STUDIES IN THE DYNAMICS OF DISINFECTION

V. THE TEMPERATURE COEFFICIENT OF THE REACTION BETWEEN PHENOL AND *BACT. COLI*, DERIVED FROM DATA OBTAINED BY AN IMPROVED TECHNIQUE

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(With 3 Figures in the Text)

The manner in which the activity of a disinfectant varies with temperature is very important both from the theoretical and the practical points of view, yet rarely is any information supplied concerning the temperature coefficients of the commonly used germicides. Chick (1930) surveyed the general position, and it appears that the usually accepted formula relating activity and temperature requires that the rate of disinfection, i.e. the bacterial death-rate calculated in the usual manner, shall be proportional to a constant (known as the temperature coefficient) raised to the power of the temperature employed. The use of such a formula is quite justified so long as it is accepted that the bacterial death-rate is truly constant and then no level of mortality need be specified, since obviously, if the death-rate is in fact constant, its value will be independent of the degree of mortality chosen. But evidence is accumulating that this assumption is often unwarranted. In particular, the manner in which the death-rate changes during the disinfection of *Bact. coli* cultures with phenol under various conditions of temperature and concentration has recently been analysed in detail (Jordan & Jacobs, 1944*a*, 1945). The results of these experiments can be used to reveal the effect of temperature on the rate of this reaction, but evidently the varying death-rates necessitate a different measure of the rate of disinfection. If disinfection of a culture is defined, however, as the process of the reduction of the original number of cells to a definite percentage of survivors, then the rate of disinfection becomes simply the reciprocal of the time needed to kill a percentage of the initial population, and the fact that the death-rates of the cultures, in the usual bacteriological sense, may have varied during the disinfections becomes irrelevant. This way of measuring rate of disinfection is analogous to that in which the rates of many other biological processes are estimated, e.g. the rate of heart-beat when, although several consecutive movements of unequal duration are involved in each beat, the reciprocal of the time for a whole beat forms a useful measure of the rate at which the heart is functioning.

Such an 'over-all' death-rate has been used in the following analysis of the effect of temperature on the rate of disinfection of *Bact. coli* cultures by phenol. The degree of mortality at which activities are compared is, however, of some importance, and the present authors (Jordan & Jacobs, 1944*b*) have recently urged that, for several reasons, the time taken to attain the highest practicable degree of mortality is to be preferred to 50 or 99% mortality times for use in dynamic studies of germicidal activities. With the improved technique that they employed it was possible to determine with considerable accuracy the 99·999999% mortality time (called the virtual sterilization time or *v.s.t.*), and the reciprocal of this mortality time has been chosen here as the most suitable measure of the 'over-all' rate of disinfection.

ANALYSIS OF RESULTS

The *v.s.t.*'s obtained for several temperatures at each of five phenol concentrations are given in Table 1 and Fig. 1. The full details of these experiments have already been published (Jordan & Jacobs, 1944*a*, 1945). Since a more general biological conception has been invoked in the use of these times as measures of the rate of disinfection, it has been thought desirable to test the agreement of the data with as many as possible of the formulae which from time to time have been used to express the relation between temperature and the rates of biological processes, rather than to confine attention to the formula usually employed when bacterial disinfections are concerned. Bělehrádek (1935) lists these formulae, and it is clear that they fall into two groups. First, there are those in which the rate of reaction is considered to be proportional to some power of the temperature, and secondly, those in which the rate (or time) is proportional to a constant raised to the power of the Centigrade temperature

or the reciprocal of the Absolute temperature. The usual bacteriological formula belongs to the second group.

In the first group the formulae have the general form $t(T-\alpha)^b = a$ for any given concentration of germicide, where t is the reaction time, T the Centigrade temperature, and α, b and a are constants. The graphs of these formulae are all hyperbolae differing only in the values of the constants, and the several apparently distinct formulae in the group correspond to special cases, e.g. when $\alpha = 0$ or $b = 1$. The constant α represents a minimum temperature, or biological zero, at which the rate of reaction

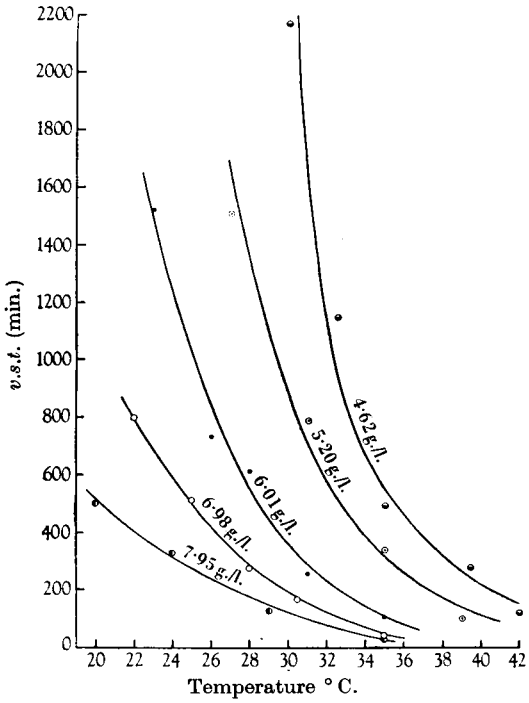

Fig. 1. Showing relationship between temperature and *v.s.t.* at various phenol concentrations.

becomes infinitely small and its value will obviously depend on the drug concentration. The idea that there should be a minimum or threshold temperature for the rate of bacteriological disinfections does not seem to have been used before, although in other fields of biology the minimum temperature is a familiar concept. The existence of such a minimum temperature and its variation with phenol concentration are strongly suggested by the curves in Fig. 1, which all rise increasingly steeply as the temperature decreases. Nevertheless, it does not necessarily follow that these curves correspond to this general formula, and to test the agreement log (*v.s.t.*) may be plotted against log $(T-\alpha)$ when straight lines should result if the graphs are of the above type. Approximate values for α may be derived from Fig. 1 by inspection, and Fig. 2 shows the results obtained with the values of α given in the legend. Although these graphs may be regarded as linear over short ranges, it is clear that over the whole temperature range each graph increases in slope with rising temperature. The increasing slopes may be reduced by making α very much smaller than the values chosen, but the tendency to increase is such that no amount of alteration of α will actually eliminate it. The alternative method for straightening these lines involves plotting log (*v.s.t.* + *constant*) instead of log (*v.s.t.*) against log $(T-\alpha)$, but

Table 1. *Virtual sterilization times and their logarithms for five phenol concentrations at various temperatures*

Phenol conc. g./l.	Temp. °C.	*v.s.t.* min.	$\log_{10}$ (*v.s.t.*)
4·62	30	2165 ± 51·27	3·3355 ± 0·0103
	32·5	1146 ± 20·22	3·0592 ± 0·0077
	35	494·3 ± 22·96	2·6940 ± 0·0202
	38	293·9 ± 9·21	2·4682 ± 0·0136
	39·5	279·1 ± 10·65	2·4458 ± 0·0166
	42	119·4 ± 7·32	2·0770 ± 0·0266
5·20	27.	1506 ± 14·80	3·1778 ± 0·0043
	31	787·3 ± 72·80	2·8962 ± 0·0402
	35	341·1 ± 3·04	2·5329 ± 0·0039
	39	101·2 ± 5·88	2·0051 ± 0·0252
6·01	23	1520 ± 18·77	3·1818 ± 0·0054
	26	733·7 ± 9·92	2·8655 ± 0·0059
	28	609·6 ± 63·04	2·7850 ± 0·0449
	31	256·2 ± 7·45	2·4085 ± 0·0126
	35	107·8 ± 0·15	2·0327 ± 0·0006
6·98	22	800·4 ± 9·42	2·9033 ± 0·0051
	25	512·2 ± 17·96	2·7095 ± 0·0152
	28	272·9 ± 8·27	2·4360 ± 0·0132
	30·5	162·7 ± 4·80	2·2113 ± 0·0128
	35	43·6 ± 3·68	1·6395 ± 0·0366
7·95	20	501·5 ± 6·58	2·7002 ± 0·0057
	24	329·5 ± 26·20	2·5179 ± 0·0345
	29	124·4 ± 0·77	2·0948 ± 0·0027
	35	28·9 ± 1·37	1·4609 ± 0·0205

this is inadmissible, since it introduces the absurdity that the *v.s.t.*-temperature curves in Fig. 1 are required to become asymptotic to a negative value of time. This type of formula, therefore, does not lead to the derivation of a satisfactorily constant temperature coefficient for the data under consideration.

The formulae of the second group have the general form $t \times \theta^T = A$, where t is the reaction time, T the Centigrade temperature, and A and θ are constants. This form may be considered to include the Van't Hoff-Arrhenius equation which can be written $t = A \exp.(\mu/2T_{\text{abs.}})$, where μ is the 'energy of activation' and $T_{\text{abs.}}$ the Absolute temperature since, as Bělehrádek (1935) has emphasized, the reciprocal of the Absolute temperature is almost exactly a

linear function of the Centigrade temperature over the biologically important temperature range. To test the agreement of the present data with this type of formula it is necessary to plot log (*v.s.t.*) against temperature. The results are shown in Fig. 3, where evidently the required linear relationship definitely is not realized at the higher concentrations. There is an approximation to linearity at 6·01 g./l., and possibly the relationship for 4·62 g./l. may also be regarded as linear, though there is apparently a tendency for the slope of the latter graph to be smaller in the middle than at the two ends. Thus, this type of formula would seem to have only a limited applicability to the present data.

the values of four common temperature coefficients, calculated between successive determinations of the *v.s.t.* at the five phenol concentrations used. The formula yielding the temperature coefficient *b* has not apparently been applied previously to bacteriological data, so values for direct comparison are not available. Bělehrádek (1935) quotes many cases of other biological processes in which *b* is satisfactorily constant and states that it varies in magnitude between 0·6 and 4·0, usually lying between 1 and 3. The values of *b* in Table 2 are almost all greater than 4 except where both phenol concentration and temperature are low. The strong tendency for *b* to rise with increase in temperature is clearly shown

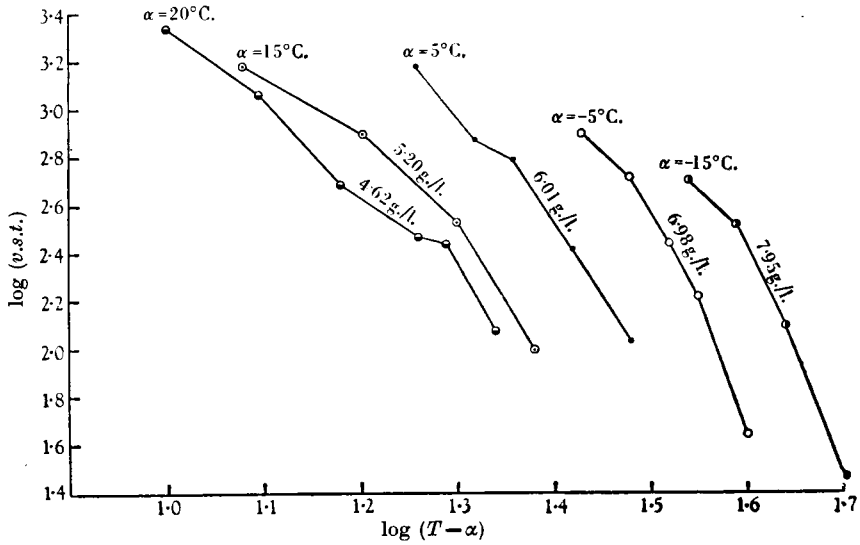

Fig. 2. Showing relationship between log $(T-\alpha)$ and log (*v.s.t.*) for the values of α and concentrations of phenol shown.

DISCUSSION

It is evident that neither of the two general types of formula which have previously been employed to describe the relationship between temperature and rate of reaction in biological processes has yielded a satisfactorily constant temperature coefficient for the rate of disinfection of *Bact. coli* cultures by phenol from the new data under discussion. This is perhaps hardly surprising in view of the large volume of literature concerning variation of biological temperature coefficients with change of temperature, concentration, etc., and the fact that neither type of formula has been found to be universally applicable (Bělehrádek, 1935). It may, however, be of value to examine the manner in which the more commonly used coefficients derived from the present data vary with temperature and concentration and to compare the values obtained with those of previous workers. In Table 2 are given at all phenol concentrations. However, Fig. 2 suggests that if fixed limits of reaction time are taken, *b* may remain roughly constant irrespective of the concentration of phenol.

The temperature coefficient θ is the one usually employed in bacteriology, where it expresses the relative increase in reaction rate per °C. rise in temperature. The corresponding coefficient Q_{10} expresses the relative increase in rate for a 10°C. rise in temperature. Both values are given in Table 2 calculated between successive determinations of the *v.s.t.* At 6·01 g./l. the values of θ may be regarded as fluctuating about a mean, and possibly this view may also be taken of the values at 4·62 g./l., although here the variations are considerable and the values tend to be lowest in the middle of the temperature range. At the other three concentrations θ rises consistently with increasing temperature. The values of Q_{10} show the same tendency, but, naturally, the

differences are exaggerated, and where θ varies markedly and consistently with temperature the values of Q_{10} can have little real meaning. The variations in Q_{10} are considerable and, being systematic, no significance could be attached to mean values, but it may be pointed out that all except three values exceed that of 2–3 which is typical of many chemical reactions. This is in agreement with the findings of Chick (1908), who obtained the value of 5·5 for the Q_{10} of phenol for the destruction of *B. anthracis* spores and values of 7–15 for *B. paratyphosus*. Chick's values of Q_{10} are well within the range covered by those in Table 2. The change in

Table 2. *Values of the temperature coefficients b, θ, Q_{10} and μ calculated between successive determinations of the v.s.t. at five phenol concentrations*

Phenol conc. g./l.	Temp. range °C.	b*	θ	Q_{10}	μ
4·62	30 –32·5	2·85	1·29	12·75	47,100
	32·5–35	4·61	1·40	28·89	63,300
	35 –38	2·85	1·19	5·66	33,200
	38 –39·5	0·65	1·04	1·41	6,700
	39·5–42	7·04	1·41	29·86	66,900
5·20	27 –31	2·56	1·18	5·06	29,600
	31 –35	3·75	1·23	8·10	39,200
	35 –39	6·66	1·36	20·86	58,400
6·01	23 –26	4·73	1·28	11·33	43,000
	26 –28	2·04	1·10	2·53	16,700
	28 –31	7·06	1·34	17·99	52,900
	31 –35	6·05	1·24	8·70	40,500
6·98	22 –25	4·24	1·16	4·43	26,200
	25 –28	6·61	1·23	8·16	37,700
	28 –30·5	7·09	1·23	7·92	37,800
	30·5–35	11·02	1·34	18·65	54,700
7·95	20 –24	3·88	1·11	2·86	18,300
	24 –29	9·98	1·22	7·02	35,000
	29 –35	11·42	1·28	11·39	45,300

* The values of b are based on threshold temperatures (α) of 20° C. at 4·62 g./l., 15° C. at 5·20 g./l., 5° C. at 6·01 g./l., −5° C. at 6·98 g./l. and −15° C. at 7·95 g./l.

Q_{10} with phenol concentration observed by Chick cannot be detected with certainty in the present data by the method of presentation adopted.

In Table 2 are also given values of the temperature velocity constant μ (the 'energy of activation') calculated from the Van't Hoff-Arrhenius equation. There is, of course, a close relation between Q_{10} and μ, so that the variations in the latter, like those of Q_{10}, are considerable and systematic. When μ varies with temperature, as it frequently does in biological data, the idea that the reaction being investigated is due to a series of catenary reactions, each with its own characteristic value of μ, has been introduced. The rate of the reaction as a whole is imagined to change suddenly at various critical temperatures as each member of the series of reactions becomes in turn the limiting factor. The values of μ might then serve to identify the various reactions. Bělehrádek (1935) has fully discussed the objections to this view and has also adduced evidence that the value of μ is influenced by many environmental factors, so that the identification of a reaction from the μ value could scarcely be hoped for. Here it may be pointed out, however, that the values of μ in Table 2 are very much larger than those usually associated with oxygen absorption by living organisms including bacteria, which commonly cluster around 12,000 or 16,000 according to the temperature employed and rarely exceed 22,000.

Temperature coefficients which vary markedly with temperature are naturally very unsatisfactory and are of little practical use, but certain considerations can be advanced concerning the theoretical relationship between θ and temperature which may be of assistance in an attempt to discover a more satisfactory coefficient. Empirically it is often found that θ increases as the temperature is reduced. For example, Ames & Smith (1944) found that the bactericidal action of chlorine had a Q_{10} which rose from 1·5 between 30 and 40° C. to 3·0 between 10 and 20° C. The following considerations show that this is a necessary consequence of the method of calculating this temperature coefficient. The slope of the graph of log (*reaction time*) plotted against temperature is equal to log θ, and since in biological processes θ lies in the range 1·0–1·9, the slope of the graph may be from zero to 0·28, according to the nature of the process and the kind of organism under investigation. Usually values near 0·04 are encountered corresponding to a Q_{10} of 2·5. At all events, the slope of this graph is quite small. However, if a threshold temperature exists the reaction rate must become zero at that temperature, and at temperatures near the threshold the logarithms of the reaction times must accordingly become very large and increase rapidly with small decrements in temperature. In other words, the slope of the graph, and hence the value of θ, must increase as the temperature approaches the threshold value no matter how nearly constant it may have been at higher temperatures. No formula which requires a linear relation to exist between temperature and the logarithm of the reaction time can yield a temperature coefficient which will be constant both for temperatures near to and distant from the threshold value. As Bělehrádek (1935) points out, the formula $t(T-\alpha)^b=a$ often yields a constant temperature coefficient b in cases where the value of θ varies. This is undoubtedly because the formula provides for t to become infinitely large as T approaches the threshold value α, whereas the formula for the calculation of θ makes no such provision. The existence of a threshold temperature at all phenol concentra-

tions is strongly indicated by the graphs in Fig. 1, and in the data for 4·62 g./l. the increase in θ as the temperature falls is clearly shown (Table 2). The fact that the increase in θ is not shown at any other concentration is no doubt due to the temperatures employed having been too far above the threshold values quoted in the legend of Fig. 2.

Although θ usually rises as temperature falls, cases are not unknown in which the reverse occurs (Bělehrádek, 1935). Also, in the present case, it is evident that at four of the five concentrations used θ increased in the upper part of the temperature range employed. Even in the remaining case a slight tendency to increase can be detected. Now as the temperature is increased and the reaction time becomes very small the logarithm of the reaction time must decrease very rapidly so that it is not at all improbable that the slopes of the log (*reaction time*) – temperature graphs would tend to increase again at high temperatures. However, the formula $t(T-\alpha)^b=a$ does not and cannot compensate for this increase as it does for the comparable increase at the lower part of the temperature range as can be seen by comparing Figs. 2 and 3. Therefore, that formula also cannot be expected to give a fully satisfactory temperature coefficient where θ increases towards the upper part of the temperature range employed.

According to the arguments set out above the complete graph of log (*reaction time*) against temperature should be sigmoid, rising steeply at both ends. It should be of small and almost constant slope in the centre portion because θ is often apparently constant over a fairly considerable temperature range. In fact, this type of curve has been realized fairly completely in the graph for 4·62 g./l. (Fig. 3). The other graphs in that figure probably represent parts of similar sigmoid curves which are incomplete because the temperatures employed have not been sufficiently close to the threshold values. Possibly these considerations might be extended to biological processes in general. If so, the conflicting views as to whether θ falls, remains constant, or rises as the temperature is increased become reconciled and are to be recognized as different aspects of the same truth. However, the problem of determining a satisfactorily constant temperature coefficient for the present disinfection data remains, and in the next paper of this series an attempt will be made to derive such a coefficient.

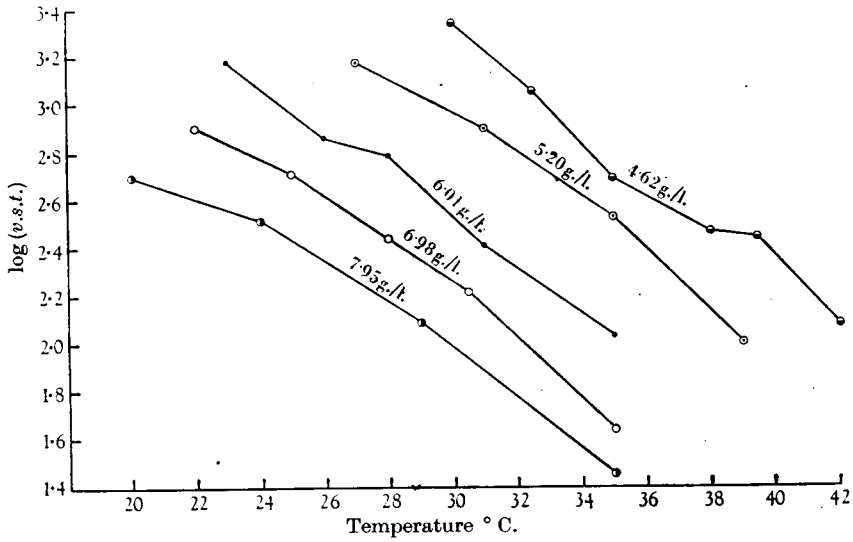

Fig. 3. Showing relationship between temperature and log (*v.s.t.*) at various phenol concentrations.

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, and the relationship between this rate and temperature at five phenol concentrations has been examined.

2. The graphs of log (*v.s.t.*) against temperature reveal that the formula $t\times\theta^T=A$, as usually employed for the calculation of the temperature coefficient of the rate of disinfection, has only a limited applicability for these data. θ and Q_{10} increased with temperature especially at high concentrations, and at the lowest concentration there was a tendency for them to increase again as the temperature was reduced below a certain point.

3. The other general type of formula, $t(T-\alpha)^b=a$, used for the calculation of biological temperature

coefficients, is also of very limited value when applied to these data since b increases with temperature at all concentrations.

4. The magnitude of these temperature coefficients and their manner of variation are considered in relation to previously published data by other authors and the way in which θ should theoretically vary with temperature is discussed.

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