

STUDIES IN THE DYNAMICS OF DISINFECTION

VI. CALCULATION OF A NEW AND CONSTANT TEMPERATURE COEFFICIENT FOR THE REACTION BETWEEN PHENOL AND *BACT. COLI*

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(With 4 Figures in the Text)

In the previous paper of this series (Jordan & Jacobs, 1946) the effect of temperature on the rate of the reaction between phenol and *Bact. coli* was analysed in detail, and it was shown that none of the formulae commonly employed to describe the relation between temperature and rate of reaction of biological processes yielded a satisfactorily constant temperature coefficient. The experimental evidence showed clearly that at all phenol concentrations Q_{10} increased markedly as the temperature rose above a certain value. Further, it was argued that because of the existence of a minimum or threshold temperature for any given phenol concentration, at which the rate of reaction becomes infinitely small, Q_{10} must of necessity rise as the temperature approaches the threshold value. There was experimental evidence for this at the lowest phenol concentration used. Variation of Q_{10} with temperature seems to be the rule rather than the exception in biological processes, and the position is obviously unsatisfactory. The temperature coefficient b of Bělehrádek (1935) is generally more constant than Q_{10} , as the formula from which it is derived takes account of the existence of a threshold temperature. However, that formula cannot yield a constant value of b in cases where Q_{10} increases as the temperature rises above a certain value, as is the case in these disinfection data. In view of the need for a truly constant temperature coefficient, a method of treating the present data has been developed which not only allows for an increase of Q_{10} at each end of the temperature range but also leads to the derivation of a true constant which partakes of the nature of a temperature coefficient. This method, developed to suit the special case under consideration, may have a wider application in biology.

RESULTS AND METHOD OF TREATMENT

As in the previous paper, cited above, the 99.999999% mortality time, called the virtual

sterilization time or *v.s.t.*, has been used as a measure of the rate of the reaction between phenol and *Bact. coli* under various conditions of temperature and concentration in spite of the fact that the death-rate of the cells of the cultures, in the usual bacteriological sense, was not constant throughout any one experiment. These times, with their standard errors, are given in Table 1 and shown graphically in Fig. 1, from which it is clear that at

Table 1. *The relation between v.s.t. and log₁₀ (v.s.t. - 10) and temperature at five phenol concentrations*

Phenol conc. g./l.	Temp. °C.	<i>v.s.t.</i> min.	log ₁₀ (<i>v.s.t.</i> - 10)
4.62	30	2165 ± 51.27	3.3334
	32.5	1146 ± 20.22	3.0554
	35	494.3 ± 22.96	2.6851
	38	293.9 ± 9.21	2.4532
	39.5	279.1 ± 10.65	2.4300
	42	119.4 ± 7.32	2.0391
5.20	27	1506 ± 14.80	3.1750
	31	787.3 ± 72.80	2.8906
	35	341.1 ± 3.04	2.5199
	39	101.2 ± 5.88	1.9600
6.01	23	1520 ± 18.77	3.1790
	26	733.7 ± 9.92	2.8595
	28	609.6 ± 63.04	2.7778
	31	256.2 ± 7.45	2.3913
	35	107.8 ± 0.15	1.9903
6.98	22	800.4 ± 9.42	2.8978
	25	512.2 ± 17.96	2.7009
	28	272.9 ± 8.27	2.4198
	30.5	162.7 ± 4.80	2.1838
	35	43.6 ± 3.68	1.5263
7.95	20	501.5 ± 6.58	2.6915
	24	329.5 ± 26.20	2.5045
	29	124.4 ± 0.77	2.0584
	35	28.9 ± 1.37	1.2765

each phenol concentration the *v.s.t.* tends to increase more and more rapidly as the temperature falls, so

that the existence of a minimum or threshold temperature at which the *v.s.t.* will become infinitely long can be postulated. The threshold temperature will vary with the phenol concentration, falling as the concentration rises, and will be well defined because of the rapid increase in slope of the curves as that temperature is approached. On the other hand, although the *v.s.t.* must tend towards zero as the temperature rises, the shape of the curves in Fig. 1 suggests that the approach is asymptotic. Therefore, no well-defined temperature at which the *v.s.t.* actually becomes zero can be fixed. However, for most practical purposes the *v.s.t.*-temperature curves may be assumed to terminate at some low

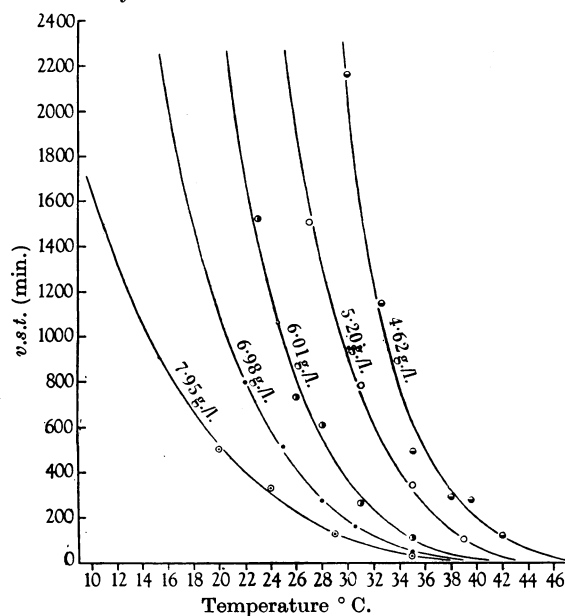


Fig. 1. Showing relationship between *v.s.t.* and temperature at five phenol concentrations.

value of the *v.s.t.*, because of the difficulty of determining such values accurately when they are very small. In these experiments values of less than 10 min. would certainly be inaccurate and the shape of the curves below that point of little significance. If the curves are truncated at the point where *v.s.t.* = 10 min. it becomes possible to fix a well-defined 'maximum' temperature for each phenol concentration, i.e. a temperature at which the *v.s.t.* becomes equal to 10 min. It may then be said that the curves of (*v.s.t.* - 10) plotted against temperature must fall from infinity to zero as the temperature rises from the minimum to the maximum value, and it follows that the graphs of $\log(v.s.t. - 10)$ against temperature must pass from plus infinity at the minimum temperature to minus infinity at the maximum temperature. The values of $\log(v.s.t. - 10)$ are given in Table 1, and in Fig. 2 the graphs of

$\log(v.s.t. - 10)$ against temperature are shown. The five curves are evidently all of the same form in the upper parts of their temperature ranges. They increase markedly in slope as the maximum temperature is approached and may be considered to be asymptotic to the ordinate at that temperature. At the other end of the temperature scale the curves for the higher concentrations of phenol are incomplete because temperatures sufficiently close to the threshold values have not been used, but the curve for the lowest phenol concentration shows the expected tendency to rise sharply as the minimum temperature is approached. It may then be assumed that at all concentrations the complete curves would also be asymptotic to the ordinate at the appropriate minimum temperature. In each case the existence of a central flatter portion in the curve is clearly apparent from the experimental data. The type of curve which would fit the modified data is, therefore, sigmoid in shape, with $\log(v.s.t. - 10)$ varying between plus and minus infinity between two temperature limits to each of which the curve is asymptotic. The whole curve or even the major portion of it may not be realizable in practice at any one phenol concentration, since the maximum temperature may be so high that thermal death would occur in the absence of phenol or the minimum temperature too low to be experimentally practicable. Nevertheless, the lower and middle portions of the curves should be obtainable at high phenol concentrations and the upper portions at low concentrations and this is actually what has occurred.

The logistic curve, described by the Pearl-Verhulst equation (Pearl, 1930), is of the required form. It is a very flexible formula and can be made to fit both symmetrical and asymmetrical sigmoid curves. There appears to be no way of deciding whether the curves under discussion here are symmetrical or not, and very extensive data would be needed to decide this point. Consequently, for the sake of simplicity, the symmetrical form only has been examined. With symbols appropriate to the present case, the formula for a given phenol concentration, is

$$T - T_{\min.} = \frac{T_{\max.} - T_{\min.}}{1 + e^{a+r \log_{10}(v.s.t.-10)}} \quad (1)$$

where T is the experimental temperature, $T_{\min.}$ and $T_{\max.}$ are respectively the postulated minimum and maximum temperatures (all in $^{\circ}\text{C}.$), and a and r are constants, the latter partaking of the nature of a temperature coefficient as will appear below. The above formula may be rearranged thus

$$\frac{T_{\max.} - T}{T - T_{\min.}} = e^{a+r \log_{10}(v.s.t.-10)},$$

which, on taking logarithms, becomes converted to

$$\log_{10} \left[\frac{T_{\max.} - T}{T - T_{\min.}} \right] = 0.4343 [a + r \log_{10}(v.s.t. - 10)]. \quad (2)$$

Evidently, in order to test the fit of the data to this formula $\log_{10} \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ must be plotted against $\log_{10} (v.s.t. - 10)$. Straight lines should result at each phenol concentration if this formula is adequate, but a difficulty arises because the closeness of the fit to a straight line depends on the values chosen for T_{\max} and T_{\min} . Fortunately, an approximately correct value of T_{\max} for each phenol concentration can be obtained by inspection of the graphs of *v.s.t.* against temperature. This having been done, the procedure adopted for finding T_{\min} .

was then considered to be the best minimum temperature obtainable from the data to match the corresponding chosen value of T_{\max} . The slopes of the best straight lines corresponding to the pairs of 'best' values of T_{\max} and T_{\min} , having already been determined, it only remained to calculate the complete equations of regression of $\log \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ on $\log (v.s.t. - 10)$ together with the standard errors of the slopes of these lines. In Table 2 are given the values of T_{\max} and T_{\min} for each phenol concentration with the other constants of the logistic

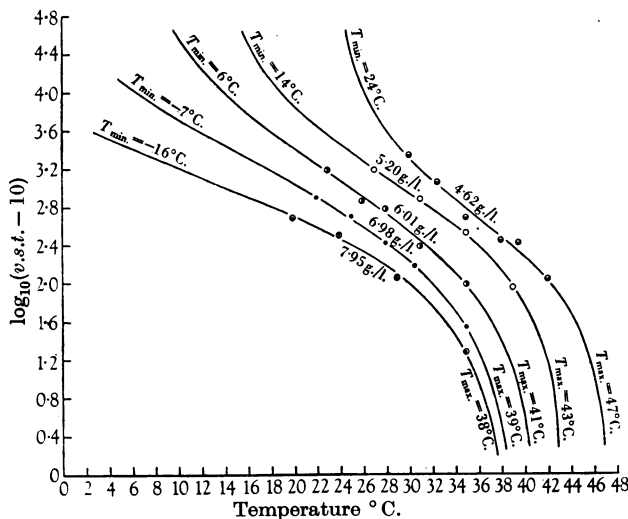


Fig. 2. Showing relation between $\log_{10}(v.s.t. - 10)$ and temperature. Curves fitted to maximum and minimum temperatures shown.

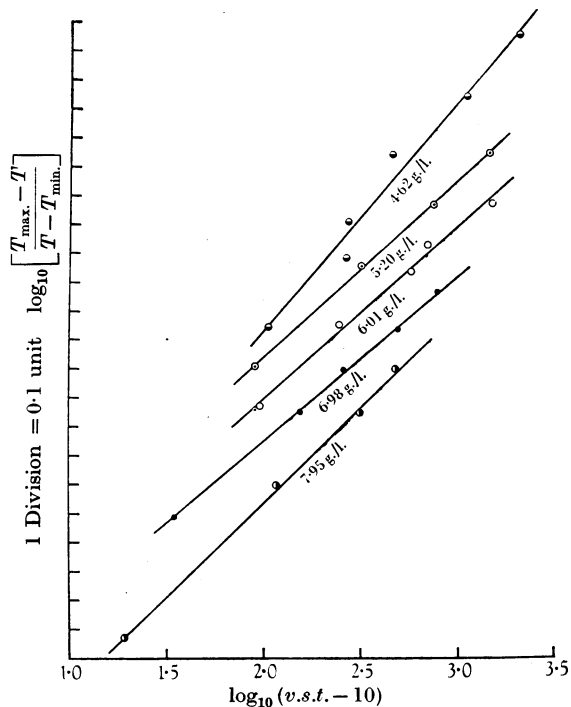


Fig. 3. Showing relation between $\log_{10}(v.s.t. - 10)$ and $\log_{10} \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ (see text).

has been to calculate, for each chosen T_{\max} , the values of $\log \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ for each of a series of likely values of T_{\min} . For each set of data thus obtained the slopes of the best fitting straight lines relating $\log \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ to $\log (v.s.t. - 10)$ were calculated. Then in each case the closeness of the fit to a straight line was estimated in the usual way by determining the sum of the squares of the deviations of the y values from the corresponding calculated values where $y = \log \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$. It was found that this sum was smallest at a certain definite value of T_{\min} , which

formulae based on these values. It will be seen that the standard errors of the slopes of these lines are all satisfactorily small, and it is apparent that the logistic formula can be used to express the data as a whole very adequately. In order to emphasize how effective the use of the logistic formula has been in reducing the curved lines of $\log (v.s.t. - 10)$ against temperature (Fig. 2) to the linear form, the actual graphs of $\log \left[\frac{T_{\max} - T}{T - T_{\min}} \right]$ against $\log (v.s.t. - 10)$ are shown in Fig. 3. Since the slopes of the five lines and the values of the intercepts on the abscissa are all so nearly equal (see Table 2) the graphs, if plotted in the usual way on one diagram, would very nearly

coincide, so the lines have been spaced out in the figure by shifting the ordinates. The scale of the ordinate is the same for each graph, but the origins have been displaced sufficiently to prevent the lines from overlapping. Here it should be mentioned that the curves of *v.s.t.* against temperature in Fig. 1 and of $\log(v.s.t. - 10)$ against temperature in Fig. 2 correspond to the calculated straight lines in Fig. 3 whose constants are given in Table 2 except those for 5.20 g./l. which, for reasons to be discussed below, have been based on a minimum temperature of 14°C. instead of on -10°C., which appears to

given germicide concentration. The logistic equation has the additional advantage of including the minimum or threshold temperature, which again is an important characteristic of a disinfection process and one which hitherto has received insufficient attention. This formula thus appears to have a rational basis for application to these data. The practical value of the formula, however, lies largely in the fact that it provides a close fit to the experimental data. In Table 3 are given the values of the *v.s.t.* derived from the logistic equations whose constants are set out in Table 2, together with the differences from the experimental values given in

Table 2. The constants of the logistic equation as fitted to the $\log(v.s.t. - 10)$ - temperature data

Phenol conc. g./l.	Chosen values of T_{max} °C.	'Best' values of T_{min} °C.	0.4343r	0.4343a	-a/r
4.62	47	24	0.7851 ± 0.0552	-2.1499	2.7385
5.20	43	-10	0.5952 ± 0.0030	-2.2532	3.7854
6.01	41	6	0.5910 ± 0.0342	-1.8421	3.1169
6.98	39	-7	0.5717 ± 0.0070	-1.8928	3.3111
7.95	38	-16	0.6457 ± 0.0220	-2.0488	3.1728
		Mean	0.6377	Mean	-2.0374
		s.d.	0.0865	s.d.	0.1721

be aberrant inasmuch as it is not in accord with the other minimum temperatures.

DISCUSSION

It is not contended that the Pearl-Verhulst logistic equation is necessarily the one which would fit most accurately the data under discussion here. The justification for the use of this formula rests on the fact that it provides a sigmoid curve of the general type which theoretical considerations require should be obtained when $\log(v.s.t. - 10)$ is plotted against temperature. That its use necessitates a limitation of the data to certain temperature ranges, which vary with the concentration of phenol, is obvious, since T_{max} is the temperature at which the *v.s.t.* is 10 min. and, whereas the formula breaks down at higher temperatures, in practice *v.s.t.*'s shorter than 10 min. might be realizable. However, this limitation is not unduly objectionable, since it leads only to the rejection of data which, even with the refined technique employed here, could at best be only of doubtful accuracy. If by further refinements in technique accurate *v.s.t.*'s shorter than 10 min. were obtained, T_{max} could be redefined to correspond to any suitable value of the *v.s.t.* and, if this value were low enough, T_{max} could be regarded as the temperature for 'instantaneous' disinfection. In any case it is advantageous to have a value for T_{max} in the temperature coefficient formula, as it gives an idea of the temperature required for very rapid action at a

Table 3. Values of the *v.s.t.* calculated from the best fitting logistic equations, with the differences from the experimental values given in Table 1

Phenol conc. g./l.	Temp. °C.	Calculated <i>v.s.t.</i> min.	Calculated minus experimental
			<i>v.s.t.</i> min.
4.62	30	2074	- 91.0
	32.5	1091	- 55.0
	35	621.9	+ 127.6
	38	322.0	+ 28.1
	39.5	227.2	- 51.9
	42	117.2	- 2.2
5.20	27	1501	- 5.0
	31	784.5	- 2.8
	35	345.3	+ 4.2
	39	100.7	- 0.5
6.01	23	1452	- 68.0
	26	814.6	+ 80.9
	28	547.5	- 62.1
	31	287.7	+ 31.5
	35	101.4	- 6.4
6.98	22	814.1	+ 13.7
	25	492.0	- 20.2
	28	280.3	+ 7.4
	30.5	162.6	- 0.1
	35	43.5	- 0.1
7.95	20	518.9	+ 17.4
	24	302.9	- 26.6
	29	133.1	+ 8.7
	35	28.5	- 0.4

Table 1. Clearly, the differences are, in general, quite small, and there is thus good practical as well as theoretical justification for this application of the logistic formula.

It would be anticipated that the four constants of the logistic equation would vary with phenol concentration in an orderly and regular manner, and these relationships have, therefore, been examined in the hope of finding a single formula to describe all the data. With the material available at present this has not proved to be completely possible, but certain interesting trends are apparent. The five chosen values of $T_{\max.}$ (Table 2) vary in an orderly manner with concentration as is shown in Fig. 4, curve *A*. A sixth point can be added since the relation between *v.s.t.* and concentration at 35° C. (Jordan & Jacobs, 1944, 1945) indicates that at that temperature a *v.s.t.* of 10 min. would occur at about 9.4 g. phenol per l., and hence $T_{\max.}$ for that concentration must be 35° C. These six points all fall on a curve which may be a hyperbola with an equation of the form $(T_{\max.} - \alpha)(C - \beta)^n = K$, where C is the phenol concentration in g./l. and α , β , n and K are all constants. If α is put equal to 10° C. and β is taken to be 3.0 g./l., both reasonable values, and $\log(T_{\max.} - 10)$ is plotted against $\log(C - 3)$ for all six values, a very close approximation to a straight line is obtained. The value of n indicated by the calculated best straight line is 0.2632 ± 0.0183 and $K = 41.53$. The standard error of n is satisfactorily small, and graph *A* in Fig. 4, which is that corresponding to the calculated equation $(T_{\max.} - 10)(C - 3)^{0.2632} = 41.53$, illustrates the closeness of fit to the experimental data. It is important to remember, however, that the values of $T_{\max.}$ were chosen in the first instance from freehand curves.

Turning to the values for $T_{\min.}$, it seems that the value for 5.20 g./l. is much out of line with the rest, and that it is a reflexion of the abnormally large curvature of the graph of $\log(v.s.t. - 10)$ against temperature at that concentration. It is hardly surprising that one value of $T_{\min.}$ should be aberrant since, as the data usually refer to half of the logistic curve only, an error in one value of the *v.s.t.* could easily give an exaggerated curvature and hence an aberrant value for $T_{\min.}$. When the remaining four values of $T_{\min.}$ are plotted against phenol concentration (Fig. 4, *B*) there is a strong indication of a linear relationship between these factors. Moreover, the *v.s.t.*-concentration curve at 35° C., referred to above, indicates that $T_{\min.}$ is 35° C. when the phenol concentration is approximately 3 g./l. If this point, which cannot be greatly in error, be plotted on the graph (Fig. 4, *B*), the impression of linearity is considerably strengthened. The best straight line fitted to these five points has a slope of 10.75 ± 0.744 , the standard error being satisfactorily small. The com-

plete equation relating $T_{\min.}$ to phenol concentration is $T_{\min.} = 69.83 - 10.75C$, where C is the concentration in g./l. Line *B* in Fig. 4 corresponds to this formula and illustrates the close agreement with the data. When $C = 5.20$ the value for $T_{\min.}$ given by this formula is 13.91° C., which is a much more reasonable value than the aberrant figure of -10° C. previously obtained. If the relationship between $\log\left[\frac{T_{\max.} - T}{T - T_{\min.}}\right]$ and $\log(v.s.t. - 10)$ for this concentration be recalculated, using 14° C. for $T_{\min.}$, the constants of the logistic formula for 5.20 g./l. become: $0.4343r = 0.7233 \pm 0.0252$; $0.4343\alpha = 2.0488$ (cf. previous values given in Table 2). The standard error of the slope of the line, which as originally

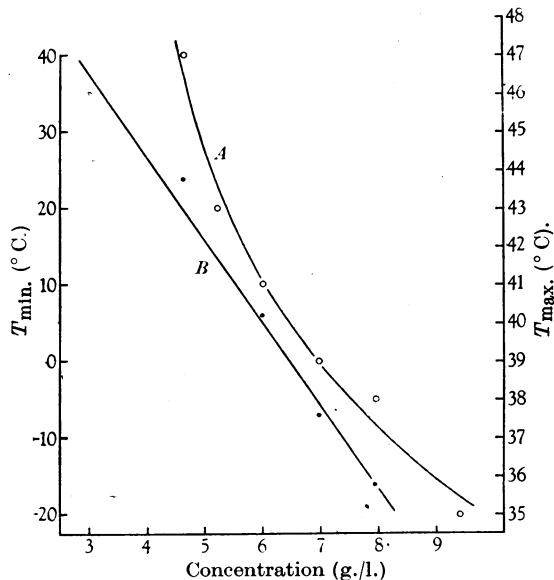


Fig. 4. Showing relation between $T_{\max.}$ and concentration (curve *A*) and $T_{\min.}$ and concentration (curve *B*).

calculated was remarkably small, is now naturally increased, but it still remains low and compares favourably with those of the slopes for other phenol concentrations. The new calculated values of the *v.s.t.* are also less close to the experimental values, but the differences are still small and it has not been considered necessary to tabulate them. The graphs for 5.20 g./l. as actually drawn in Figs. 1 and 2 correspond to the 14° C. value for $T_{\min.}$, and the experimental data evidently do not deviate greatly from the calculated curve.

The constant r is of very considerable importance, as it partakes of the nature of a temperature coefficient. This becomes clear when equation (2) above is written in the form

$$v.s.t. = 10 + A \left[\frac{T_{\max.} - T}{T - T_{\min.}} \right]^{(0.4343r)^{-1}}, \quad (3)$$

where $\log_{10} A = -a/r$. The constancy of the value of r for each phenol concentration can be gauged from the magnitude of its corresponding standard error which in each case is small (see Table 2) and from the graphs in Fig. 3. The importance of r may, however, be even greater, since, if its value could be shown to be constant for all phenol concentrations, it would then become a unique temperature coefficient for the reaction under consideration. It is true that the experimental values of $0.4343r$ could be regarded as scattered about a mean which, as shown in Table 2, has a value of 0.6377 with a standard deviation of 0.0865 and a coefficient of variation of 13.6% . Alternatively, substituting the 'improved' value of 0.7233 at 5.20 g./l., the mean becomes 0.6634 with a standard deviation of 0.090 and the coefficient of variation is unchanged. On the other hand, there is a suggestion that r may rise as the concentration falls, and if the 'improved' value for 5.20 g./l. be taken into consideration this impression is strengthened. But much of the evidence for a rise in r with decreasing concentration depends on the one high value at 4.62 g./l., and it should be pointed out that the value of $0.4343r$ is sometimes rather sensitive to small changes in $T_{\min.}$, an alteration of from 0.01 to 0.02 or more units per °C. change in minimum temperature being encountered. For instance, the value of 0.7851 ± 0.0552 for $0.4343r$ at 4.62 g./l. falls to 0.6802 ± 0.0558 when $T_{\min.}$ is decreased from 24 to 20° C. and the loss of accuracy is not great. In point of fact, the lower value for $T_{\min.}$ fits much better than the higher value to the calculated linear relation between minimum temperature and concentration. The best way of attempting to decide whether r remains constant or rises as concentration falls would be to obtain a $v.s.t.$ -temperature curve for the lowest phenol concentration with which it is possible to work, since the value of r so derived should, if it does change in the manner suggested, then have its highest possible value. At present it would be better to say only that r does not change greatly within the concentration range studied, and that there is a possibility that it may prove to be a true constant for all concentrations. Similar considerations apply to the values of the constant a and to the ratio $-a/r$, the latter being the value of $\log_{10} (v.s.t. - 10)$ when

$$\log \left[\frac{T_{\max.} - T}{T - T_{\min.}} \right] = 0, \text{ i.e. at the point of inflexion of}$$

the curve, where the experimental temperature is midway between $T_{\min.}$ and $T_{\max.}$. Again, further experiments at low phenol concentrations are desirable.

If it can be established that r and a are constant or, if not, the manner in which they vary with temperature, substitution for $T_{\max.}$, $T_{\min.}$, r and A can be made in equation (3) above, each in terms of phenol concentration, so as to lead to the derivation

of a single formula relating $v.s.t.$ with temperature and phenol concentration. At present it is not possible to do this precisely since the exact relationships between a and r and phenol concentration are not clear. However, assuming that $0.4343a$ and $0.4343r$ are both constant and have the mean values shown in Table 2, and substituting the calculated equations for $T_{\max.}$ and $T_{\min.}$ given above, equation (3) becomes

$$v.s.t. = 10 + 1566 \left[\frac{41.53 (C - 3)^{-0.2632} - (T - 10)}{T + 10.75C - 69.83} \right]^{1.5681} \quad (4)$$

The values of the $v.s.t.$ calculated from this equation agree quite well with the experimental values at 7.95 g./l. This was to be expected, since the constants $0.4343a$ and $0.4343r$ for that concentration (see Table 2) are very near the mean values used to form equation (4). For 6.98 and 6.01 g./l. agreement is fairly good, probably because the values of $0.4343a$ and $0.4343r$ are both somewhat lower than the respective means with the result that the values of $-a/r$, which so greatly affect the calculated $v.s.t.$'s, are not far removed from the ratio of the mean values. The data at 5.20 g./l. are exceptional, as explained above, but although the value of $-a/r$ is so much above the ratio of the mean values, the calculated $v.s.t.$'s are in fair agreement with the experimental data. At 4.62 g./l., however, the situation is different, as both the constants and their ratios differ markedly from the mean values. Consequently, agreement between calculated and observed values of $v.s.t.$ is poor. Also, if equation (4) be used to calculate $v.s.t.$'s corresponding to phenol concentrations outside the range considered above, values are obtained which are greatly in excess of the experimental values obtained previously at 35° C. (Jordan & Jacobs, 1944, 1945) for concentrations ranging from 3.48 to 4.25 g./l. It is probably true, therefore, to conclude that both a and r cannot remain constant for all phenol concentrations. Also, it may be that the calculated relationship between $T_{\max.}$ and phenol concentration does not hold for concentrations below 4.62 g./l. In further work, designed to investigate these points at low phenol concentrations, it will not be possible to work at temperatures near the calculated values of $T_{\max.}$, since for low concentrations these are so high that thermal death would occur. It will be necessary to reverse the procedure adopted above and deduce from the experimental data values for $T_{\min.}$ from which the appropriate values of $T_{\max.}$ can be calculated.

SUMMARY

1. The virtual sterilization time ($v.s.t.$) has been used as a measure of the rate of disinfection of *Bact. coli* cultures by phenol under carefully standardized conditions. The relationship of this time to tem-

perature at each of five phenol concentrations has been examined from a fresh point of view, since it has previously been shown (Jordan & Jacobs, 1946) that none of the commonly accepted temperature coefficients was satisfactorily constant.

2. The relationship is such that a minimum or threshold temperature exists for each concentration. A corresponding 'maximum' temperature has been fixed, defined as the temperature at which the *v.s.t.* is 10 min. The value of (*v.s.t.* - 10) thus varies from infinity to zero between these temperature limits.

3. Sigmoid curves are obtained when $\log(v.s.t. - 10)$ is plotted against temperature for given phenol concentrations. These may be regarded as asymptotic to ordinates at the minimum and maximum temperatures.

4. The Pearl-Verhulst logistic equation gives a curve of the required sigmoid type, and this formula has been shown to fit the curves of $\log(v.s.t. - 10)$ against temperature very satisfactorily over the range of concentrations studied.

5. One of the constants of this formula partakes of the nature of a temperature coefficient, and it has, therefore, been possible to derive a truly constant temperature coefficient for each phenol concentration.

6. The values of this new temperature coefficient do not vary greatly with phenol concentration within the range studied, but it is not yet possible to establish whether it is essentially constant for all phenol concentrations with which it is possible to work.

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