STUDIES IN THE DYNAMICS OF DISINFECTION

X. THE EFFECT OF LETHAL TEMPERATURES ON STANDARD CULTURES OF *BACT. COLI*. III. ON THE VARIATION OF THE RATE OF DISINFECTION WITH TEMPERATURE AT *p*H 7·0, INCLUDING THE CALCULATION OF A NEW AND CONSTANT TEMPERATURE COEFFICIENT.

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(With 5 Figures in the Text)

The temperature coefficient is a most important characteristic of any disinfection process, providing as it does a means of calculating the acceleration in rate of reaction to be expected from a given rise in temperature. In the destruction of moist bacteria by heat, temperature coefficients can also be calculated, the lethal agent in this case being presumably the molecules of the water in which the organisms are suspended, or ionic products of the water or the substances dissolved therein. Chick (1930) speaks of disinfection by hot water, but this is really only a convenient term to use, since it would be difficult if not impossible to obtain bacteria suspended in pure water. Also, in the unbuffered system of bacteria in water small amounts of solutes added with the cells might markedly affect the *p*H, and hydrogen ions can be actively germicidal.

Certain experiments on the disinfection of whole cultures of *Bact. coli*, the details of which have already been reported (Jordan, Jacobs & Davies, 1947), have yielded results which can be used for calculation of the temperature coefficient of the rate of destruction of this organism in its own buffered culture fluid. These disinfections should, therefore, be regarded as having been accomplished by hot phosphate solutions at *p*H 7·0, containing also the products of the metabolism by *Bact. coli* of Difco nutrient broth. This point is important since the lethal effects of heat (so-called) may vary considerably with the nature of the medium in which the organisms are suspended, and in experiments designed to test the effect at relatively high temperatures of an added germicide, any lethal action due to the other components of the environment must be borne in mind.

In the following discussion it is shown how these particular results fit the various formulae proposed for the calculation of temperature coefficients for biological systems, including that recently suggested by Jordan & Jacobs (1946*b*) and successfully applied to the disinfection of similar standard cultures of *Bact. coli* by phenol.

RESULTS AND DISCUSSION

In the experiments to be discussed here the temperature of a large volume of *Bact. coli* culture was rapidly raised to the required level, and then the course of the disinfection was followed by making bacterial counts at intervals with the usual plating technique. The cultures were grown under standard controlled conditions as described previously (Jordan & Jacobs, 1944), at a *p*H of 7·0 obtained by the use of a phosphate buffer. It is known that in these cultures no significant change in *p*H occurs in the period of about 40 hr. allowed for the population to reach its stable level of approximately 330 million viable cells per ml. As has already been shown (Jordan *et al.* 1947), the logarithmic death-rate varied with time during the disinfections, and accordingly it is not feasible to use the actual death-rates for the calculation of the temperature coefficient. A similar situation existed in the experiments with phenol, and in that case the time taken to reach a mortality of 99·999999 % was taken as a measure of the over-all death-rate (Jordan & Jacobs, 1946*a*). The use of such a high percentage mortality was only possible because a large initial population was employed, and because there was a progressive increase in mortality towards complete sterilization. In the heat disinfections now under discussion, although equally large numbers of cells were used, the mortality never reached such a high level since an apparently permanent population of survivors became established (Jordan *et al.* 1947). In fact, the highest mortality which could with safety be used for the comparison of all the experiments was 99·99 %, and the time taken to reach this point is here used as a measure of the over-all rate of

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reaction for the present purpose of calculating the temperature coefficient. In the paper cited above it was shown that a linear relationship existed between the logarithms of the numbers of survivors and time over the mortality range of approximately 90–99·99 %, and the equations for the regressions of log survivors on time were given. From these equations the 99·99 % mortality times have been calculated together with their standard errors. For convenience, these times will hereafter be called the disinfection times, and their values at different temperatures are given in Table 1, with their logarithms. The manner in which the disinfection time varied with temperature is shown graphically in Fig. 1, from which it is evident that small changes in temperature had a profound effect on the disinfection time, which was roughly doubled by a decrease in temperature of 1°. From the shape of the graph in Fig. 1 it is obvious that a minimum temperature for the reaction must exist at which the disinfection time becomes infinitely large. This needs no emphasis since growth must occur if the temperature be reduced sufficiently, provided that the organisms have access to food, a condition

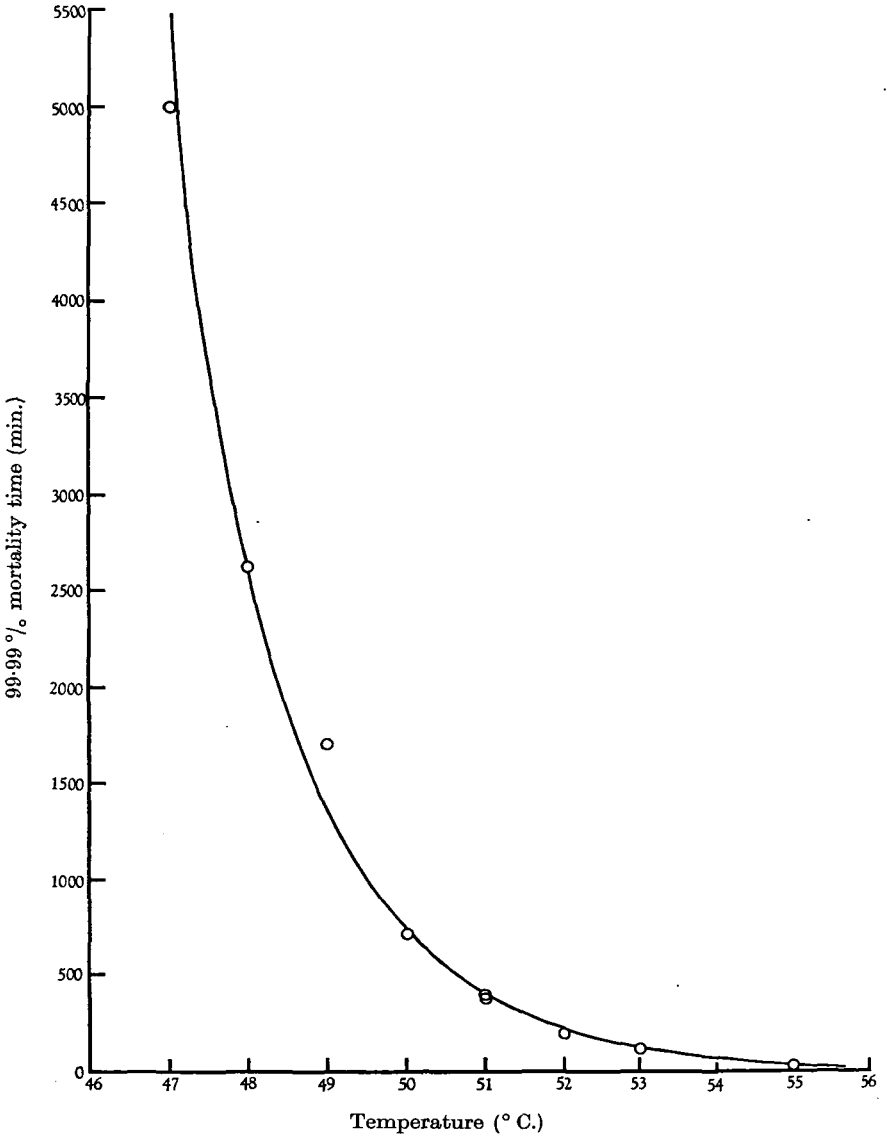

Fig. 1. Showing the relationship between 99·99°/₀ mortality time and temperature for *Bact. coli* at *p*H 7·0.

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Table 1. *The relation between temperature and* 99·99 % *mortality time in cultures of* Bact. coli *at* pH 7·0

Temp. ° C.	99·99 % mortality time: min.	99·99 % mortality time: $\log_{10}$ min.
47	5004·0 ± 74·29	3·6993 ± 0·0064
48	2612·0 ± 31·71	3·4169 ± 0·0053
49	1705·0 ± 25·31	3·2316 ± 0·0064
50	724·1 ± 15·80	2·8598 ± 0·0095
51 (*a*)	357·7 ± 3·28	2·5536 ± 0·0040
51 (*b*)	389·9 ± 12·41	2·5909 ± 0·0138
52	192·0 ± 6·68	2·2833 ± 0·0151
53 (*a*)	110·9 ± 8·52	2·0448 ± 0·0334
53 (*b*)	113·2 ± 2·22	2·0539 ± 0·0085
55	18·6 ± 1·17	1·2695 ± 0·0273

particular experimental conditions employed, although in richer media and under other conditions growth with gas production from lactose occurs at this temperature.

To test the agreement of the disinfection times with this formula, the logarithms of the times may be plotted against log $(T-\alpha)$, when a straight line should result if the formula is satisfactory. Fig. 2 shows the result obtained when α is taken as 44° C., and evidently the formula cannot be regarded as an accurate expression of the data. It happens that a closer approach to linearity can be obtained if α is reduced in value, but even with α reduced to zero the curvature of the line is not wholly eliminated. Moreover, this device is in any case unsatisfactory

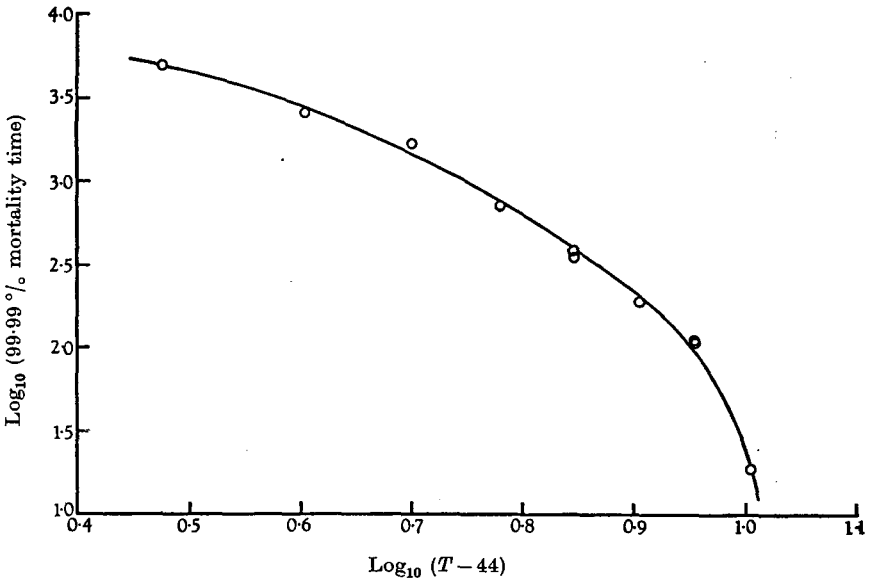

Fig. 2. Showing the relationship between $\log_{10}$ (99·99% mortality time) and $\log_{10}$ $(T-44)$ for *Bact. coli* at pH 7·0.

which in the present experiments was fulfilled. In view of the existence of this minimum temperature it appeared that the formula $t\,(T-\alpha)^b = a$ might fit the data, where t is the disinfection time, T the Centigrade temperature and a, b and α are constants. This type of formula has often been used to describe the relation between reaction time and temperature in biological processes (Bělehrádek, 1935), and in it α represents the minimum temperature referred to above and b is the temperature coefficient. From the form of the graph in Fig. 1 it appears that the minimum temperature for the heat disinfection of these cultures would be about 44° C., and it may be mentioned here that the results of other experiments (Jordan & Jacobs, 1947) have indicated approximately 44° C. as the maximum temperature for growth of this strain of *Bact. coli* under the since the formula would then yield calculated disinfection times for temperatures at which active growth is known to occur.

The more usual formula applied to bacteriological disinfection data is of the form $t \times \theta^T = A$, where t is the reaction time, T the Centigrade temperature, θ the temperature coefficient and A a constant. The agreement of the data with this formula can be tested by plotting the logarithm of the disinfection time against the temperature when a straight line should result if the formula provides a good fit. That there is a good approximation to linearity is clear from Fig. 3, and this formula can, therefore, be considered reasonably satisfactory. The linear relationship between log disinfection time (Y) and temperature (T) is expressed by the equation

$$Y = \bar{y} + m\,(T-\bar{T}),$$

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where $\bar{y}$ is the mean of the logarithms of the disinfection times, m ($=\log_{10}\theta$) is the slope of the line, and $\bar{T}$ the mean value of the temperatures. In the present case $\bar{T}=50{\cdot}9$, $\bar{y}=2{\cdot}6004\pm0{\cdot}0207$ and $m=-0{\cdot}2953\pm0{\cdot}0088$, and the line drawn in Fig. 3 corresponds to these values. The standard errors of both $\bar{y}$ and m are seen to be small, the ratio of m to its standard error being satisfactorily high at 33·5, showing that this formula fits the data closely.

Table 2 (col. 2) gives the values of the disinfection times at the various temperatures calculated from this formula, together with their standard errors and their differences from the experimental values. Exact agreement would naturally not be expected, owing not only to the uncertainty inherent in the actual determinations of the disinfection times but also to the fact that the effect of changes in temperature on the disinfection time was so great that

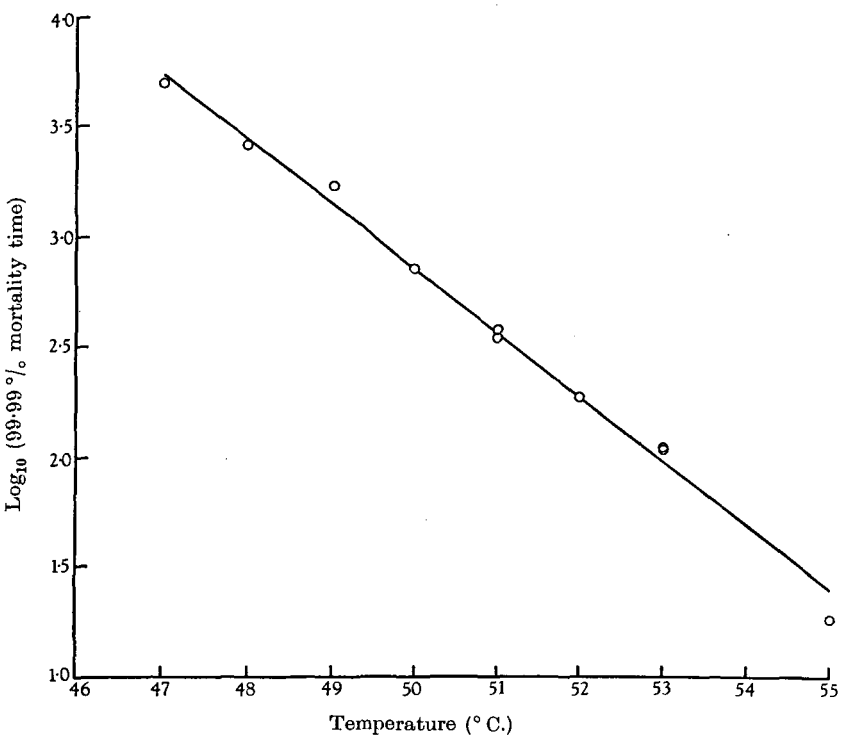

Fig. 3. Showing the relationship between $\log_{10}$ (99·99 °/$_o$ mortality time) and temperature for *Bact. coli* at *p*H 7·0.

Table 2. *Disinfection times (min.) calculated from two different formulae, with their differences from the experimental values*

Temp. ° C.	Time calculated from $t\times1{\cdot}973^{T}=42{\cdot}76\times10^{6}$	Difference from experimental value*	Time calculated from $t-10=723{\cdot}6\left[\frac{56-T}{T-44}\right]^{1{\cdot}8413}$	Difference from experimental value*
47	5648 ± 521·5	+644	5479	+475
48	2862 ± 216·5	+250	2602	− 10
49	1450 ± 88·8	−255	1355	−350
50	734·5 ± 37·4	+ 10·4	733·6	+ 9·5
51 (*a*)	372·1 ± 17·7	+ 14·4	399·4	+ 41·7
51 (*b*)		− 17·8		+ 9·5
52	193·0 ± 10·1	+ 1·0	211·9	+ 19·9
53 (*a*)	97·74 ± 6·24	− 13·16	105·7	− 5·2
53 (*b*)		− 15·46		− 7·5
55	25·09 ± 2·40	+ 6·49	18·75	+ 0·15

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

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any very small experimental errors in the former would lead to large differences in the latter. Actually the relatively large differences between the experimental and calculated disinfection times at 47, 48 and 49° C. could be accounted for wholly by assuming errors in the experimental temperatures of 0·15, 0·15 and 0·20° C. respectively, which are not very greatly in excess of the possible limits of error in the thermostatic temperature setting. On the other hand, the calculated time for 55° C. differs by 35% from the observed time, and to account for this an error in the temperature setting of 0·5° C. would have been required. An error of this magnitude is well outside the known limits of accuracy, and it therefore appears that the above relationship between disinfection time and temperature is not nearly so satisfactory at the higher as at the intermediate and lower temperatures. This is a point of some significance which is considered further below.

The extreme sensitivity of the disinfection time to small changes in temperature is clear from Table 1 and Fig. 1, and it is also shown by the formula for the relationship discussed above. When this is transformed from the semi-logarithmic form the equation becomes $t \times 1{\cdot}973^T = 42{\cdot}76 \times 10^6$. The temperature coefficient is thus very high, and its value of 1·973 per degree Centigrade means that the disinfection time was approximately doubled for every degree fall in temperature. The corresponding value of Q_{10} is 897, an exceedingly high figure even for a reaction in which, it is generally agreed, high coefficients are the rule. Here a brief digression may be made to emphasize that, as there is an almost linear relationship between the Centigrade temperature and the reciprocal of the absolute temperature within the biologically important temperature zone (Bělehrádek, 1935) especially over short ranges, the disinfection times must also follow closely the Van't Hoff-Arrhenius law

$$t = A \exp(\mu/2T_{\text{abs.}}),$$

where $T_{\text{abs.}}$ is the absolute temperature and μ the 'energy of activation'. When this law is tested by calculating the regression of $\log t$ on $1/T_{\text{abs.}}$, it is found that there is a very close approximation to a straight line whose slope is $30{,}870 \pm 1037$, the ratio of the slope to its standard error being 29·8. The value of μ, which can be obtained by multiplying the slope by 4·6, is therefore 142,000.

The value of 897 for the Q_{10} of the heat death of *Bact. coli* appears to be the highest recorded in the literature for the vegetative cells of bacteria, the next highest being that of 560 obtained by Watkins (1933), quoted by Rahn (1945) in the most recent review of this subject, but Smith (1923) obtained a value of 690 for the spores of *Botrytis cinerea* over the lowest temperature range tested. Most notable, however, is the extraordinary range of values quoted by different authors even for the same organism, although doubtless using different strains. Thus Chick (1910) obtained a value of 12 for the Q_{10} using *Bact. coli*, while Gage & Stoughton (1906) found 27·9 for the same organism. These values, although high in comparison with those common in non-biological reactions, are extremely low compared with that of 897 obtained in the present work. In all these three researches the temperature ranges employed nearly coincided, being 49–52, 50–55 and 47–55° C. respectively, and the different results are most likely to be ascribed to the nature of the fluid in which the cells were heated. Possibly also Skrabal's hypothesis (see Bělehrádek, 1935) is relevant. According to this, fast reactions have low temperature coefficients, whereas slow reactions have high coefficients, and in the present experiments the reaction rates were lower than those recorded by Chick.

Although the experimental data fit the formula $t \times \theta^T = A$ so closely, this cannot be regarded as a completely satisfactory expression of the change in disinfection time with temperature, partly because it does not take any account of the existence of a minimum temperature for the reaction and so yields calculated times for temperatures at which growth is known to occur. Moreover, this formula does not provide for the increase in slope of the log (disinfection time)-temperature graph, and hence the increase in θ also, which must occur as the minimum temperature is approached (Rahn, 1945; Jordan & Jacobs, 1946*a*). In addition, it was pointed out above that the formula became less satisfactory at the highest temperature employed, the divergence being such that the experimental value of t was considerably lower than that calculated from the formula. Admittedly, this is based on one observation only, but the magnitude of the divergence is relatively large and therefore probably not due to experimental error. A similar situation also occurred in the phenol disinfection experiments (Jordan & Jacobs, 1946*a*) on similar cultures, and in addition appears in the log (disinfection time)-temperature graph given by Chick (1930) for the disinfection of *Bact. typhosum* by hot water, although no specific reference is made to it by that author. The disinfection time must tend towards zero as the temperature is increased, and from Fig. 1 it appears that the approach might be asymptotic, so that no definite temperature could be fixed at which it could be said that the disinfection time was zero. But since disinfection times of less than 10 min. would certainly be inaccurate, little would be lost by truncating the graph of Fig. 1 at that time. The corresponding temperature may be defined as the 'maximum' temperature for the reaction. Thus the graph of $(t - 10)$ against temperature must pass from infinity to zero between a

minimum and a maximum temperature, while the graph of log $(t-10)$ against temperature must pass from plus infinity to minus infinity between the same temperature limits. Since the deduction of 10 min. from the disinfection times when these exceed 100 min. makes little difference to their plotted against temperature are shown in Fig. 4, and clearly they conform to the expected pattern except that the increase in slope near the minimum temperature has not been realized, probably because experimental temperatures sufficiently near the minimum were not employed. This situation recalls

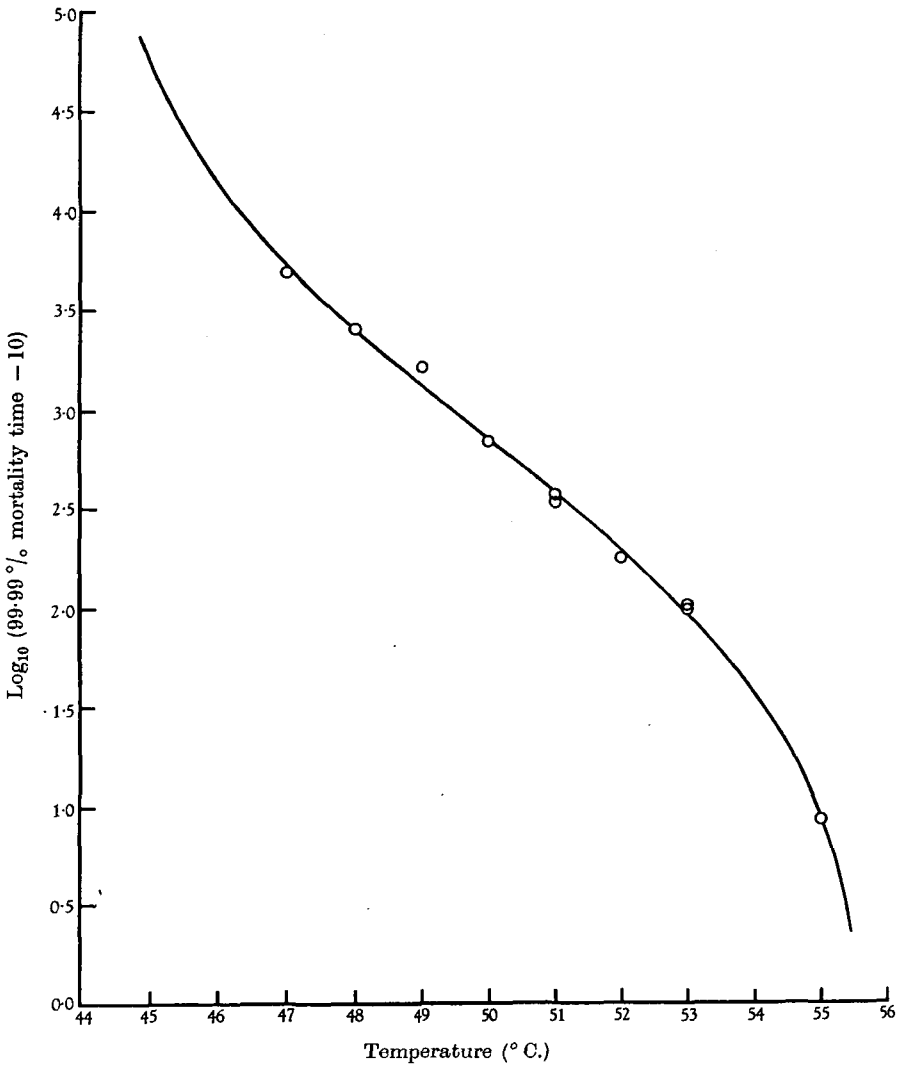

Fig. 4. Showing the relationship between $\log_{10}$ (disinfection time -10) and temperature for *Bact. coli* at *p*H 7·0.

logarithms, the graph of log $(t-10)$ against temperature will be little altered from that of log t against temperature between 47 and 53° C. and, since the latter graph is almost a straight line within this range, any formula used to describe these data must provide for this. Near the maximum and minimum temperatures, however, the slope of the graph must increase rapidly. The values of log $(t-10)$ that which occurred with the higher concentrations in the phenol disinfection of similar cultures of *Bact. coli* (Jordan & Jacobs, 1946*b*), and in view of the probability that the whole graph might be sigmoid it was decided to test whether the Pearl-Verhulst logistic formula (Pearl, 1930), which proved so successful in expressing the phenol data, would apply also to heat disinfection.

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This logistic formula has already been given, and its adaptation to the case of disinfection discussed, in the paper referred to above. The form of the adapted equation, with symbols appropriate to the present case, is $t-10=B\left[\frac{T_{max.}-T}{T-T_{min.}}\right]^n$, where t is the disinfection time, T the experimental temperature, $T_{max.}$ and $T_{min.}$ the postulated maximum and minimum temperatures (all Centigrade) and B and n are constants, the latter partaking of the nature of a temperature coefficient. To test the agreement of the disinfection times with this formula, $\log(t-10)$ may be plotted against $\log\left[\frac{T_{max.}-T}{T-T_{min.}}\right]$, when a straight line, whose slope is equal to n in the formula given above, should result. The closeness of the fit to a straight line will obviously depend on the values chosen for $T_{max.}$ and $T_{min.}$. These may be fixed roughly by inspection from Fig. 1, from which 56° C. has been chosen for $T_{max.}$ and 44° C. for $T_{min.}$, the choice of the latter figure also being influenced by indications from the results of other experiments (Jordan & Jacobs, 1947) that the minimum temperature for growth of this strain of *Bact. coli* is 44° C. in the particular nutritional conditions employed.

Using these limits of 56 and 44° C., the graph shown in Fig. 5 is obtained when $\log(t-10)$ is plotted against $\log\left[\frac{56-T}{T-44}\right]$, and the near approach to linearity is evident. The equation of the best-fitting straight line is

$$Y=2{\cdot}8595+1{\cdot}8413\log_{10}\left[\frac{56-T}{T-44}\right],$$

where Y is the calculated value of $\log_{10}(t-10)$ corresponding to an experimental temperature T within the range 44–56° C. The slope of this line has a standard error of $\pm 0{\cdot}0361$, and the ratio of the slope to its standard error is 51·0. The fit is thus very good, better even than that to the formula $t\times\theta^T=A$, so the logistic formula can be regarded as very satisfactory for expressing the manner in which the disinfection time changes with temperature. The above equation, after transformation from the logarithmic form, becomes

$$t-10=723{\cdot}6\left[\frac{56-T}{T-44}\right]^{1{\cdot}8413},$$

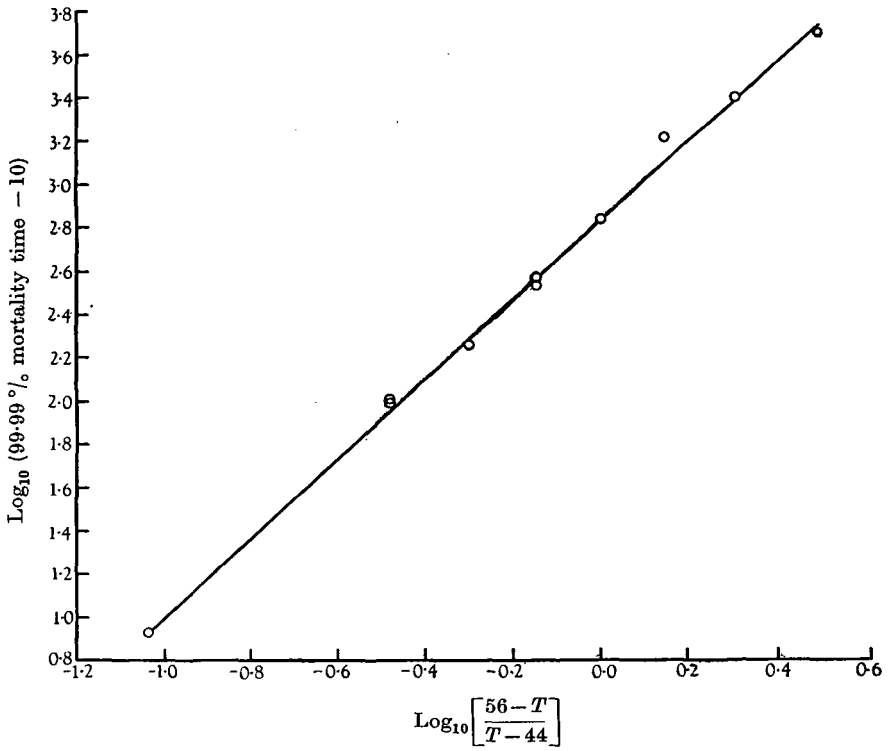

Fig. 5. Showing the relationship between $\log_{10}(99{\cdot}99\ \%$ mortality time $-10)$ and $\log_{10}\left[\frac{T_{max.}-T}{T-T_{min.}}\right]$ for *Bact. coli* at *p*H 7·0 when $T_{max.}=56°$ C. and $T_{min.}=44°$ C.

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and the actual line drawn in Fig. 4 corresponds to this equation. Further, the graph shown in Fig. 1 has been drawn to run through the values of the disinfection time calculated from the above formula, and it is clear that the experimental values lie very close to it. In Table 2 the values of the disinfection times calculated from this formula are given, together with their differences from the experimental values. It will be seen that in seven of the ten cases the logistic formula has given a better fit to the observed values than the formula more usually employed ($t \times \theta^T = A$), the times calculated from which are also given in the table. In the remaining three cases the logistic formula proved less satisfactory, but not seriously so. Regarding the fit given by the logistic formula in another way, it appears from Fig. 1 that in all cases except one the experimental disinfection times differ from the calculated values by amounts which could have been accounted for wholly by errors in the experimental temperature setting not exceeding 0·05° C. In the one exceptional case (49° C.) the corresponding temperature error would have been 0·3° C. This situation is considerably better than that existing when the formula $t \times \theta^T = A$ was used (see Fig. 3), where seven temperature errors exceeding 0·1° C., one of which must have been as high as 0·5° C., must have occurred if the differences between the experimental and calculated disinfection times are to be attributed wholly to this cause. It should be emphasized that this 'temperature error' method of estimating the goodness of fit of these two formulae is one of convenience only, since part of the difference between the experimental and calculated values is obviously due to the inevitable chance errors in the estimation of the disinfection times. The particular advantage gained by the use of the logistic formula is that the calculated value agrees well with the experimental at 55° C. where the other expression showed signs of breaking down. Also, it is singularly satisfactory that the logistic formula is successful in expressing the data when a value for the minimum temperature is employed which is not only appropriate to the present case, but which also agrees with information about the maximum growth temperature of this organism obtained from a wholly separate series of experiments where, nevertheless, the organism was subjected to similar conditions of nutrition. No advantage could have been claimed for the logistic formula if an unwarrantedly low minimum temperature had been necessary to obtain a satisfactory fit. Finally, while there can be no real claim for fundamental significance for the logistic formula to express disinfection data, since in some respects it is obviously an approximation, yet it is to be preferred to other formulae which have been employed in the past since it takes account of the existence of a minimum temperature for the reaction and provides for the increase in θ which must occur near that temperature. In fact, it provides a truly constant temperature coefficient over the whole range of temperature which can be employed. The use of a maximum temperature in the formula, even though this has been fixed arbitrarily, is of advantage as it gives an indication of the temperature required for very rapid disinfection. Its introduction is necessary to obtain that increase in θ which was actually observed to occur between the two highest temperatures used.

SUMMARY

1. The 99·99 % mortality time (t) has been used as a measure of the rate of disinfection of standard cultures of *Bact. coli* by heat under carefully controlled conditions, and the relationship between this rate and temperature (T) over the range 47–55° C. has been examined.

2. From the form of the relationship a minimum temperature of about 44° C. for the reaction was indicated, but the formula $t\,(T-\alpha)^b = a$, which has been used for the calculation of biological temperature coefficients in the past, was quite inadequate to express the relationship when an acceptable value for the maximum temperature (α) was employed.

3. The formula $t \times \theta^T = A$ more usually employed in bacteriological work, fitted the data reasonably well except at the highest temperature. The very high value of 897 for Q_{10} was obtained.

4. On theoretical grounds, the above formula could not apply at temperatures near the minimum, and also it appeared likely to break down when high temperatures were used.

5. It was shown that the full graph of $\log (t-10)$ against temperature should be sigmoid and asymptotic to two temperatures, a minimum and a maximum, the latter being defined as the temperature at which 99·99 % mortality would be produced in 10 min.

6. The graph of the Pearl-Verhulst logistic equation is of this type and, with 44 and 56° C. as the minimum and maximum temperatures, it provided an excellent fit to the data, especially at the highest temperature used.

The authors wish to express their gratitude to Messrs I.C.I. (Pharmaceuticals) Ltd. for their valuable financial assistance, which has enabled one of us (H.E.F.D.) to devote his full time to this work.

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(*MS. received for publication* 26. XI. 46.—Ed.)

CORRIGENDUM

STUDIES IN THE DYNAMICS OF DISINFECTION

VIII. THE EFFECT OF LETHAL TEMPERATURES ON STANDARD CULTURES OF *BACT. COLI*. I. A DETAILED ANALYSIS OF THE VARIATIONS OF DEATH-RATE WITH TIME

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Table 3 (p. 142).

The heading of col. 2 should read

$$\log_{10} \underline{S} = \overline{\log_{10} S} + \underline{b(t-\bar{t})}$$

and the heading of col. 3 should read

Standard error of $\overline{\log_{10} \underline{S}}$