STUDIES IN THE DYNAMICS OF DISINFECTION

II. THE CALCULATION OF THE CONCENTRATION EXPONENT FOR PHENOL AT 35°C. WITH BACT, COLI AS TEST ORGANISM

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(With 3 Figures in the Text)

The present system of expressing the activity of germicides in terms of the phenol coefficient is, obviously, very unsatisfactory. Apart from criticism that can be made of the method itself, it is manifestly impossible to attempt to sum up in a single ratio the behaviour of a germicide at all temperatures and concentrations, when the activity of the substance on dilution or on changing the temperature may not vary in the same way as that of the standard germicide, phenol. This fallacy has long been recognized although the practice of determining phenol coefficients still persists.

Chick (1908) formulated an empirical relationship between concentration and disinfection time, and Watson (1908) showed that the relation could be expressed in the form $C^n \times t = constant$ (K). Phelps (1911) proposed that a method of standardization should comprise the determination of the true velocity coefficient (K), the concentration exponent n and the temperature coefficient θ for each disinfectant. It appears, however, that the reaction velocity is often far from constant, and it may be that it is never really so when the experimental conditions are so designed that the death of the bacteria is due to the germicide alorle and not to a combination of adverse influences. This point has been discussed in an earlier paper (Jordan & Jacobs, 1944), in which an improved technique is described, and the results of the action of phenol on Bact. coli are analysed from the aspect of the distribution of resistance among the cells. In the present paper the same results are used for the calculation of the concentration exponent for phenol. Withell (1942 a, b) has recently stressed the importance of determining the manner in which the activity of a germicide varies with concentration, at the same time emphasizing the necessity for the adoption of 'counting' instead of 'end-point' methods in the assay of germicidal activity. With this opinion the present authors are in complete agreement.

RESULTS

It should be emphasized that the death-rate of *Bact. coli*, under the special experimental conditions used here, was, at any one phenol concentration, far from constant throughout the germicidal process and showed signs of rising to a peak value from which it subsequently declined. The evidence for this has already been discussed (Jordan & Jacobs, 1944), but it was decided that, because

J. Hygiene 43

of the excessive variation between replicate plates which tends to occur in counts made at high mortalities, this peak and subsequent fall in the death-rate cannot be regarded as firmly established. Accordingly, the course of the disinfection is treated below as if the death-rate had risen to a maximum value which was maintained until the mortality was virtually complete. With the limited data available this would appear to be the most satisfactory presentation of the germicidal process.

Table 1 shows the times required, at the several phenol concentrations, to produce various degrees of mortality at 35° C. The 'virtual sterilization time' (v.s.t.) is the time required for the mortality to reach 99.9999999 % of the initial number of organisms present (generally about 330×10^6 per ml.), and this time was obtained from the calculated line of regression of log survivors on time during the phase of constant maximum death-rate, by making the appropriate substitutions in the formulae given in the earlier paper of this series. As the experiments were often prolonged until mortalities in excess of 99.99999 % had been recorded, the extent of the extrapolation required was not great. The times for 99.9 % and for 99% mortalities have also been calculated from these regression formulae, except in the latter case at the lowest phenol concentration when this time, and also the times to reach 50 % mortality, were read off from the smoothed log survivors-time curves.

The disinfection time of previous workers may be regarded as equivalent to the *v.s.t.* of this research, or to some fixed very low percentage of survivors. The results here presented are in good agreement with the established empirical relation $C^n \times t = K$, where *t* is the *v.s.t.*, since, if the logarithms of the *v.s.t.* are plotted against the logarithms of the phenol concentrations a straight line is obtained. This is shown in Fig. 1, graph *A*, where the line drawn is the best straight line calculated to fit the points, its equation being $\log_{10} t + 5.8421 \log_{10} C = 6.6064$, where *t* is the *v.s.t.* in min. and *C* the concentration of phenol in g. per l. Transformed, this becomes

$$C^{5\cdot8421} \times t = 4\cdot040 \times 10^{6}.$$

The value 5.8421 for n has a standard error of ± 0.1876 . Graph B in Fig. 1 shows that a satisfactory linear relation between $\log_{10} t$ and $\log_{10} C$ is also obtained when the 99 % mortality times are used, but the new value of n, 6.9638 ± 0.2164 , differs significantly from that derived

 $\mathbf{25}$

from the *v.s.t.* Applying the 't' test (Fisher, 1938) a value of 3.916 is obtained, which for 16 degrees of freedom gives P < 0.01. There is also a linear relationship between the logarithms of the phenol concentrations and the logarithms of the 99.9 % mortality times, but this graph has not been included in Fig. 1 as it runs too close to graph *B*. The value of *n* derived from these times is 6.6062 ± 0.2034 , and it differs significantly from the

from the value of n based on the 99 % mortality times (P, 0.5 approx.). Above 4.62 g. per l. graph C increases in slope. When a straight-line is fitted above this point the value of n obtained is 8.9835 ± 1.662 .

Although the death-rates were not constant throughout each experiment in the present series, it appears that phenol concentration is related in a similar manner to both maximum death-rate (see Jordan & Jacobs, 1944)



Fig. 1. Showing relationship between \log_{10} phenol concentration and \log_{10} virtual sterilization time (curve A), $\log_{10} 99$ % mortality time (curve B) and $\log_{10} 50$ % mortality time (curve C).



Fig. 2. Showing relationship between log phenol concentration and log maximum death-rate for Bact. coli.

value 5.8421 (P between 0.01 and 0.02) but not from the value 6.9638 (P, 0.2-0.3). Graph C (Fig. 1) shows the logarithms of the 50 % mortality times. It is evident that here a single straight line no longer fits the data adequately and a curve is indicated. The upper portion of this curve is almost straight and over the concentration range 3.48-4.62 g. phenol per l. gives a value for n of 6.4438 ± 0.3122 . This value does not differ significantly from that derived from the v.s.t. (P, 0.3 approx.) nor

and v.s.t. Table 2 and Fig. 2 illustrate this relation, the line shown being the calculated best straight line whose formula is $\log_{10} k_m' = 5.0752 \log_{10} C - 2.0376$, where k_m' is the maximum death-rate (×1000 for convenience). The standard error of the slope of this line is ± 0.2110 . Alternatively, $k_m = 9.1743 \times 10^{-6} \times C^{5.0752}$, where k_m is the actual maximum death-rate per min. It should be observed that, whereas at 7.0 g. phenol per l. and over, the maximum death-rate occurred during the deaths, of

364

a considerable proportion of the total organisms, at 3.48 g. per l. it covered only a very small fraction of the total deaths, in fact, less than 0.2 %.

Table 2. Maximum death-rates of Bact. coli for various phenol concentrations at 35° C.

| Phenol concen- tration g. per l. | Log ₁₀ phenol concen- tration | Maximum death-rate per min. (×1000) | Log ₁₀ maximum death-rate |
|---|---|--|--|
| 3 · 4 8 | 0.5416 | 3.59 | 0.5551 |
| 3.76 | 0.5752 | 8.50 | 0.9294 |
| 3.96 | 0.5977 | 10.70 | 1.0294 |
| 4 ·00 | 0.6021 | 12.30 | 1.0899 |
| 4.25 | 0.6284 | 14.60 | 1.1644 |
| 4-62 | 0.6646 | $25 \cdot 40$ | 1 4048 |
| 5.09 | 0.7067 | 30.10 | 1.4786 |
| 6·04 | 0.7810 | 84.70 | 1.9279 |
| 6.98 | 0.8439 | 194.10 | $2 \cdot 2880$ |
| 8.00 | 0.9031 | 311.10 | $2 \cdot 4929$ |

DISCUSSION

It is important to remember that the formula relating concentration and time is an empirical one based on disinfection times, i.e. those times required for the production of some low level of survivors such that either by chance no viable organisms are removed in the small volume of suspension used for subculturing, or the organisms removed are too few to initiate growth. The formula is not necessarily applicable to the times required to produce other (intermediate) degrees of mortality. Supposing the law were applicable to intermediate mortalities and that at concentrations C_1 and C_2 times t_1 and t_2 were required for the production of this high degree of mortality, while times T_1 and T_2 were needed for the production of a lesser mortality, then $C_1^n t_1 = C_2^n t_2$ and $C_1^n T_1 = C_2^n T_2$. The necessary relation between the four times is thus that $t_2/t_1 = T_2/T_1$ when n is constant. This condition would clearly be fulfilled in either of two cases, (a) if the bacteria had constant death-rates, i.e. if the logarithms of the numbers of survivors plotted against time gave straight lines at all concentrations, and (b) if the probit-log survival time curves were parallel at all concentrations, since then.

$$\log t_2 - \log t_1 = \log T_2 - \log T_1.$$

Concerning the former case, the log survivors-time curves were, in all the present series of experiments, so far from being straight lines that it need not be dealt with here. With regard to the latter case, the relationship between probits and log survival times for these data has already been analysed in detail (Jordan & Jacobs, 1944), where it was shown that the results appear to be fitted best by intersecting straight lines. As far as the comparison of intermediate mortalities is concerned, the existence of these changes in slope of the probit-log survival time graphs would not be of significance, provided that they always occurred at a fixed probit value and that the two intersecting straight lines were parallel to their counterparts at all phenol concentrations. These conditions were not always satisfied.

Withell (1942a) proposed that the times required to produce a 50 % mortality (L.T. 50) should be used in the formula $C^n \times t = K$ for calculation of the concentration exponent, claiming that 'this preserves the sense of the equation', but without proving that the use of the L.T. 50 as an alternative to the disinfection time was justified. Later, the same author (1942b) showed that, with Bact. coli as the test organism, the probit-log survival time graph for phenol at 5 g. per l. was parallel to that at 6 g. per l. Parallel lines were also given by parachlormetacresol at 0.7 and 0.75 g. per l. Having proved the existence of parallelism over these small concentration ranges, Withell proceeded to determine the concentration exponent for each germicide over much wider ranges, namely, 5-9 g. per l. for phenol and 0.5-0.75 g. per l. for

parachlormetacresol, although the use of the L.T. 50, or any other mortality time, can only be justified if it has been shown that the probit-log survival time graphs are parallel over the full range of concentrations concerned. The results of the present authors, previously reported, show that these graphs are not parallel at all concentrations of phenol, although from 3.76 to 4.62 g. per l. there is very little difference in the slope of the upper line of each pair of graphs, which in all cases covers the probit value 5 (50 % mortality). In agreement with this, the graph of the logarithms of the 50 % mortality times plotted against the logarithms of phenol concentrations (Table 1, Fig. 1, C) runs almost parallel to those for the v.s.t. and 99 % mortality times over this range (Fig. 1, A, B). Above 4.62 g. phenol per l. the graphs A and C cease to lie parallel, and clearly the 50 % mortality time is not so satisfactory as the v.s.t. for calculating the concentration exponent. Indeed, if the former were chosen the value of n for phenol would vary considerably according to the concentration range over which it was determined. Using the 50 % mortality times, the value of n is 6.4438 ± 0.3122 over the concentration range 3.48-4.62 g. per I., but it rises to 8.9835 ± 1.662 over the range 4.62-8.00 g. per l. However, the difference of over 2.5, although large, cannot be considered significant since P is between 0.2 and 0.3. Nevertheless the latter value of n has a standard error more than five times as large as that of the former. This error may well have been large when the phenol concentrations were high and the 50 % mortality times short because it is difficult to determine the latter with sufficient accuracy and the results are likely to be irregular. Assuming that the value for n (6.4438) for the concentration range 3.48-4.62 g. per I. had held for all concentrations, calculation of the 50 % mortality time at 8.00 g. per l. gives 4.8 min., whereas the experimental value was 1.9 min. The latter value may have been a low estimate since the end of the 5 min. occupied by the addition of the phenol solution was taken as zero time and at high concentrations some cells must have died before the addition was complete (see Jordan & Jacobs, 1944). Clearly, with the technique employed here, very short times cannot be regarded as satisfactory for the calculation of the concentration exponent. It is interesting to find that if the logarithms of the L.T. 50 times given by Withell (1942b) are plotted against the logarithms of phenol concentrations a graph very similar to that of Fig. 1, C is obtained (see Fig. 3), thus affording further evidence that the 50 % mortality times give values of the concentration exponent for

Table 1. Relation between phenol concentration and times of exposure of Bact. coli required to produce various degrees of mortality at 35° C.

| rtality time | | { | log ₁₀ min. | 3.0212 | 2.7597 | 2.6561 | 2.6454 | 2.4871 | 2.1987 | 1.7160 | 1.0792 | 0.0414 | 0-2788 |
|--|-----------------|----------------------|------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------------------|---------------------------------|---------------------|---------------------|---------------------|
| | 20 % moi | | min. | 1050 | 575 | 453 | 442 | 307 | 158 | 52 | 12 | 1.1 | 1-9 |
| | rtality time | | log ₁₀ min. | $3 \cdot 2923$ | 2.9750 ± 0.0019 | 2.8156 ± 0.0171 | 2.8089 ± 0.0074 | 2.6609 ± 0.0147 | 2.3674 ± 0.0180 | 2.0334 ± 0.0489 | 1.5051 ± 0.0430 | 1.0000 ± 0.0678 | 0.8633 ± 0.0694 |
| | 10m % 66 | au % mor | min. | 1960 | 944 ± 4.2 | 654 ± 25.7 | 644 ± 11.0 | 458 ± 15·5 | 233 ± 9.7 | 108 ± 12.2 | 32 ± 3.2 | 10 ± 1.6 | 7.3±1.17 |
| Virtual sterilization time 99.9 % mortality time | ortality time | | log ₁₀ min. | 3.3659 ± 0.0030 | 3.0257 ± 0.0014 | 2.8733 ± 0.0117 | 2.8603 ± 0.0062 | 2.7210 ± 0.0102 | $2 \cdot 4362 \pm 0 \cdot 0122$ | $2 \cdot 1523 \pm 0 \cdot 0307$ | 1.6335 ± 0.0274 | 1.1761 ± 0.0384 | 1.0212 ± 0.0390 |
| | om % 6.66 | | min. | 2322 ± 15.9 | 1061 ± 3.3 | 747 ± 20.2 | 725 ± 10.4 | 526 ± 12.4 | 273 ± 7.7 | 142 ± 10.0 | 43 ± 2.7 | 15 ± 1.3 | 10.5 ± 0.94 |
| | rilization time | srilization time | log ₁₀ min. | 3.5700 ± 0.0060 | 3.2175 ± 0.0005 | 3.0845 ± 0.0059 | 3.0539 ± 0.0084 | 2.9390 ± 0.0117 | 2.6721 ± 0.0131 | 2.4886 ± 0.0222 | 2.0086 ± 0.0195 | 1.6128 ± 0.0155 | 1.4314 ± 0.0187 |
| | Virtual ster | | min. | 3715 ± 50.9 | 1650 ± 2.05 | 1215 ± 16.5 | 1132 ± 21.8 | 869 ± 23.4 | 470 ± 14.1 | 308 ± 15.8 | 102 ± 4.6 | 41 ± 1.5 | 27 ± 1.2 |
| tenol stration | , | log ₁₀ g. | per l. | 0.5416 | 0.5752 | 0.5977 | 0.6021 | 0.6284 | 0.6646 | 0.7067 | 0.7810 | 0.8439 | 0.9031 |
| Concen | | g. per l. | 3.48 | 3.76 | 3.96 | 4·00 | 4.25 | 4.62 | 5.09 | 6.04 | 6.98 | 8·00 | |

Table 3. Comparison of experimental and calculated values of v.s.t. and log₁₀ (v.s.t.). Values calculated from regression of log₁₀ (v.s.t.) on log₁₀ (phenol concentration)

| | ence | " | mental | ue* | 'n. | I | 32 | 6 | 2 | 60 | 35 . | 16 | 6 | œ | 4 |
|---------------------|------------|--------------|-------------------|--------------|-----------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| ion | Diffe | fro | experii | valı | Ĩ | 1 | í | 1 | + | Ĩ | ÷ | Ì | + | + | 1 |
| ol concentrati | | | | v.s.t. | min. | ł | 1618 | 1206 | 1139 | 809 | 505 | 292 | 111 | 49 | 23 |
| ue for lowest pheno | • | Difference | from | experimental | value* | I | -0.0087 | -0.0030 | +0.0027 | -0.0312 | +0.0309 | -0.0239 | +0.0357 | +0.0755 | -0.0781 |
| Omitting ve | | | | | $\operatorname{Log}_{10}(v.s.t.)$ | I | 3.2088 ± 0.0238 | 3.0815 ± 0.0215 | 3.0566 ± 0.0211 | 2.9078 ± 0.0188 | 2.7030 ± 0.0166 | 2.4647 ± 0.0158 | 2.0443 ± 0.0195 | 1.6883 ± 0.0277 | 1.3533 ± 0.0329 |
| | Difference | from | experimental | value* | min. | -946 | +112 | + 87 | + 95 | so I | + 59 | 80 | 6 + | 9 + | 9 |
| 702 | | | | v.s.t. | min. | 2769 | 1762 | 1302 | 1227 | 861 | 529 | 300 | 111 | 47 | 21 |
| Including all value | | • Difference | from | experimental | value* | -0.1277 | +0.0285 | +0.0300 | +0.0349 | 0.0038 | +0.0516 | - 0-0109 | +0.0351 | +0.0634 | -0-1011 |
| | | | | | $Log_{10} (v.s.t.)$ | 3.4423 ± 0.0344 | 3.2460 ± 0.0298 | 3.1145 ± 0.0271 | 3.0888 ± 0.0266 | 2.9352 ± 0.0241 | 2.7237 ± 0.0220 | 2.4777 ± 0.0221 | 2.0437 ± 0.0282 | 1.6762 ± 0.0368 | 1.3303 ± 0.0464 |
| | | | Log ₁₀ | (g. phenol | per l.) | 0.5416 | 0.5752 | 0.5977 | 0.6021 | 0.6284 | 0.6646 | 0.7067 | 0.7810 | 0.8439 | 0-9031 |

* The sign is positive when the calculated value exceeds the experimental.

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phenol which rise for high concentrations. The same author's data for *parachlormetacresol* yield a straight line when plotted in this way, but this may be because the effect was very rapid (many of the L.T. 50 values were less than 1 min.) so that the log survivors-time curves were approximately straight lines. As mentioned above, when this is the case the times to reach any fixed level of survivors can be used in the formula $C^n \times t = K$.

Consideration of the times taken to reach 99, 99.9 and 99.999999 % mortality (v.s.t.) shows that they can all be used for the calculation of satisfactory (i.e. constant) concentration exponents over the full range of phenol



Fig. 3. Showing relation between logarithms of L.T. 50 times (Withell's data, see text) and logarithms of phenol concentrations.

concentrations. The values obtained, however, vary according to the degree of mortality chosen, being 6.9638 ± 0.2164 , 6.6062 ± 0.2034 and 5.8421 ± 0.1867 respectively. The last value differs significantly from both the others, but the difference between the first two values is not significant. Which then of these values of nis the most important? It is, of course, complete and not partial sterilization which is desirable in practice, and in view of this it would seem that the value of *n* derived from the v.s.t. should take precedence over the others. Thaysen (1938) has shown that any method for determining these times which depends on obtaining sterile subcultures is very unreliable and counting methods should, therefore, be adopted. If, as a result of further work, it should prove that variation of the value of the concentration exponent with degree of mortality is a general phenomenon, then it will be desirable to adopt a method of the type used in

the present work which allows such variations to be determined. In order that interfering factors should be minimized, as was stressed in the previous paper of this series, it is suggested that the technique of choice should involve the direct addition of the germicide to a standard bacterial culture maintained in an otherwise constant environment. A difficulty is that virtual sterilization as defined above is a percentage mortality, so that standardization of the initial number of viable cells is required if comparable results are to be obtained by different workers. The exact value of the percentage mortality which should be regarded as representing virtual sterilization is an arbitrary matter. Levine, Buchanan & Lease (1926-7), who used caustic soda as germicide, suggested that the times to reach 99.9 % mortality might be used for the calculation of the concentration exponent, but Myers (1929) contended that these were unsatisfactory, as they involved low counts and large errors. Hobbs & Wilson (1942) also came to the conclusion that in the presence of caustic soda the times to reach high mortalities could not be determined accurately. Concerning this point, the percentage mortality which it is possible to determine without the number of colonies per plate falling too low depends on the initial number of viable cells, and in the experiments reported here extremely high percentage mortalities were recorded. The present authors are of the opinion that the degree of mortality representing virtual sterilization should be as high as can be made practicable. At the same time the tendency to excessive variation between replicate plates of counts made at high mortalities must be borne in mind, since it may not be possible to eliminate this in all cases by improvements in technique. It is intended to use these new experimental methods to obtain more data with other germicides in the hope of throwing further light on the problem of the variations in the concentration exponent.

The virtual sterilization times have afforded a satisfactory basis for the calculation of the exponent for phenol, the points for the data lying very closely on graph A in Fig. 1. Since the *v.s.t.*'s were obtained by calculation from one end of a regression line they may be expected to have fairly large standard errors, and it may be questioned whether the goodness of fit of the data to a straight line may not be an artefact. However, the standard errors of the v.s.t.'s were in fact not large (see Table 1). They never exceeded 5.13 % of the time, and in five cases the percentage error was 3 or less. The points have thus been fixed with some precision. Looking at the matter in another way, since in all cases the variance of the v.s.t. was small compared with the square of the v.s.t., the quantity $V(v.s.t.)/(v.s.t.)^2$ can be taken as a measure of the variance of $\log_{e} (v.s.t.)$. The values given in Table 1 for the standard errors of the logarithms of the virtual sterilization (and other) times have been calculated in this way. The standard errors of the logarithms of the v.s.t.'s are all quite small, the values thus possess a considerable degree of accuracy and the good fit of the data to a straight line when plotted against log phenol concentration must be regarded as genuine. In Table 3 are given the values of $\log_{10} (v.s.t.)$ calculated from the regression of $\log_{10} (v.s.t.)$ on \log_{10} (phenol concentration) and also the corresponding v.s.t.'s, together with the differences from the experimental values given in Table 1.

In spite of the close fit of the data to a straight line, the calculated v.s.t. for the lowest phenol concentration differs from the experimental value by 946 min., although the logarithms differ by only 0.1277. At other concentrations the differences are not unduly large. Evidently when the v.s.t. is long quite large deviations from calculated values may be obscured through the use of the logarithmic scale for plotting. The difference between the actual and calculated values is regarded as being too great to be due entirely to experimental errors, although the possibility exists that in prolonged experiments significant amounts of phenol may have been lost in the air-stream, resulting in an increase in the v.s.t. A divergence of this kind from the empirical law is, however, to be expected at low concentrations, since with all poisons there is a threshold value below which no lethal effect is produced. In other words, t becomes infinite while Cis still measurable, so that n must increase when C is very small. If this aberrant value at the lowest phenol concentration be omitted from the calculations, the formula of the best straight line becomes

$$\log_{10} t + 5.6588 \log_{10} C = 6.4638$$

368

$$C^{5\cdot6588} \times t = 2 \cdot 909 \times 10^6.$$

This value of n has a standard error of ± 0.1422 . Table 3 also shows the differences between the experimental values and the calculated values of the v.s.t. and $\log_{10}(v.s.t.)$ based on this new value of n. As would be anticipated, the agreement between the calculated and experimental values is closer than before. These two values of n do not differ significantly, P being between 0.4and 0.5, and both values for the concentration exponent are very close to that of 5.5 calculated by Watson (1908) from Chick's data, although the experimental conditions were very different. Tilley (1939) obtained a value of 5.7, but Reichel (1909) found the much lower figure of 4.0. Withell (1942b) gives the value 4.3, but this was derived from the 50 % mortality times which in our data were not completely satisfactory for this calculation.

The general acceptance of the idea that bacteria have constant death-rates (k) when exposed to lethal agencies has led to the adoption of a method of calculating concentration exponents from the death-rates by the substitution of 1/k for t in the formula connecting concentration and time. This method is obviously satisfactory so long as the death-rate is effectively constant, but when this varies markedly during the germicidal process the value of k used is, in effect, the over-all death-rate. In that case there is no advantage in using the over-all death-rate rather than the time itself, and it is merely a roundabout way of calculating the exponent. In the calculations above, the use of the times to reach various degrees of mortality has been equivalent to using the over-all death-rates (over the corresponding portions of the disinfections), but it is not justifiable to use the actual death-rates when these vary considerably. Other workers who have obtained varying rates have come to the same conclusion, e.g. Levine et al. (1926-7) working with caustic soda. Finally, it must be stressed that it is necessary to determine the complete disinfection curves at a number of concentrations of germicide if information is needed as to the actual progress of the disinfection process since, clearly, the fact that the law $C^n \times t = K$ applies to the times for any particular degree of mortality is no guarantee that the death-rate has been constant throughout each experiment. It is important that this should be realized, since calculations of the time required to produce a given mortality, or of the number of survivors after a given time, assuming a constant death-rate, would give very misleading results. The supposed constant deathrate would, in fact, be an estimate of the apparent overall death-rate, whereas, in the conditions used here, considerable variations in the death-rates have been encountered. For instance, at the lowest phenol concentration used in these experiments, the calculated 50 % mortality time, assuming a constant death-rate, is 136 min. as against the actual time of 1050 min. Again, after 1000 min. the calculated number of survivors is about 2×10^6 per ml. compared with the actual figure of 180×10^6 . The importance of determining the shape of the disinfection curve, particularly at low concentrations of germicide, is obviously great and such investigations should on no account be omitted.

SUMMARY

1. Disinfection curves obtained from data on the action of phenol on Bact. coli at 35° C. under conditions such that unfavourable circumstances, other than the presence of the germicide, were as far as possible eliminated, have been used for the calculation of the concentration exponent for phenol, i.e. n in the formula $C^n \times t = K$. The death-rate was not constant throughout the germicidal process but showed initially a phase of slow but increasing death-rate which merged gradually into a phase which was treated (for reasons given) as one of constant rate. This was also the maximum rate for any given phenol concentration.

2. The virtual sterilization times (v.s.t.'s), i.e. the times in min. required for the mortality to reach 99.999999 % as determined by slight extrapolation of the log survivorstime curves, the 99.9% mortality times and the 99% mortality times could all be used for the calculation of values of n for phenol as they all gave satisfactory linear relations between log concentration and log time.

3. The 50 % mortality times did not show a satisfactory linear relation between log phenol concentration and log time over the full concentration range, and at this mortality level the concentration exponent appeared to increase for concentrations above 4.62 g. phenol per l.

4. The value of n varied according to the mortality level chosen. It was 5.8421 ± 0.1876 , 6.6062 ± 0.2034 and 6.9638 ± 0.2164 when the v.s.t.'s, 99.9 % mortality times or 99 % mortality times were used. The differences between the first and second and first and third values are significant, but that between the second and third values is not. The value calculated from the v.s.t.'s is regarded as being the most important.

5. Evidence was obtained that, as expected on theoretical grounds, n increases for very low concentrations of phenol. If the aberrant value obtained at the lowest phenol concentration be omitted from the calculations, the value of n calculated from the v.s.t.'s becomes $5.6588 \pm$ 0.1422, but the decrease is not significant.

6. The maximum death-rate was related to the phenol concentration according to the expression $k_m = 9 \cdot 1743 \times 10^{-6} C^{5 \cdot 0752}$, where k_m is the maximum (logarithmic) death-rate per min. and C the concentration of phenol in g. per l.

REFERENCES

| CHICK, H. (1908). J. Hyg., Camb., 8, 92. FISHER, R. A. (1938). Statistical Methods for Research Workers, 7th ed. Edinburgh: Oliver and Boyd. HOBBS, B. C. & WILSON, G. S. (1942). J. Hyg., Camb., 42, 436. JORDAN, R. C. & JACOBS, S. E. (1944). J. Hyg., Camb., 43, 275. | MYERS, R. P. (1929). J. Agric. Res. 38, 521. PHELPS, E. B. (1911). J. Infect. Dis. 8, 27. REICHEL, H. (1909). Biochem. Z. 22, 149. THAYSEN, A. C. (1938). J. Hyg., Camb., 38, 558. TILLEY, F. W. (1939). J. Bact. 38, 499. WATSON, H. E. (1908). J. Hyg., Camb., 8, 536. WITHELL, E. B. (1942a). J. Hyg., Camb., 42, 339. |
|--|---|
| LEVINE, M., BUCHANAN, J. H. & LEASE, H. (1926-7). Iowa St. Col. J. Sci. 1, 379. | WITHELL, E. R. (1942b). Quart. J. Pharm. 15, 301. |

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CORRIGENDUM

STUDIES ON AIR-BORNE VIRUS INFECTIONS

II. THE KILLING OF VIRUS AEROSOLS BY ULTRA-VIOLET RADIATION

By D. G. ff. EDWARD, DORA LUSH AND R. B. BOURDILLON

We regret that in the above paper (vol. 43, p. 11) we referred to an ultra-violet lamp used in experiments on the killing of influenza virus aerosol as a G.E.C. 'Sterilamp'. We now learn that this was incorrect, and that the word 'Sterilamp' is a

registered trademark which should be used only for describing a germicidal lamp made by the Westinghouse Electric Company. This Company has asked us to publish this correction.

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