The regression obtained by plotting the percentage survivors as probits against log. survivor time could be assumed linear over a limited (but useful) range without causing serious error[3,4]; further, when probit-log. time regressions were taken as linear, it could be shown[5] that parallelism existed between the regressions of different concentrations as well as the same concentration of a compound, thereby enabling a characteristic coefficient to be assigned to each disinfectant-organism reaction. The values of the coefficient varied with the temperature of the experiment[6,7].

Sufficient experimental data have now been accumulated to enable the usefulness and accuracy of a chosen level of mortality for assessing the bactericidal activity of the disinfectant compounds to be decided with some confidence.

SELECTION OF AN INTERMEDIATE MORTALITY LEVEL FOR THE COMPARISON OF BACTERICIDAL ACTIVITY

The inaccuracies of an end-point technique for estimating bacterial death times have led to the selection of intermediate mortality levels, determinable with greater precision, for comparing germicidal activities. Adoption of this principle involves the counting of viable organisms during the course of the disinfection process. Until a sound statistical analysis of the results could be developed and thereby afford a means of calculating the limits of error of the experimental technique, it was not possible to compare, on a mathematical basis, the merits of the different mortality levels proposed by different authors.

The Fallacies of Using Reaction Velocities as a Means of Comparison. Counting techniques enable the reaction velocity to be determined at different stages during the disinfection process. Since it was at first believed that the overall death rate for a particular concentration of a disinfectant was constant, the mean of intermediate death rates was used as a criterion of comparison of different disinfectant solutions. Furthermore, the overall reaction velocity was taken as representative of the efficiency of the disinfection process and was recommended by Phelps[8] as the basis for the evaluation of bactericidal activity. However, when it is realised that a constant death rate is often fortuitous

and that the death rate of the disinfection process does indeed vary along its course, comparison of overall death rates must be criticised as being misleading and uninformative.

The mortality levels chosen by previous workers. In bactericidal problems, the comparison of activity by the times taken for mortalities of less than 100 per cent. appears to have been first suggested by Levine, Buchanan and Lease[9], who recommended the use of the time for a 99·9 per cent. kill. Myers[10] based his comparison on a 99 per cent. mortality since he believed that this could be determined with greater accuracy than higher levels; Weber and Levine[11] also used this degree of mortality, whereas Baker and McClung[12] calculated the time for the death of 99·99 per cent. of the initial inoculum. Hobbs and Wilson[13] expressed doubt as to the accuracy of the computation of the times for a 99·9 per cent. mortality and employed reaction velocities to compare the bactericidal activities of the disinfectants used by them.

The choice of a 50 per cent. mortality level. (*a*) *In pharmacological assay problems.* In pharmacological problems the comparison of the potencies of therapeutically active substances has long been made by utilising the dose affecting 50 per cent. of the test animals. Trevan[14] had shown that the slope of the mortality-dose curve was steepest in the neighbourhood of the dose causing 50 per cent. mortality and this was also shown to be true when the logarithm of the dose was used (Gaddum[15]). Trevan coined the expression "LD50" (the dose which caused 50 per cent. mortality); because of the normal characteristics of the mortality curves, statistical methods could be used to determine the LD50 and the error of its estimation with great precision. Technical faults were thereby detectable, which when rectified enabled the accuracy of the biological assay to be improved enormously.

(b) *In bacteriological and insecticidal assay problems.* In microbiological problems of this nature, Henderson Smith[16] had used the time to kill 50 per cent. of the initial inoculum as a means of comparison in determining the temperature coefficient of hot water against *Botrytis* spores. Withell[17] conceived the idea of an "LT50" (i.e., the time to kill 50 per cent. of the initial inoculum) as the basis of comparison of germicidal activity. By means of the statistical techniques developed by Gaddum[15] and Bliss[18,19,20,21,22] he was able to demonstrate an approximate rectilinear relationship between the probit (a function of the percentage mortality) and the logarithm of the time, thereby facilitating the accurate estimation of LT50.

The conditions necessary for the selection of a convenient arbitrary mortality level. When the mortality curve can be transformed to a straight line along its complete course, the level chosen for comparison of activity is of little importance, for in these circumstances any percentage mortality can be computed from the regression with equal facility. Although the greatest accuracy might be obtained at the 50 per cent. mortality, Bliss[20] preferred to use a 97.725 per cent. mortality (corresponding to probit 7) for comparison of insecticides, as he asserted that

this conveyed more useful information to the entomologist than comparisons made on a 50 per cent. kill. Moore and Bliss[23] used a 95 per cent. mortality for a similar work.

The "virtual sterilisation time." Jordan and Jacobs[24,25] used arguments similar to those of Bliss, when comparisons of bactericides were to be made; they used the exceedingly high mortality level of 99·999999 per cent., which they called the "virtual sterilization time" (v.s.t.). This they were able to determine with very little extrapolation of the experimental data, by using a large initial inoculum (approximately 330 million organisms per ml.) and an extremely specialised experimental technique. They believed that for a proper conception of disinfection potentialities, the comparison of activity should be made at the stage nearest to complete disinfection concomitant with accurate determination, since in practice it was the absolute extinction of the organisms which was sought after.

The mortality level adopted for the experiments. One of the objects of this work was to develop the statistical technique of examining disinfection data. So long as a rectilinear regression could be established over a reasonable range, all the refinements of modern statistical methods could be applied usefully. The use of the LT50 as a basis of comparison of the activity of disinfectant solutions greatly simplifies the calculations and has been exploited for this purpose. Provided that a rectilinear probit-log. time regression may be assumed, the mathematical treatment of the disinfection data will be exactly similar for any desired level of mortality within the probit range under investigation. Comparisons based on levels outside this range must be rejustified before they are used. The results in this thesis have shown that the

TABLE I

CALCULATION OF THE SUMS OF SQUARES FOR DEVIATIONS OF LT50 OR LOG.LT50 FOR CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 20°C.

	Concentrations of ethylene glycol monomethyl ether									
	42·5 per cent.		45·0 per cent.		47·5 per cent.		50·0 per cent.		52·5 per cent.	
	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit
	208a	2·7895	208d	2·3895	209c	2·2562	209d	1·4185	210g	0·3712
	209f	2·9373	209e	2·7922	211e	2·2340	210f	2·4706	211g	0·6497
	210c	2·5255	210d	2·1268	213c	1·8588	211f	1·3843	212e	1·0784
	211c	2·6837	211d	2·1033	214c	1·6837	212d	1·8706	213d	0·9229
$S(LT\,50)$		10·9360		9·4118		8·0327		7·1438		3·0222
No. of expts.		4		4		4		4		4
$LT\,50$		2·7340		2·3529		2·0082		1·7859		0·7556
$S(LT\,50)^2$		29·989437		22·453240		16·371178		13·530870		2·574591
$\frac{S^2(LT\,50)}{n}$		29·899024		22·145495		16·131067		12·758470		2·283423
$S(LT\,50-LT\,50)^2 =$ $S(LT50)^2-\frac{S^2(LT50)}{n}$ $=SS$		0·090413		0·307745		0·240111		0·772400		0·291168

probit-log. time relationship is not strictly linear but may be assumed to be so between probits 4 and 6 without incurring any serious error.

Calculation of Results from Experiments at 20°C.

1. *Calculation of log. LT50.* Since the probit-log. time regression between probits 4 and 6 may be assumed linear, the function takes the form of

$$y = \bar{y} + b(x - \bar{x}) \qquad (1)$$

from which the value of x (the log. time) may be calculated for any value of y (the probit). By assigning y the value of 5 (the probit corresponding to a 50 per cent. response) x may be computed, since y, b and $\bar{x}$ are all known. For example, in Experiment 164b (the disinfection of *Bact. coli* by 75 per cent. ethylene glycol at 20°C.), the following data were obtained:

Log.time (x)	Probit (y)
1·301	4·102
1·699	4·447
2·255	4·874
2·477	5·418
2·631	5·431
$\bar{x}$=2·073	$\bar{y}$=4·854

The mean slope (b) for 75 per cent. ethylene glycol at 20°C. is 1·2025 (Table X, Part V[5]). Equation (1) may be transposed to

$$x = \frac{y - \bar{y} + b(\bar{x})}{b} \qquad (2)$$

Substituting in equation (2)

$$x = \frac{5 - 4{\cdot}854 + 1{\cdot}2025\ (2{\cdot}037)}{1{\cdot}2025}$$

$$= 2{\cdot}1945 = \text{log. LT50.}$$

The calculations of the log. LT50's for all the individual tests are too numerous to publish (321 separate equations are involved). However, since the mean LT50's for each concentration will be needed to calculate the empirical variance, these have been set out in Table II.

2. *Calculation of the standard errors of the LT50's.* This calculation is essentially the same as that used in the computation of the standard errors of the probit-log. time regressions (Part VIII[8]). The sum of squares for the deviations of each log. LT50 from its mean log. LT50 (the mean value of the mean LT50 for all the tests at a particular concentration) is computed for every concentration of all the compounds (for experiments performed at 20°C.). Table I shows the calculations for the monomethyl ether; the calculations for the other compounds are precisely the same. The sums of squares for the deviations of LT50 for the other compounds are included in Table II.

The next stage is to calculate the mean squares; this is achieved by dividing each sum of squares by the appropriate number of degrees of freedom. In the experiments with 42·5 per cent. monomethyl, for example, the mean square will be $\frac{0{\cdot}090413}{3} = 0{\cdot}030138$. The total sum of squares of the deviations for all the experiments at 20°C. is seen to be 6·243986 (Table II) which for 165 degrees of freedom has a mean square of 0·037842.

TABLE II

THE EMPIRICAL VARIANCE OF THE INDIVIDUAL MEANS FROM THEIR MEAN LT50'S OF EXPERIMENTS WITH CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 20°C.

Compound	Concentration	SS	N	Mean square	$V_{\log.LT50} = V_T$	$T = \sqrt{V_T}$	Mean LT50 = t min.	$S_t = t.s_T$ min.
	per cent.							
Ethylene glycol	72·5	0·160464	11	0·014583	0·003154	±0·05616	173	±9·72
	75·0	0·735303	31	0·023719	0·001183	0·03438	128	4·40
	77·5	0·219221	14	0·015659	0·002523	0·05023	65	3·26
	80·0	0·143436	9	0·015937	0·003784	0·06152	35	2·15
	82·5	0·447628	8	0·055953	0·004205	0·06485	10	0·65
	85·0	0·074278	9	0·008253	0·003784	0·06152	24	1·48
	90·0	1·111313	9	0·123479	0·003784	0·06152	6·21	0·38
Monomethyl ether	42·5	0·090413	3	0·030138	0·009461	0·09721	542	42·93
	45·0	0·307745	3	0·102582	0·009461	0·09721	225	17·82
	47·5	0·240111	3	0·080037	0·009461	0·09721	102	8·08
	50·0	0·772400	3	0·257467	0·009461	0·09721	60	4·83
	52·5	0·291168	3	0·097056	0·009461	0·09721	57	4·51
Monoethyl ether	25·0	0·013826	2	0·005913	0·012614	0·11230	311	34·93
	27·5	0·005202	1	0·005202	0·018921	0·13760	106	14·59
	30·0	0·072759	3	0·024253	0·009461	0·09721	55	5·35
	32·5	0·274433	4	0·068858	0·007568	0·08700	17	1·48
	35·0	0·171692	3	0·057231	0·009461	0·09721	9·5	0·92
Monopropyl ether	7·8	0·038138	3	0·012711	0·009461	0·09721	126	12·25
	9·0	0·049307	3	0·016436	0·009461	0·09721	55	5·35
	10·0	0·071354	3	0·023785	0·009461	0·09721	27	2·62
	11·0	0·049113	3	0·016371	0·009461	0·09721	14	1·36
	12·0	0·056922	3	0·018974	0·009461	0·09721	8	0·77
Monobutyl ether	3·50	0·036101	3	0·012034	0·009461	0·09721	133	12·93
	3·75	0·164006	3	0·054669	0·009461	0·09721	70	6·80
	4·00	0·029107	3	0·009702	0·009461	0·09721	30	2·92
	4·25	0·006001	3	0·002000	0·009461	0·09721	24	2·33
	4·50	0·021083	3	0·007028	0·009461	0·09721	10	0·97
Monohexyl ether	0·400	0·219795	4	0·054949	0·007568	0·08700	247	21·49
	0·425	0·171177	4	0·042794	0·007568	0·08700	85	7·40
	0·450	0·001404	1	0·001404	0·018921	0·13760	45	3·92
	0·475	0·089286	3	0·029762	0·009461	0·09721	37	3·22
	0·500	0·109805	4	0·027451	0·007568	0·08700	25	2·18
Total		6·243986	165	0·037842*				

*$\frac{6{\cdot}243986}{165} = 0{\cdot}037842$

The variance of log. LT50 (V_T) at a particular concentration is obtained by dividing the average mean square (0·037842) by the number of experiments performed at that concentration; in the instance cited it will be $\frac{0{\cdot}037842}{4} = 0{\cdot}009461$. Hence the greater the number of tests performed at a particular concentration the smaller will be the value of V_T. The standard error of log. LT50, (S_T) equals $\sqrt{V_T}$. The standard error

of LT50, (S_T), is given from the relationship $S_t = t.S_T$.* The standard errors of the mean values of the LT50's at each concentration have been calculated and included with their mean LT50's in Table II.

3. *Construction of limits of error curves for the estimation of LT50.* The limits of error for one estimation will depend on the mean value of LT50, determined from a large number of experiments, and on the probability level at which it is desired to work. The following examples illustrate the method of calculating the limits at three probability levels for two widely separated values of LT50.

(a) *Calculations.*

(i) *When the mean LT50 is* 100 *minutes.* The average mean square for the deviations of log. LT50 is given in Table II as 0·037842; its standard error (S_T) will be $\sqrt{0{\cdot}037842} = \pm 0{\cdot}1945$. This is the standard error for one experiment, which in terms of arithmetic time will be $100 \times \pm 0{\cdot}1945 = \pm 19{\cdot}45$ minutes (from $S_t = t.S_T$). When the mean of n experiments is taken,

$$s_t = \pm 100 \sqrt{\frac{0{\cdot}037842}{n}} = \pm \frac{0{\cdot}1945}{\sqrt{n}} \qquad (3)$$

The limits of the estimation are $\pm cs_t$ (where c = normal deviate). Hence, when one experiment is performed, the limits will be as follows:

at $P = 0{\cdot}01$, $\pm 2{\cdot}576 \times 19{\cdot}45 = \pm 50{\cdot}09$ minutes
at $P = 0{\cdot}05$, $\pm 1{\cdot}96 \times 19{\cdot}45 = \pm 38{\cdot}13$ minutes
at $P = 0{\cdot}325$, $\pm 1{\cdot}00 \times 19{\cdot}45 \pm = 19{\cdot}45$ minutes

This means that when the result from only one estimation is taken and the correct value should be 100 minutes, at $P = 0{\cdot}01$, the LT50 in one instance out of every 100, should fall outside the limits $100 \pm 50{\cdot}09$ minutes; at $P = 0{\cdot}5$, the limits will be $\pm 38{\cdot}13$ minutes, i.e., only 5 results out of every 100 fall outside the range $100 \pm 38{\cdot}13$ minutes, whereas at $P = 0{\cdot}325$, one result out of every three should fall outside the limits $100 \pm 19{\cdot}45$ minutes.

When the mean of several tests is taken, the limits of error will be proportionally smaller. The limits at the three probability levels up to 40 experiments have been calculated from equation (3) and set out in Table III(a).

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* Let t = LT50 and T = log. LT50, then T = log. t

$$V_T = V_t \frac{(dT)^2}{(dt)}$$

hence $s_T = s_t \frac{dT}{dt}$ (since $S = \sqrt{V}$)

But $\frac{dT}{dt} = \frac{1}{t}$

therefore $s_T = \frac{S_t}{t}$ or $s_t = t.s_T$.

TABLE III

RELATION BETWEEN THE LIMITS OF ERROR OF ESTIMATION OF LT50 AT DIFFERENT PROBABILITY LEVELS AND THE NUMBER OF REPLICATE TESTS PERFORMED

(*a*) WHEN LT50=100 MINUTES

	Number of replicate tests (*n*)												
	1	2	3	4	5	9	10	16	20	25	30	36	40
Limits at $P=0{\cdot}01$	50·09	35·41	28·89	22·05	22·40	16·69	15·82	12·53	11·19	10·02	9·14	8·35	7·92
Limits at $P=0{\cdot}05$	38·13	26·91	22·10	19·07	17·02	12·69	12·11	9·54	8·51	7·61	6·94	6·35	6·02
Limits at $P=0{\cdot}325$	19·45	13·76	11·22	9·73	8·71	6·48	6·15	4·87	4·35	3·89	3·55	3·24	3·08

(*b*) WHEN LT50=20 MINUTES

	Number of replicate tests (*n*)												
	1	2	3	4	5	9	10	16	20	25	30	36	40
Limits at $P=0{\cdot}01$	10·02	7·09	5·79	5·01	4·48	3·34	3·17	2·51	2·24	2·01	1·83	1·67	1·59
Limits at $P=0{\cdot}05$	7·63	5·39	4·40	3·81	3·41	2·54	2·41	1·91	1·70	1·55	1·39	1·27	1·21
Limits at $P=0{\cdot}325$	3·89	2·75	2·24	1·95	1·74	1·30	1·26	0·97	0·87	0·78	0·71	0·65	0·62

(ii) *When the mean LT50 is 20 minutes.*

$$\text{Here } s_t = \pm 20\sqrt{\frac{0{\cdot}037842}{n}} = \pm \frac{3{\cdot}98}{\sqrt{n}} \qquad (4)$$

Limits of the estimation. When one experiment is performed, the limits will be as follows:

at $P = 0{\cdot}01$, $\pm 2{\cdot}576 \times 3{\cdot}89 = \pm 10{\cdot}02$ minutes
at $P = 0{\cdot}05$, $\pm 1{\cdot}96 \times 3{\cdot}89 = \pm 7{\cdot}63$ minutes
at $P = 0{\cdot}325$, $\pm 1{\cdot}00 \times 3{\cdot}89 = \pm 3{\cdot}89$ minutes

The limits at the three probability levels up to 40 experiments have been calculated from equation (4) and set out in Table III (b).

From the sets of results in Table III curves have been constructed (Figure I) to illustrate how the limits of error of the estimation diminish as the number of tests from which the mean LT50 is calculated, is increased.

(b) *The use of the limits of error curve.* When a number of limits of error curves have been constructed to cover the range of LT50's expected in a series of experiments, the error of the estimations at the different probability levels can be deduced rapidly.

For most of the mean LT50's for different concentrations of the compounds investigated in this thesis, four estimations have been used. In some instances, e.g., ethylene glycol at 20°C., many more tests were performed at each concentration. However, it is seen from Figure 1 that if an LT50 of 100 minutes is expected, then from the mean of four experiments the experimental times may be expected to fall outside the limits of 81 and 119 minutes 5 times out of every 100 estimations at $P = 0{\cdot}05$). These limits become narrower as the number of tests is increased; in fact to halve the deviation (i.e., to double the accuracy) requires quadruple the number of tests. For example, when the mean of 20 experiments is taken, the limits are 91·5 and 108·5 minutes; yet for 30 experiments they are 93 and 107 minutes, and for 40 experiments only 94 and 106 minutes. It is necessary to decide on the limits of error required in an assay and then to perform the required number of tests to procure this accuracy; although greater precision is obtainable by carrying out a larger number of experiments from which to compute the mean, it may be decided that the benefits of the smaller additional accuracy so obtained is not in keeping with the nature of the assay.

Calculation of Results from Experiments at 30°C.

Calculation of the log. LT50's and the standard errors of LT50's. The log. LT50's for each experiment and the mean figure at each concentration of a substance was calculated as before from the probit-log. time regression equation.

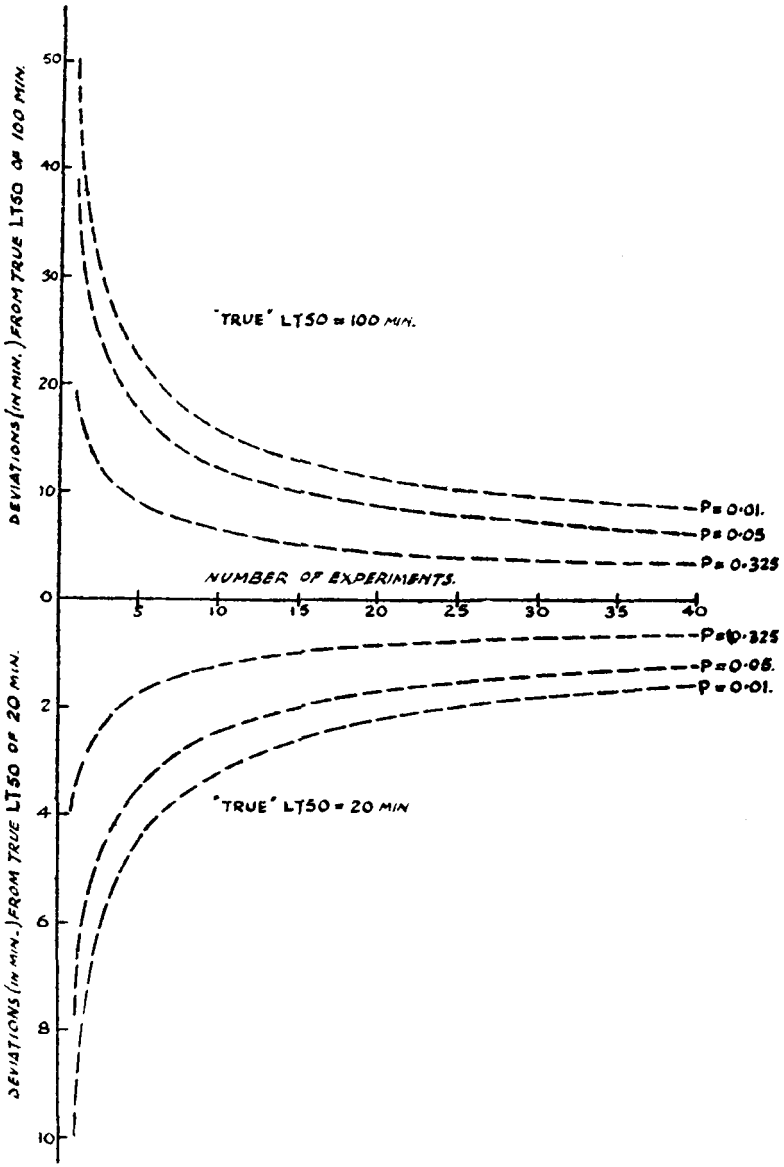

FIG. 1.—Relation between the limits of error of the estimation of LT50 at different probability levels and the number of replicate tests performed.

The sum of squares for the deviations of each log. LT50 from its log. LT50 (the mean value of LT50 for all the tests at a particular concentration) was computed for every concentration of all the compounds. These have been set out in Table IV together with their mean squares. The total sum of squares of the deviations of all the experiments is 3·184845, which for 74 degrees of freedom has a mean square of 0·043038.

The variance of log. LT50 (i.e., V_T) at a particular concentration is obtained by dividing the average mean square (0·043038) by the number of experiments performed at that concentration. Table IV presents a

TABLE IV

THE EMPIRICAL VARIANCE OF THE INDIVIDUAL MEANS FROM THEIR MEAN LT50'S OF EXPERIMENTS WITH CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 30 C.

Compound	Concentration	*SS*	*N*	Mean Square	$V_{\text{log. LT50}} = V_T$	$s_T = \sqrt{V_T}$	Mean LT50 = *t* min.	$S_t = ts_T$ min.
	per cent.							
Ethylene glycol	62·5	0·021650	2	0·010825	0·014346	0·1098	±101	±11·09
	65·0	0·425643	3	0·141881	0·010759	0·1038	113	11·73
	67·5	0·779288	5	0·155858	0·007173	0·0847	28	2·37
	70·0	0·021981	3	0·007327	0·010759	0·1038	16	1·66
Monomethyl ether	35·0	0·099914	3	0·033305	0·010759	0·1038	90	9·34
	37·5	0·050939	3	0·016980	0·010759	0·1038	54	5·61
	40·0	0·056315	3	0·018772	0·010759	0·1038	25	2·60
	42·5	0·051599	3	0·017200	0·010759	0·1038	13	1·35
Monoethyl ether	12·5	0·103907	3	0·034636	0·010759	0·1038	156	16·19
	15·0	0·142569	3	0·047523	0·010759	0·1038	87	9·03
	17·5	0·241659	3	0·080556	0·010759	0·1038	37	3·84
	20·0	0·305846	3	0·101949	0·010759	0·1038	8	0·83
Monopropyl ether	3·0	0·028459	3	0·009486	0·010759	0·1038	159	16·50
	4·0	0·034941	3	0·011647	0·010759	0·1038	91	9·45
	5·0	0·021521	3	0·007174	0·010759	0·1038	55	5·71
	6·0	0·008830	3	0·002943	0·010759	0·1038	27	2·80
Monobutyl ether	1·5	0·405014	3	0·135005	0·010759	0·1038	294	30·52
	2·0	0·086614	3	0·028871	0·010759	0·1038	50	5·19
	2·5	0·026305	3	0·008768	0·010759	0·1038	22	2·28
	3·0	0·079045	3	0·026348	0·010759	0·1038	8	0·83
Monohexyl ether	0·325	0·021501	3	0·007167	0·010759	0·1038	98	10·17
	0·350	0·008527	3	0·002842	0·010759	0·1038	64	6·64
	0·375	0·028385	3	0·009462	0·010759	0·1038	41	4·26
	0·400	0·122318	3	0·061159	0·010759	0·1038	18	1·98
	0·425	0·012075	2	0·006038	0·014346	0·1038	9	0·99
Total		3·184845	74	0·043038*				

$$*\frac{3\cdot184845}{74} = 0\cdot043038$$

summary of the bactericidal activities at 30°C. of all the concentrations of the different compounds, together with the standard errors of the mean values of the LT50's at each concentration.

It is seen that the mean square at 30°C. (0·043038) and at 20°C. (0·037842) are of the same order; this indicates that the technique is constant and sound.

THE EFFECT OF VARIATION IN THE INITIAL NUMBER OF ORGANISMS ON THE VALUE OF LT50

Experimental part. The experiments in Part VI[6], designed to show the effect of variation in the initial number of organisms on the value of the slope of the regression, are also suitable to demonstrate the effect on the value of the LT50.

Results and calculations. Table V sets out the LT50's obtained for the experiments, which were carried out with 75 per cent. ethylene glycol at 20°C. Log. LT50 was calculated from the equation $y = y + b(x - \bar{x})$

where $y=5$, $y=$the mean value of y for a test, $b=$the mean slope of the regression (1·2025, Table X Part V[5]), $x=$log. LT50 and $\bar{x}=$the mean value of x for a test.

TABLE V

VALUES OF LT50 FROM THE DISINFECTION OF *BACT. COLI* BY 75 PER CENT. ETHYLENE GLYCOL AT 20°C. FROM EXPERIMENTS USING DIFFERENT INITIAL NUMBERS OF ORGANISMS

Group	Expt. No.	Initial inoculum. No. of organisms per ml.	Log. LT50	LT50 minutes
A	186f	114,000	2·126	134
	186d	221,000	2·039	109
	186b	379,000	2·005	101
B	199e	156,600	2·109	129
	199g	1·491 millions	2·035	108
	199j	12·83 ,,	2·118	131
	199b	13·30 ,,	2·062	115
C	184b	1·247 ,,	1·984	96
	184d	2·484 ,,	1·933	86
	184f	5·295 ,,	1·985	97
	184h	12·64 ,,	2·000	100
D	168b	9·44 ,,	1·903	80
	167b	16·54 ,,	2·151	142
	169b	28·10 ,,	1·963	92
E	202d	15·25 ,,	2·018	104
	202f	81·27 ,,	2·182	152
	202f	143·1 ,,	2·140	138
	202k	299·6 ,,	2·387	244
F	200b	462·9 ,,	2·290	195
	200c	462·9 ,,	2·375	237
	200d	462·9 ,,	2·324	211
	200e	462·9 ,,	2·490	309

CONCLUSION

There was no correlation betwen the LT50 and the initial number of organisms over a very large range (114,000 organisms per ml. to 143·1 millions per ml.); the results from experiments with still heavier initial inocula, however, gave larger LT50's. The experimental technique was not sufficiently sensitive to detect differences in the values of LT50 over a certain range, and it would appear that there is considerable latitude in the numbers of organisms which should be added to disinfectant solutions when comparing their bactericidal activities under the standardised conditions.

SUMMARY

1. The advantages of using intermediate mortality levels instead of end-points and reaction velocities for the comparison of bactericidal activity have been discussed.

2. The time to kill 50 per cent. of the initial inoculum (LT50) has been employed and its logarithm computed mathematically from the probit-log. time regression equation of the disinfection data between *Bact. coli* and ethylene glycol and its monoalkyl ethers, for experiments at 20°C. and at 30°C.

3. The standard errors of the LT50's for experiments at 20°C. and 30°C. have been computed; from the former, the limits of the estimations at three probability levels (at $P=0{\cdot}01$, $0{\cdot}05$ and $0{\cdot}325$) have been calculated and limits of error curves constructed.

4. The experimental technique was not sufficiently sensitive to detect differences in the values of LT50's over a large range of different initial numbers of organisms under standardised conditions. After a certain stage had been reached, however, heavier initial inocula resulted in larger LT50's.

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