STUDIES IN THE DYNAMICS OF DISINFECTION

XIV. THE VARIATION OF THE CONCENTRATION EXPONENTS FOR HYDROGEN AND HYDROXYL IONS WITH MORTALITY LEVEL, USING STANDARD CULTURES OF *BACT. COLI* AT 51° C.

By R. C. JORDAN, Ph.D. and S. E. JACOBS, Ph.D.

From the Physiology Department, University College of South Wales and Monmouthshire, Cardiff, and the Bacteriological Laboratory, Imperial College of Science and Technology, London

(With 2 Figures in the Text)

Originally, the concentration exponent (n) of a disinfectant was calculated from the empirical relationship $C^n \times t = constant$ between concentration C and disinfection time t, but as further knowledge was gained of the process of disinfection changes in the method of calculation have been introduced. Increased accuracy has resulted from the introduction of 'counting' instead of 'end-point' methods, while on the assumption that bacteria exposed to lethal agencies die according to a logarithmic law, the reciprocal of the logarithmic death-rate has been substituted in the equation for the disinfection time. Recognition of the fact that the logarithmic death-rate is often not constant led to the proposal to substitute for the disinfection time the period elapsing before a definite percentage mortality had occurred. Levine, Buchanan & Lease (1926–7) suggested that the 99·9 % mortality time should be used, but this was challenged by Myers (1929) on the ground that such high mortality times were likely to be very inaccurate because of the low counts and consequent high errors involved. The 99 % mortality time was proposed as an alternative. Withell (1942) advocated the use of the 50 % mortality time. Hobbs & Wilson (1942) also came to the conclusion that the errors involved in the estimation of small numbers of survivors were too large to permit of sufficient accuracy. They suggested the use of the reciprocal of the death-rate prevailing in the region of 50 % mortality, where the errors were less. Jordan & Jacobs (1944b) pointed out that the percentage mortality which can be determined accurately depends to some extent on the initial number of cells employed. These authors (1944a) were able to obtain very regular results in experiments with phenol and *Bact. coli*, and because of this, as well as through employing very large cell populations, exceedingly high percentage mortalities were satisfactorily determined. The question then arose, whether the different procedures proposed would all give the same value of the concentration exponent. Jordan & Jacobs (1944b) emphasized that the same result would be arrived at by the use of any mortality time, provided that either the logarithmic death-rate was constant or that the probit-log survival-time curves for all concentrations were parallel over the requisite mortality range. Neither of these conditions was fulfilled in carefully controlled experiments with phenol and *Bact. coli* at 35° C., and the lower the percentage mortality chosen the higher was the value of n. Also, when the 50 % mortality time was used the concentration range giving a constant value of n became smaller. The concentration exponent increased at the higher concentrations, i.e. when the times were short. Later it was shown that when the virtual sterilization (99·999999 % mortality) time was employed, a much higher value of n was obtained when the times were shortened by increasing the temperature to 39° C. (Jordan & Jacobs, 1946). Rahn (1932) pointed out that because of the existence of a threshold concentration for the activity of a disinfectant, n must increase as that value is approached, i.e. when disinfection times are very long.

In view of the variations in the concentration exponent which have been shown to occur and which can be anticipated on theoretical grounds, the usual formula can have only a limited applicability. Jordan & Jacobs (1946) showed that the Pearl-Verhulst logistic equation provided a very good expression of the relationship between phenol concentration and virtual sterilization time over a wide range of concentration and at different temperatures. One of the constants of the formula could be regarded as a concentration exponent which was not only applicable to the whole concentration range at any one temperature but might also be constant for all temperatures. Evidently, more information is needed concerning the relationship between the concentration of disinfectants and the intensity of their

19-2

action. Further experiments have now been carried out on the disinfection of standard cultures of *Bact. coli* under rigidly controlled conditions, in which the effect of variation in pH at 51° C. was examined. The results of these experiments are used below for the calculation of the concentration exponents of hydrogen and hydroxyl ions.

RESULTS AND DISCUSSION

In the experiments to be discussed, standard cultures of *Bact. coli* were exposed at 51° C. under carefully controlled conditions to pH values of 2·8, 3·9, 4·8, 5·7, 6·05, 6·25, 6·4, 6·65, 6·8, 7·0, 7·3, 7·7, 8·2 and 8·8, the numbers of survivors being determined at

The general shape of the log survivors-time curves in these experiments has already been examined (Jordan & Jacobs, 1948 *a*). From these the times needed to reach mortalities of 50–99·99 % were obtained. Mortalities higher than 99·99 % could not be considered because permanent surviving populations became established. Those mortality times could, of course, be used for the present purpose of calculating the concentration exponents, but this has not been done because subsequently better calculated values became available. These did not, however, alter the general picture of the relationship between pH and mortality time. Owing to the nature of the log survivors-time curves, 50 and 90 % mortality times could not as a rule be calculated, but

Table 1. *The relation between* pH *and the logarithms of the times (in minutes) taken to reach various percentage mortalities in disinfections of standard cultures of* Bact. coli *at 51° C.*

pH	$\log_{10}$ 50 % mortality time	$\log_{10}$ 90 % mortality time	$\log_{10}$ 99 % mortality time	$\log_{10}$ 99·9 % mortality time	$\log_{10}$ 99·99 % mortality time
8·8	0·8681 ± 0·0025	1·2816 ± 0·0017	1·5377 ± 0·0133	1·6836 ± 0·0096	1·8037 ± 0·0125
8·2	1·1626 ± 0·0303*	1·6379 ± 0·0313	1·7905 ± 0·0191	1·9021 ± 0·0194	1·9939 ± 0·0261
7·7	1·4745 ± 0·0395*	1·9135 ± 0·0146	2·0938 ± 0·0086	2·2256 ± 0·0076	2·3341 ± 0·0100
7·3	1·7538 ± 0·0131	2·1908 ± 0·0135	2·3247 ± 0·0094	2·4226 ± 0·0124	2·5031 ± 0·0170
7·0 (*a*)	1·6198 ± 0·0033	2·0567 ± 0·0177	2·3089 ± 0·0045	2·4430 ± 0·0041	2·5533 ± 0·0060
7·0 (*b*)	1·5682†	2·0728 ± 0·0116	2·2953 ± 0·0068	2·4580 ± 0·0070	2·5919 ± 0·0097
6·8	2·0105 ± 0·0209	2·4659 ± 0·0135	2·5974 ± 0·0078	2·6935 ± 0·0099	2·7726 ± 0·0144
6·65	2·1182 ± 0·0133	2·5733 ± 0·0080	2·6656 ± 0·0049	2·7331 ± 0·0063	2·7886 ± 0·0090
6·4	1·9128 ± 0·0147	2·2139 ± 0·0088	2·4594 ± 0·0069	2·6389 ± 0·0086	2·7866 ± 0·0112
6·25	2·0847 ± 0·0195	2·4784 ± 0·0064	2·5663 ± 0·0044	2·6306 ± 0·0050	2·6835 ± 0·0066
6·05	1·9936 ± 0·0411	2·4883 ± 0·0077	2·5806 ± 0·0052	2·6480 ± 0·0065	2·7035 ± 0·0089
5·7	1·8806 ± 0·0486	2·3017 ± 0·0272	2·4182 ± 0·0142	2·5033 ± 0·0122	2·5735 ± 0·0179
4·8	1·4932 ± 0·0197	1·7577 ± 0·0119	2·0173 ± 0·0071	2·2071 ± 0·0070	2·3633 ± 0·0094
3·9	0·9031†	1·2041†	1·6549 ± 0·0088	2·0294 ± 0·0096	2·1530 ± 0·0101
2·8	0·0828 ± 0·0395‡	0·5944 ± 0·0083‡	0·8932 ± 0·0039‡	1·0686 ± 0·0033‡	1·1931 ± 0·0034‡

* Values from graph of probits against time, calculated.
† Values from graph of $\log_{10}$ survivors against time, not calculated.
‡ Values from graph of $\log_{10}$ survivors against time, calculated.

frequent intervals by the plating method (Jordan & Jacobs, 1948*a*). The apparatus and technique have been fully described in earlier papers of this series, and here it is only necessary to recall that the exposure of the cells was made in the original culture fluid, the reaction of which was kept at pH 7·0 with phosphate buffer, and that the necessary alterations in pH were made by the addition of phosphoric acid or caustic soda solution as required. By this means the introduction of ions of a different type from those already present was avoided. The experiments were intended to reveal the effects of changing the concentrations of hydrogen and hydroxyl ions only, but some alteration of the concentrations of ions other than these, e.g. sodium and potassium, was inevitable. Also, the relative proportions of ions such as HPO_4'' and H_2PO_4' must have been different at the various pH values employed.

had to be read from the freehand curves. In a subsequent examination of the probit-log survival-time relationships in these experiments (Jordan & Jacobs, 1948*b*), it was established that a bilinear treatment fitted the data with considerable exactitude. This enabled calculated values to be obtained in the great majority of cases even for the 50 and 90 % mortalities, and consequently the data obtained in this way have been preferred. Another, though less important, reason for employing the values obtained from the probit-log survival-time relationships was that the logarithms of the mortality times are obtained directly, and it is these values which are needed for the calculation of the concentration exponent.

The logarithms of the various mortality times, with their standard errors, are given in Table 1. It will be seen that in a few cases the probit-log

survival-time data were not sufficient to provide all the required values. The experiment at pH 2·8 furnishes the principal instance, and here the log survivors-time relationship provided calculated values. At pH 3·9 and 7·0 certain values could still be obtained only from freehand curves and consequently in these instances no standard errors can be attached. At pH 8·2 and 7·7 use was made of a bilinear relationship, probably fortuitous, between probits and time to give calculated 50 % mortality times. In general, the standard errors are small, as was anticipated from the close fit provided by the bilinear treatment of the probit-log time graphs, but on the whole the logarithms of the lower mortality times have the larger errors. This follows from the

calculated. The slopes of these lines are the concentration exponents and they are given, with their standard errors, in Table 2. The lines drawn in Fig. 1 correspond to the calculated regression formulae. From the smallness of the standard errors there is no doubt that the exponents are satisfactory, but it is also clear that there is a distinct tendency for the values to fall as higher mortality times are chosen for the calculation. The slight increase in the value at 90 % mortality over that for 50 % is not statistically significant ($P = 0·5–0·6$). The only case in which the decrease between successive values of the exponent is significant is that of 90–99 % mortality ($P < 0·01$), but the values derived from both the 99·9 and 99·99 % mortality times are also
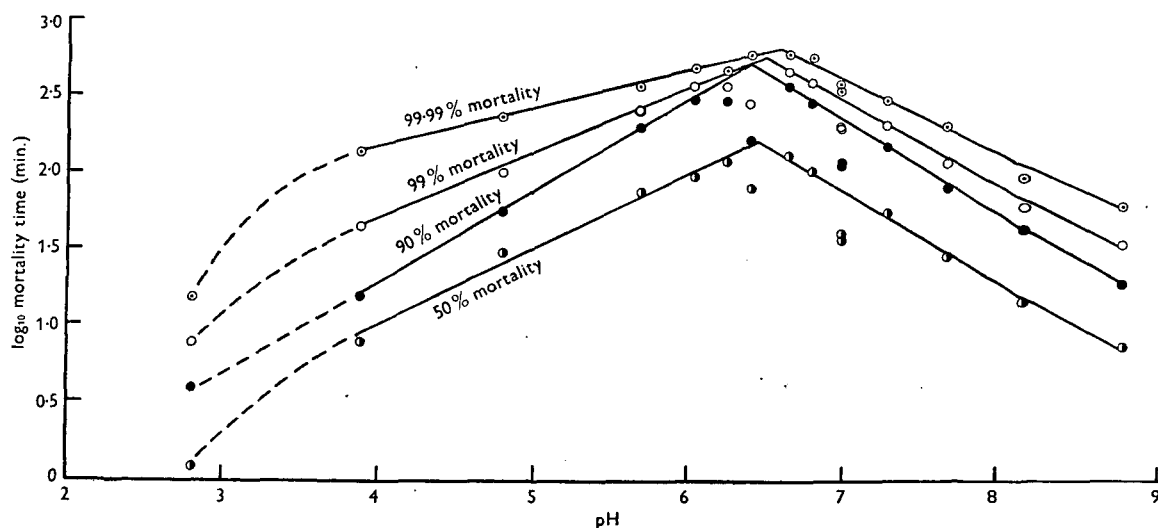

Fig. 1. Showing the relationship between log mortality time and pH for various degrees of mortality in standard cultures of *Bact. coli* at 51° C.

fact that the portions of the probit-log time graphs, from which these times were obtained, were the least satisfactory, but there are theoretical reasons why this should be so (Jordan & Jacobs, 1948b).

The relationship between $\log_{10}$ mortality time and pH is shown graphically in Fig. 1, the data for 99·9 % mortality having been omitted in order to avoid confusion in the diagram. On the alkaline side of true neutrality, which at 51° C. is at about pH 6·6 (International Critical Tables), there are very strong indications of linearity at all mortality times, except at pH 7·0 where, as shown previously (Jordan & Jacobs, 1948a), the more easily killed cells of the populations studied had heightened sensitivity. Accordingly, the data for pH 7·0, with the exception of the values for 99·99 % mortality where the divergence from the linear relationship is not marked, were excluded when from the remainder the regressions of log mortality time on pH were

significantly different from that obtained from the 90 % mortality times ($P < 0·01$ in each case) in spite of the gradually increasing size of the standard error as the mortality is increased. This is regarded as sufficient proof of the reality of the diminution in the value of the exponent.

On the acid side of neutrality there is also a strong indication of a linear relationship between pH and log mortality time, but here the data for pH 6·4 are distinctly out of place at all mortalities except the highest, while those for pH 6·25 are aberrant at the intermediate mortalities and possibly at the highest also. The situation at these two pH values thus shows some resemblance to that at pH 7·0. Again, the values for pH 2·8 are lower than the linear relationship would indicate, except at 90 % mortality, but little reliance can be placed on the lower mortality times in this case, owing to their shortness and the scantiness of the data on which they are based

However, it is clear that the logarithm of the 99·99 % mortality time at least is undoubtedly lower than the linear relationship requires. To accord with that relationship a value of 1·9 approx. should have been obtained, whereas 1·19 was actually recorded. Translating these values into times, the required value is 80 min. and the recorded value 15·6 min., i.e. a more than five-fold increase. This is wholly outside the possible experimental error and, moreover, is quite at variance with the full experimental records (see Jordan & Jacobs, 1948a). In view of this general divergence, the results for pH 2·8 have been disregarded in the calculation of the concentration exponents. So also have the results for pH 6·4 except at the highest mortality, and those for pH 6·25 except at the highest and lowest mortalities. The remaining data were used to calculate the concentra-

pH 6·65, which is very close to the neutral point for water at 51° C. The pH values at the intersections of the acid and alkaline regression lines are also all close to that point. The mean of the values is 6·51 which, therefore, could be regarded as a calculated value of the pH for maximal resistance of the standard cultures studied, irrespective of the percentage mortality used as the criterion. However, if the actual data are consulted, this conclusion appears to be tenable only when the times for the two highest mortalities are considered. At 99 % and lower mortalities there is instead a heightened susceptibility in this region, or just below it, and it is felt that in view of the irregularity of the results within the pH range 6·25–7·0 the regression lines there may not truly reflect the activities of the hydrogen and hydroxyl ions.

Table 2. *The variation in the concentration exponents of hydrogen and hydroxyl ions according to the level of mortality used to measure the rate of reaction, together with the* pH *for maximal survival thereby indicated*

Percentage mortality level	pH range	Concentration exponent with its S.E.	Ratio of exponent to its S.E.	pH of intersection of regressions
		Acid conditions		
50	3·9–6·25	0·4920 ± 0·0351	14·0	6·46
90	3·9–6·05	0·6002 ± 0·0081	74·1	6·40
99	3·9–6·05	0·4309 ± 0·0088	49·2	6·48
99·9	3·9–6·05	0·2876 ± 0·0288	10·0	6·60
99·99	3·9–6·4	0·2454 ± 0·0134	18·3	6·60
		Alkaline conditions		
50	6·65–8·8*	0·5890 ± 0·0147	40·0	
90	6·65–8·8*	0·5993 ± 0·0081	73·6	
99	6·65–8·8*	0·5390 ± 0·0143	37·8	
99·9	6·65–8·8*	0·5096 ± 0·0191	26·7	
99·99	6·65–8·8	0·4695 ± 0·0259	18·1	

* The values for pH 7·0 have been omitted (see text).

tion exponents shown in Table 2, while in Fig. 1 the lines drawn correspond to the resulting regression equations. The standard errors of the concentration exponents are small and the values therefore satisfactory, but they are not constant. The situation is similar to that encountered at alkaline reactions, namely, a tendency for the value to rise between 50 and 90 % mortality and to fall as the mortality chosen is further increased. In this case all the differences between successive values of the exponent are significant, whether they are positive or negative, except that between the values derived from the 99·9 and 99·99 % mortality times. The values of P are: 50–90 %, 0·02–0·05; 90–99 %, <0·01; 99–99·9 %, 0·01–0·02; 99·9–99·99 %, 0·1–0·2.

It is interesting to observe that experimentally maximal survival occurred at all mortality levels at

Clearly, hydrogen and hydroxyl ions are both toxic at 51° C. Considering either by itself there ought, if a threshold concentration exists, to be a tendency for the slope of the pH-log mortality-time graph to increase as that concentration is approached. Since, however, the concentrations of these ions are interdependent, evidence for this increase could be secured only if the pH corresponding to the threshold value for hydrogen ions was below, or not greater than, that of the threshold for hydroxyl ions. Also, other lethal agencies must not be operating at the same time. Now the curves obtained by plotting mortality time against pH (see Jordan & Jacobs, 1948a, and Fig. 2, where two of the graphs are shown) are regular from pH 2·8 to 6·05 and from pH 7·3 to 8·8, and such that if extrapolated each might become asymptotic to an ordinate in the pH

zone 6·5–7·0. In other words, there is strong evidence for the existence of threshold values of pH both for hydrogen and hydroxyl ions. Actually, the extrapolated portions of both the curves might legitimately be regarded for a given mortality time as asymptotic to the ordinate at pH 6·6, the neutral point at this temperature, but this does not constitute proof that the threshold values coincide at that point. The extrapolated curves may or may not overlap. In the former case there must be a pH zone

for hydroxyl ions, a non-toxic zone should exist within which there should, in the absence of other lethal agents, be little difficulty in obtaining high mortality times. However, the pH values employed were so close together that it is unlikely that the critical zone was missed and therefore, whatever the respective threshold values may have been, it seems certain that under the conditions employed some factor or factors other than the hydrogen and hydroxyl ions was at work.
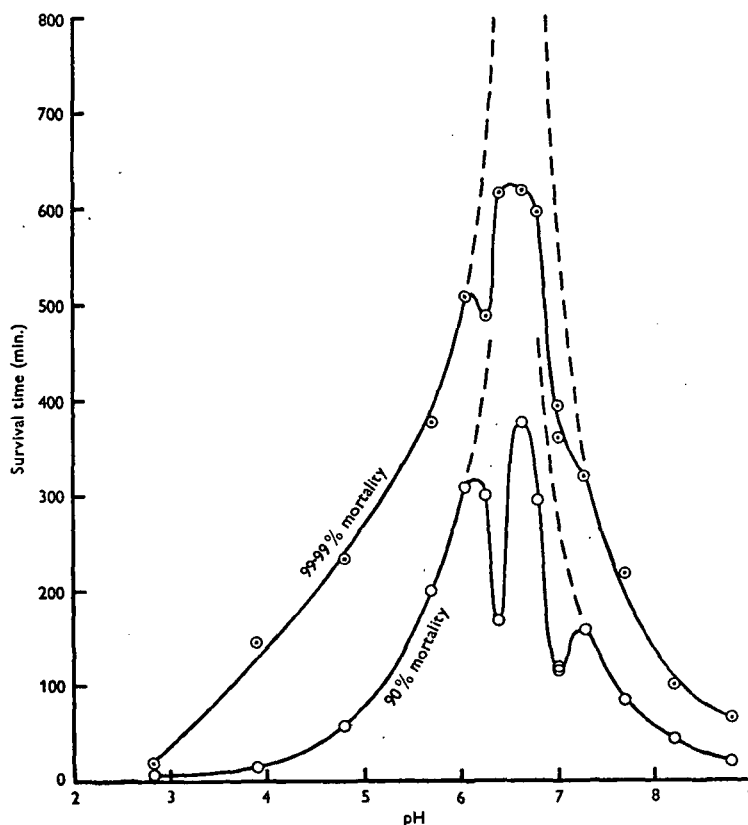

Fig. 2. Showing the relationship between survival time and pH for two levels of mortality in standard cultures of *Bact. coli* at 51° C.

in which both hydrogen and hydroxyl ions are active simultaneously, and this could account for part at least of the irregularity of the results at pH 6·25–7·0. However, if these two ions were the only lethal agents at work, the effect would probably be to produce a single dip in the pH-time curve with the minimum at the point where the separate curves, if they could be realized experimentally, would intersect. The presence of two minima, clearly shown at all mortalities except the highest (Table 1), suggests that the actual situation is more complex. If the extrapolated curves do not overlap, and the threshold pH value for hydrogen ions is below that

It is highly probable that, apart from the possible toxicity at this relatively high temperature of other ions present in the culture fluid in which these disinfections were carried out, a considerable effect was produced by the undissociated water molecules. Since the ionization of water is small, the concentration of these can be regarded as effectively constant under all the conditions of pH employed. The rate of action due to the water molecules must, therefore, also be constant, unless catalysed in some way by the hydrogen, hydroxyl or other ions, in which case the effect may properly be regarded as a property of the ions rather than of the water molecules. How-

ever, the proportion of the net effect produced by the water molecules must depend on the rapidity of action of the ions at any given concentration. Where the effects of the ions are small, as in the region of pH 6·6, the observed effect may be attributable very largely to the undissociated water molecules. This is suggested by the graph for the 99·99 % mortality times where the times are almost constant at pH 6·4–6·8. The experimental results are thus consistent with the theory that the threshold pH for the disinfectant activity of hydrogen ions is below that for hydroxyl ions and that within the non-toxic pH zone thus defined disinfection is carried out mainly by water molecules. Still to be explained is the heightened sensitivity at all mortality levels in the regions of pH 6·25–6·4 and 7·0. Experimental error is unlikely to have been responsible, since the phenomenon involves a radical change in the course of the whole disinfection. It is difficult to account for the findings by a combined action of hydrogen and hydroxyl ions with water molecules unless there is synergism at critical pH values. It is possible that alterations in cell permeability may have occurred or that the effect is in some way connected with the isoelectric points of bacterial proteins. It may be remarked that the plateau in the pH-mortality time curves (see Jordan & Jacobs, 1948a) is much less regular at low than at high mortalities. Possibly the threshold pH values are not identical for all mortality times, i.e. it might be possible to find a concentration which would kill 50 % of the cells of a culture but which would not kill 99 %, no matter for how long the exposure was made. The existence of such a concentration would be strong evidence in favour of the view that the death of bacteria exposed to lethal agencies is dependent on a real distribution of resistance among the organisms and not solely on the chance involved in an ordinary chemical reaction.

Such information as is available in the older literature points to values of about 0·5 for the concentration exponents of both hydrogen and hydroxyl ions (Rahn, 1932), though the evidence is rather conflicting. The present results, obtained at 51° C., are in general agreement with this value so far as hydroxyl ions are concerned. Recently, Hobbs & Wilson (1942) obtained the value of 2·7 approx. for the concentration exponent of caustic soda at 20 and 30° C. using *Bact. coli.* At 40° C. a much lower value of 0·7 was secured, and though this was disregarded as being unreliable, it may be that the exponent decreases with rising temperature. Those authors also obtained a lower value at the upper end of the temperature range employed (30–70° C.) when *B. subtilis* spores were used. This effect was attributed to the heat itself having a significant influence on the organisms. However, as the concentration range which in practice can be employed

varies according to the temperature, the decrease in *n* with rising temperature may really mean an increase with rising concentration. Hobbs & Wilson do not state the pH values of the caustic soda solutions which they employed. The results of Myers (1929), which were obtained with bacterial spores at 60° C., show clear evidence for an increase in the value of *n* at the highest concentrations of hydroxyl ions (pH 12–13) employed.

Under acid conditions the concentration exponent for the higher mortality times, which corresponds to that obtained by the techniques of earlier investigators, is considerably smaller than 0·5. It is, however, evident that it increases to about that value below pH 3·9. Disinfection with acids is complicated by the fact that the undissociated molecules play an important part in the reaction, and under the experimental conditions employed the ionization of weak acids present in the culture medium may have been suppressed at the more acid reactions and their germicidal effect thus enhanced.

In conclusion, it may be said that in spite of the evidence for the existence of threshold concentrations of hydrogen and hydroxyl ions, the present results are not suitable for fitting to the logistic formula previously found useful for describing disinfection by phenol (Jordan & Jacobs, 1946). This is because of the enormous concentration range covered which makes it impossible, with the data available, to fix with any approach to accuracy the 'maximum' concentrations at which the mortality times would be 10 min. Many more experiments at closely spaced intervals of hydrogen and hydroxyl-ion concentration are needed before this can be attempted.

SUMMARY

1. Data obtained by the disinfection at 51° C. of standard cultures of *Bact. coli* at pH values ranging from 2·8 to 8·8 have been used for the calculation of the concentration exponents of hydrogen and hydroxyl ions. The times taken to reach various degrees of mortality from 50 to 99·99 % have been taken as measures of the activities of the ions.

2. The formula $C^n \times t = constant$ proved satisfactory for describing the data for all the mortality levels and at all the pH values tested except pH 2·8, 6·25, 6·4 and 7·0. In these instances the cultures exhibited heightened sensitivity, though the results at all, except pH 2·8, could be included when the 99·99 % mortality times were used. The exponents increased sharply at pH values below 3·9.

3. The values of the concentration exponents varied similarly under both acid and alkaline conditions, according to the mortality level chosen. The largest value was obtained from the 90 % mortality times, there being a decrease when both higher and lower levels were employed. The changes were greater under acid conditions, when the values

ranged from 0·60 to 0·25, compared with 0·60–0·47 for the alkaline reactions.

4. The mean of the pH values for maximal survival at the various mortality levels, derived from the regressions of log mortality time on pH, was 6·51, but this was close to a point of greater susceptibility. Experimentally, maximal survival occurred at pH 6·65, which is very near to the neutral point of water at 51° C.

5. It is concluded that within the pH range 6·25–7·0 the hydrogen and hydroxyl ions were relatively inactive, the effects observed being due to other factors possibly including the action of undissociated water molecules.

REFERENCES

HOBBS, B. C. & WILSON, G. S. (1942). *J. Hyg., Camb.*, **42**, 436.

JORDAN, R. C. & JACOBS, S. E. (1944a). *J. Hyg., Camb.*, **43**, 275.

JORDAN, R. C. & JACOBS, S. E. (1944b). *J. Hyg., Camb.*, **43**, 363.

JORDAN, R. C. & JACOBS, S. E. (1946). *J. Hyg., Camb.*, **44**, 421.

JORDAN, R. C. & JACOBS, S. E. (1948a). *J. Hyg., Camb.*, **46**, 136.

JORDAN, R. C. & JACOBS, S. E. (1948b). *J. Hyg., Camb.*, **46**, 280.

LEVINE, M., BUCHANAN, J. H. & LEASE, H. (1926-7). *Iowa St. Coll. J. Sci.* **1**, 379.

MYERS, R. P. (1929). *J. Agric. Res.* **38**, 521.

RAHN, O. (1932). *Physiology of Bacteria.* Philadelphia: Blakiston.

WITHELL, E. R. (1942). *J. Hyg., Camb.*, **42**, 339.

(MS. received for publication 4. VI. 48.—Ed.)