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

PART X

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IN the preface to the previous communication[1] it was pointed out that the statement of a time taken to reach a certain mortality level of an inoculum, was not by itself a sufficient criterion of the bactericidal efficiency of a disinfectant substance; other considerations should be taken into account in the assessment, namely, the effects of dilution and of temperature. The dilution factor was dealt with in the last paper; in this paper the temperature factor is expounded.

THE TEMPERATURE COEFFICIENT AND ITS METHOD OF DETERMINATION

The influence of temperature on the disinfection rate. Early observers were not slow to notice that the rate of disinfection was faster at higher temperatures. Koch[2] recorded that anthrax spores were killed more quickly by phenol at elevated temperatures; Madsen and Nyman[3] found the same to be true with mercuric chloride. Experiments at different temperatures were also conducted by Henle[4], Behring[5] and Abbot[6], and the same conclusions were reached.

Chick[7], from her studies of disinfection reactions, deduced that there was a close analogy between the manner in which the reaction velocity of disinfection and that of chemical reactions, increased with temperature. She calculated that a rectilinear relationship existed between the logarithm of the disinfection rate and the temperature of the reaction; this was confirmed by her (*loc. cit.*) when studying the disinfection of bacteria by hot water. Since it was believed that the disinfection rate (k) was constant throughout its course, k was taken as being indirectly proportional to the time for disinfection; the plot of the disinfection times against the respective temperatures (for the same strength disinfectant solution) should therefore yield a straight line.

Phelps' equation. Phelps[8] likened the increase of disinfection rate with temperature to a relationship common in physical chemistry in which the velocity of a reaction increased in geometrical progression as the temperature increased in arithmetical progression. Temperature coefficients, θ *and* Q_{10}, were postulated, these being figures indicating the proportionate increase in the rate of the process (or decrease in time of completion) for a 1°C. and 10°C. increase in temperature respectively. Mathematically, the relationship may be expressed as $k_2/k_1 = Q_{10}^{(T_2 - T_1)}$ where k_2 and k_1 are the velocity constants of the disinfection processes at temperatures T_2 and T_1 respectively, differing by 10°C. (When $T_2 - T_1 = 1$, $k_2/k_1 = \theta$). When, for example, $Q_{10} = 2$, it means that the death rate is doubled by a 10°C. rise; a further increase of 10°C. will double it again, so that a total increase of 20°C. will quadruple the death rate. From the list complied by Chick[9] it is clear that θ and Q_{10} vary with the nature of the disinfectant; for example, for phenol $Q_{10}=7$ to 8, whereas for metallic salts it is 2 to 4.

The significance of the temperature coefficient. The significance of θ (or Q_{10}) does not make itself apparent in a phenol coefficient. The comparison between the standard and the unknown substance is performed at a constant temperature, and hence the coefficient so obtained is dependent upon the temperature selected for the test. The correct relative relationship between the standard and the unknown can be calculated at other temperatures only when θ is the same for both substances. If the temperature coefficients are not the same, then the increase in rates of disinfection with temperature will be disproportionate, and then the phenol coefficient will vary when the test is performed at different temperatures.

The use of extinction times as the basis of comparison for the determination of temperature coefficients. For end point methods of investigation, Phelps' equation is interpreted as $t_1/t_2=\theta^{(T_2-T_1)}$ where t_1 and t_2 θ, it is necessary to determine the extinction times for the same strength disinfectant solutions at two temperatures.

The use of LT50 as the basis of comparison for the determination of temperature coefficients. A summary of the evidence that has accumulated to indicate that the disinfection rate varies along its course has been presented in Part II[10] of this series of communications; it has also been emphasised in Part IX that the use of disinfection rates for the comparison of bactericidal activity is not always reliable.

Throughout this work the LT50 has been used to compare activities of disinfectant solutions and it is suggested that it be substituted for t in the above expression to calculate θ for the compounds. In order to achieve this it is necessary to calculate the LT50's for the same concentration of disinfectant solution at the two temperatures employed, viz., 20°C. and 30°C.

Calculation of the Temperature Coefficients of Ethylene Glycol and its Monoalkyl Ethers

LT50's for the same concentration of disinfectant were calculated for the disinfection reactions at 20°C. and at 30°C. Suitable concentrations were selected for each compound, this being readily achieved by inspection of the data in Table I, Part IX[1]. The ratio of the two LT50's at each temperature gives Q_{10}, from which θ is calculated.

Example of a calculation

In the equation $y=\bar{y}+b(x-\bar{x})$, $y=\log$ LT50 at x, the logarithm of the selected concentration; $\bar{y}$ = mean log LT50 of the data; b = the slope of the regression (= n); $\bar{x}$ = mean log concentration of the data.

Calculation of LT50 for 70 per cent. ethylene glycol at 20°C.

$$y = 1{\cdot}550 - 15{\cdot}87(1{\cdot}845 - 1{\cdot}904) = 305 \text{ minutes.}$$

Calculation of LT50 for 70 per cent. ethylene glycol at 30°C.

$$y = 1{\cdot}678 - 18{\cdot}46(1{\cdot}845 - 1.821) = 17 \text{ minutes.}$$

Calculation of the temperature coefficient

$$Q_{10} = 305/17 = 18$$

$$\theta = \sqrt[10]{18} = 1{\cdot}334$$

Results. Table I sets out the values of Q_{10} between 20°C. and 30°C. and θ at the two concentrations for each compound. It is seen that θ falls within the range 1·0 to 1·9, the generally accepted range for biological processes (Bělehrádek[11]). Except for the monoethyl ether, Q_{10} is seen to vary between 7 and 45; the monoethyl ether appears to be anomalous and exceedingly high values have been obtained. In all the compounds except ethylene glycol the values of θ and Q_{10} are greater at the lower concentration of disinfectant used for the calculation. As Q_{10} is based on two observations only in each case, a large error of estimation is involved; there are no means of knowing whether the differences with concentration are really significant.

Discussion

Variation of the temperature coefficient with the concentration of disinfectant. In this work, temperature coefficients have been recorded at two temperatures, viz., 20°C. and 30°C. Broad generalisations only, therefore, on the nature of the coefficients, can be drawn from the results; experiments at intermediate stages involving smaller temperature ranges, should be performed before detailed inferences are made. Nevertheless, it is clear from the results in Table I that the value of θ and Q_{10} varies considerably with the concentration of the disinfectant. The variation in the values of Q_{10} is very noticeable and although θ (the tenth root of Q_{10}) appears to alter but little, this is deceptive as small differences in its value will manifest themselves when the temperature range is protracted.

Influence of the concentration exponent on the variation of the temperature coefficient with concentration of disinfectant. The variation of the temperature coefficient at different concentrations appears to be dependent on the behaviour of the concentration exponent of the disinfectant. This general phenomenon was first observed by Chick[6]. The results of Jordan and Jacobs[12] suggest a possible explanation of this effect. These authors detected that the relationship between log *v.s.t.* and log concentration of phenol was not rectilinear over a wide range of concentrations. When two regressions of this nature set up at two different temperatures, are compared in order to determine the temperature coefficient, the value of the latter will vary with the concentration selected and the trend of the variation will depend on the actual shapes of the curves.

The results obtained in this work can be used to illustrate the variation of the temperature coefficient with concentration by comparing the slopes of the log LT50-log concentration of disinfectant regressions. Figure 1 gives an example; it shows the slopes of the log LT50-log per cent. concentration regressions (i.e., the concentration exponents) of ethylene glycol monopropyl ether at 20°C. and at 30°C. It can be seen that the slopes are not parallel and hence the ratio between the values of the two LT50's at the same concentration (i.e., Q_{10}) will vary with the concentration chosen. The level of mortality selected for the calculations does not enter into the argument; the deciding factor is the concentration exponent and if this is not the same at the two temperatures then different values of Q_{10} must be obtained at different concentrations. When n does not alter, the magnitude of the slopes at different temperatures will be identical and at whatever concentration the comparison is made, the value of Q_{10} will be constant.

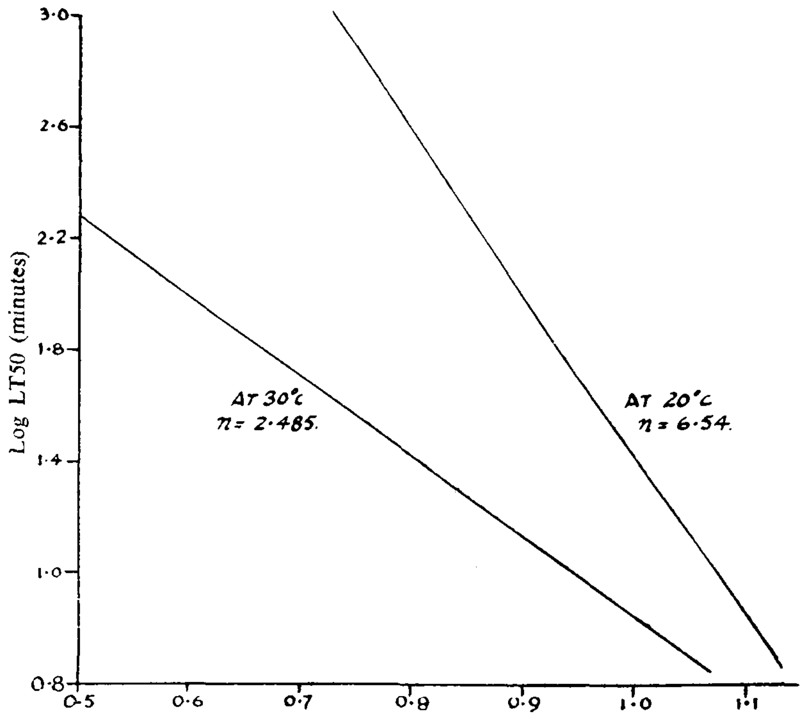

FIG. 1. Comparison of the concentration exponents (n) of ethylene glycol monopropyl ether at 20°C. and at 30°C.

Comparison of the temperature coefficients of chemical reactions and disinfection processes. The pioneer workers carried out investigations on disinfection with metallic salts and obtained Q_{10} values of between 2 and 3. Chemical reactions have a Q_{10} of the same order and the similarity of the range for the two processes gave added evidence to Chick's[6] hypothesis that a stoichiometric relationship existed between disinfectant and bacterial protein. The researches of Paul, Birstein and Reuss[13] on hydrochloric acid enhanced this conception; they obtained Q_{10} values between

1 and 3, whereas the temperature coefficient of chlorine fell between 2 and 4 (Weber and Levine[14]; Ames and Smith[15]). However, Charlton and Levine[16] found that the coefficient of this latter substance was affected by *p*H and that at *p*H 6 the temperature coefficient was between 5 and 6.

Phenol and fundamentally similar disinfectants possess higher values of Q_{10}. For phenol against *Bact. paratyphosum*, Chick[6] obtained values between 7 and 15, and against anthrax spores, between 7 and 8·6. Tilley[17] confirmed this range for phenol and also found that cresols and resorcinol were similar. Tilley's results also showed that the values of Q_{10} depended on the position on the temperature scale chosen for the determination; although a variation in the value was noticeable with different positions, no definite trend could be detected. Against *Bact. typhosum* the same author obtained extremely high values for ethyl alcohol and normal butyl alcohol; between 30°C. and 40°C., Q_{10}'s of 54 and 40 respectively were obtained, and between 20°C. and 30°C. the corresponding values were 43 and 31. The values for the compounds in the present investigation are of the same order.

High values of Q_{10} are not in line with the view that the process of disinfection is analogous to a chemical reaction; if the two processes were analogous then values of 2 to 3 for Q_{10} should be obtained consistently. Extremely high coefficients have also been recorded for the coagulation of proteins by hot water; Chick and Martin[18], for example, returned a figure of 650 for egg albumin. Henderson Smith[19] investigated the disinfection action of hot water on Botrytis spores and found that Q_{10} increased at lower temperatures. The highest value recorded by him was 690. None of these can be considered as chemical reactions.

A classification of disinfection temperature coefficients. Cooper and Haines[20] classified disinfectants into three groups according to the values of the temperature coefficient. In the first group there was no increase in the disinfection rate with increased temperature; in the second group the increase was approximately the same as that for chemical reactions, while in the third group the temperature coefficient was extremely high. Rahn[21] cast doubt on the validity of this classification because the authors had calculated the temperature coefficients from the ratio of the minimum concentrations of phenol (the standard) and the test substance required to kill the inoculum in a fixed arbitrary time; had other times been used, different temperature coefficients might have been obtained.

Limitations of the accepted formula for determination of temperature coefficients. Jordan and Jacobs[22] found that the temperature coefficient of phenol (as calculated from the usual formula) increased with temperature, especially at the higher concentrations of disinfectant. At the lower concentrations there was a tendency for the coefficients to increase again when the temperature fell below a certain level. Henderson Smith[19] had pointed out that the accepted relationship between temperature and reaction velocity was purely empirical and was devised to meet the case of chemical reaction in which the velocity of the process changed slowly over a wide temperature range; in disinfection reactions the velocity was often changing rapidly over a short range. By adaption of Arrhenius'[23] equation Henderson Smith was able to procure a rectilinear relationship between mortality times and temperature.

Proposal of a more satisfactory temperature coefficient for disinfection processes. Jordan and Jacobs[22] employed some of the established formulae commonly used in the calculation of the rate of increase of biological processes with temperature listed by Bělåerhdek[11] in the hope of deriving a constant temperature coefficient for the disinfection process. They suggested that the variation in Q_{10} at different temperature levels could be predicted on theoretical grounds, since a minimum (or " threshold ") temperature existed for any given phenol concentration and at which the disinfection reaction became infinitely small: Q_{10} must therefore rise as the temperature approaches the threshold value. Experimental evidence was secured in support of this hypothesis. From further studies[24] it was observed that the distal portion of the *v.s.t.*-temperature curve appeared to be asymptotic and it was not possible to estimate accurately the temperature at which the *v.s.t.* was zero. However, they assigned an arbitrary time of 10 minutes to the *v.s.t.* at the " maximum " temperature for each phenol concentration, i.e., the temperature at which the *v.s.t.* may be taken as equal to 10 minutes. The curves of (*v.s.t.* - 10) when plotted against temperature fell from infinity to zero as the temperature rose from the minimum to the maximum value. When log (*v.s.t.* - 10) for given concentrations was plotted against temperature, sigmoid curves were obtained which could be regarded as asymptotic to the ordinates at the minimum and maximum temperatures. The authors found that the equation known as the " Pearl-Verhulst logistic equation " (Pearl[25]), adequately described the shape of the curve. One of the constants in the formula was of the nature of a temperature coefficient and hence it was possible to derive a truly constant temperature coefficient for each phenol concentration. The values of the new temperature coefficient did not vary greatly with phenol concentrations within the range studied.

SUMMARY

1. It has been proposed that LT 50 be used in place of the extinction time in the adoption of Phelps' equation[8] for the determination of temperature coefficients.

2. The temperature coefficients of ethylene glycol and its monoalkyl ethers have been found somewhat large, the monoethyl ether exceptionally so. These high values have been used as evidence against the thesis that disinfection and chemical processes are analogous.

3. The temperature coefficients have been shown to vary with the concentrations of disinfectant used for their calculation; the variation of the concentration exponent with temperature has been suggested as an explanation of this phenomenon.

4. Reference has been made to the proposal of Jordan and Jacobs[24] of a more satisfactory temperature coefficient which does not vary with the temperature of the disinfection concentration.

TABLE I

VALUES OF THE TEMPERATURE COEFFICIENTS BETWEEN 20°C. AND 30°C. FOR CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS

Compound	Concentration	Q_{10}	θ
	per cent.		
Ethylene glycol	70·0	18·00	1·334
	80·0	24·17	1·375
Monomethyl ether	40·0	45·37	1·464
	50·0	35·83	1·430
Monoethyl ether	20·0	291·00	1·764
	25·0	113·10	1·604
Monopropyl ether	7·0	16·09	1·320
	8·0	7·44	1·222
Monobutyl ether	3·0	32·15	1·515
	3·5	27·10	1·391
Monohexyl ether	0·30	12·26	1·284
	0·45	10·25	1·262

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