STUDIES IN THE DYNAMICS OF DISINFECTION

XII. THE EFFECT OF VARIATION IN pH ON THE RATE OF DISINFECTION AT 51°C. OF STANDARD CULTURES OF *BACT. COLI*

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(With 6 Figures in the Text)

In studies on the effect of lethal temperatures on standard cultures of *Bact. coli*, Jordan, Jacobs & Davies (1947*a*) showed that a small proportion of cells survived, apparently indefinitely, exposure to temperatures which caused the majority to succumb in relatively short times. The temperature at which this effect was shown ranged from 47 to 53° C., but the level of the surviving population did not depend, so far as could be determined, on the actual temperature used. An investigation into the nature of these cells (Jordan, Jacobs & Davies, 1947*b*) gave conflicting results, and it did not appear that the property of heat resistance was inheritable in the same way that cells naturally resistant to antibiotics give rise to wholly resistant cultures. A culture grown from the permanent survivors of a disinfection showed markedly increased resistance, but this was not greatly improved when the culture was regenerated for the second time, and after further regenerations the resistance again declined. Also, in successive regenerated cultures the proportion of permanent survivors did not rise continuously. It was, however, evident from the altering shape of the disinfection curve for the successive cultures that changes in the distribution of resistance amongst the cells had occurred, and in fact after four regenerations at 51° C. the culture contained many cells whose sensitivity to heat, as judged by the logarithmic death-rate, was greater than that of any of those in the primary culture. The regenerated cultures were allowed to develop in the same flask as the primary one, so that the results were probably affected by the altering environment, in which the concentrations of organic matter and products of metabolism were continually rising and dead cells were accumulating. One change observed was that, in spite of the buffering power of the medium, the reaction drifted slowly towards the alkaline side, the pH having risen from 7·0 to 7·3 during the long experiment (lasting nearly 3 weeks) in which the culture was regenerated four times. It is by no means certain that pH 7·0 provides the optimum condition for the survival of heated cultures. Alternatively, since hydroxyl ions are highly toxic, especially at elevated temperatures, the pH change, although relatively small, might have acted so as to mask an actual continued increase in resistance in the successive regenerated cultures. It was accordingly decided to investigate the behaviour of these standard cultures of *Bact. coli* at different pH values at 51° C. The immediate object was to discover the effect of small changes in reaction, but it was also planned to cover a wide range of conditions from acid to alkaline, in order to obtain a set of data from which concentration relationships could be determined. The results of these experiments are reported below.

EXPERIMENTAL

Disinfections of standard cultures of *Bact. coli* were carried out at 51° C. under carefully controlled conditions at pH values of 2·8, 3·9, 4·8, 5·7, 6·05, 6·25, 6·4, 6·65, 6·8, 7·3, 7·7, 8·2 and 8·8, the numbers of survivors being determined at intervals by the plating method. These experiments were made in the apparatus and with the technique already described (Jordan & Jacobs, 1944; Jordan, Jacobs & Davies, 1947*a*), and the only additional matter needing description here is the method of obtaining the desired pH. The standard culture was first grown as usual at 35° C. and pH 7·0 in a phosphate buffer medium. From this culture a sample was withdrawn and titrated electrometrically with standard caustic soda or phosphoric acid as required. The calculated amount of alkali or acid to bring the whole culture to the required pH was then added to the culture flask immediately before the temperature was raised to 51° C. In order to avoid local high concentrations, the addition of alkali or acid was made slowly over a period of 10 min. and with the usual precautions to ensure rapid distribution throughout the culture. By using caustic soda or phosphoric acid the introduction of ions not originally present in the buffered medium was avoided, but it was naturally impossible to avoid changes in the relative concentrations of ions such as H_2PO_4' and HPO_4''. The actual pH was always checked at the end of each experiment. In a number of cases the plate counts were examined after 2 and 10 days' incubation in order to determine whether the shorter period gave the maximum yield of colonies from the damaged cells of the inoculum.

RESULTS AND DISCUSSION

The numbers of survivors after various periods of exposure to the different conditions of acidity and alkalinity are given in Table 1, in which for the sake of completeness the results of two earlier experiments at pH 7·0 are included. All the times recorded are from the moment when the culture temperature reached 51° C. Examination of the detailed results reveals that where the sampling was continued long after the phase of continuous decline in numbers was ended, there was evidence in all cases except one (pH 3·9) of the existence of a permanently surviving population of cells (see also Figs. 1–4), the numbers of which fluctuated greatly from time to time. This is certainly true over the pH range 8·2–4·8, though the case of pH 6·05 is doubtful as the numbers of survivors showed a steady continuous decline. At this pH viable cells were still present after more than 23 hr., though they were then very few, and probably this case is not really exceptional. At pH 8·8 and 2·8 there is also evidence of the permanent population, but here the sampling was stopped relatively soon. The experiment at pH 3·9 furnishes the only case in which absolute sterility was apparently reached, and even here the result may be misleading in view of the extreme fluctuations which normally occur in the phase of permanent population, even in a single experiment. Taking all experiments into consideration, values of less than 100 to over 40,000 viable cells per ml. occurred. The general situation recalls that encountered when disinfection at pH 7·0 at various temperatures was being investigated (Jordan *et al.* 1947*a*, *b*) and appears to be independent of the death-rate.

In earlier work on the disinfection of these standard cultures by phenol (Jordan & Jacobs, 1944), and heat (Jordan *et al.* 1947*a*), the distribution of the values of χ^2 for the counts was analysed. It was found that in phenol disinfections there was a marked tendency to excessive variation between the replicate plates of a count, the high values of χ^2 being much more frequent when the mortality was high, e.g. over 95 %. When heat at pH 7·0 was used the tendency was still present, but was much less prominent. The χ^2 analysis for the counts in the present series of experiments is given in Table 2. Clearly, there was once again an unduly large number of excessively high values. The proportion, 14 %, is similar to that encountered in the heat disinfections mentioned above, where it was 12 %, but much lower than the figure of 38 % obtained when phenol was used. It may be mentioned here that the high values of χ^2 were not associated with particular experiments, nor was there any greater incidence at pH values above or below 7·0. The experiments with phenol, however, are not strictly comparable with the others since no permanent populations were established. It has been suggested (Jordan *et al.* 1947*a*) that, on the one hand, excessively high χ^2 values are likely to be obtained when cells damaged by long exposure to adverse agencies are being counted, but that on the other hand, the cells in the permanently surviving condition can hardly be considered to be damaged. Accordingly, the values of χ^2 need to be separated into two classes, i.e. those obtained during the phase of active disinfection and those obtained from the phase of the permanent surviving population. Since during the later stages of the latter phase viable counts of 5000–10,000 cells per ml. were often obtained, numbers of this order of magnitude cannot safely be considered to belong to the phase of active disinfection, even though they may have occurred as part of the continuous decline associated with that phase. For convenience, therefore, the end of the active disinfection has been taken as *c.* 20,000 survivors per ml., i.e. a mortality of rather more than 99·99 %, notwithstanding that the continuous decline often extended to numbers much smaller than this before the fluctuating condition definitely associated with the phase of permanent population became established. When divided on this basis the values of χ^2 fall into two approximately equal groups. As shown in Table 3, each of these groups has an excess of unduly high values, but it is noteworthy that they were far more numerous in the active disinfection phase than when the permanent population was established. This suggested that there might be, as with phenol, a definite association with a certain range of mortality. An examination of the data revealed that many of these high values occurred just before and just after the end of the active disinfection phase as arbitrarily defined above, a fact which seemed to indicate that all the counts in the periods when the survivors were continuously declining really belonged to the phase of active disinfection. Therefore, a further division of the data

Table 1. *Numbers of survivors in standard* Bact. coli *cultures exposed to different conditions of* pH *at* 51° C.

Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.	Time (min.)	Survivors per ml.
	pH 8·8	295	303	235	1,271,000	450	925,100	420	414,900	430	477,500		pH 3·9
0	338,100,000	307	264	265	398,400	480	828,700	525	93,460	475	80,130	0	340,500,000
7	178,300,000	319	54	295	187,900	510	391,800	560	72,960	520*	17,100	10	124,500,000
17	44,690,000	331	29	355	35,100	540*	49,170	595	42,700	565	6,683	25	9,669,000
28	12,160,000	343	760	385*	12,020	575	13,070	630*	23,910	610	2,262	40	4,383,000
39	1,489,000	355	286	415	5,486	1395	41,600	1400	2,353	655	294	55	2,591,000
50	199,700	367	1,857	445	2,280	1440	20,460	1775	1,042	700	159	70	1,413,000
63	24,540	445	40	475	900	1485	3,895		pH 6·25	1390	4	85	770,300
73*	16,650	458	4,950	505	318		pH 6·65	0	326,600,000		pH 5·7	100	579,100
83	8,903	485	46	535	951	0	306,800,000	20	296,000,000	0	337,300,000	115	161,400
93	5,100	500	860	565	534	15	302,000,000	40	265,600,000	30	253,800,000	130	97,160
108	90	515	1,887	595	531	35	276,300,000	60	245,400,000	65	209,600,000	150*	18,810
130	3,875	530	1,755	655	510	55	241,000,000	80	209,300,000	105	142,700,000	170	6,956
150	564	1385	3,673	715	810	75	202,800,000	100	192,500,000	145	81,630,000	190	2,763
195	7,262	1411	834		pH 7·0 (*b*)	100	192,500,000	125	166,100,000	225	12,070,000	225	1,640
240	513	1450	2,584	0	355,900,000	125	178,000,000	150	154,700,000	265	2,500,000	250	336
285	275		pH 7·3	60	112,800,000	150	158,200,000	180	115,800,000	305	1,695,000	280	767
	pH 8·2	0	321,700,000	130	25,040,000	180	116,000,000	210	111,800,000	345	57,320	310	29
0	318,200,000	15	272,100,000	180	6,090,000	210	103,500,000	240	74,060,000	385*	19,410	340	21
15	161,200,000	40	204,600,000	310	163,200	270	74,500,000	270	56,920,000	425	3,157	370	8
45	23,470,000	70	133,900,000	410*	28,850	300	55,860,000	300	41,660,000	495	12,900	400	16
75	996,100	100	104,500,000	600	855	330	45,740,000	330	18,600,000	555	972	430	4
100*	17,280	130	83,660,000	780	1,082	360	32,820,000	360	4,135,000	615	9,972	460	0
125	3,436	170	14,140,000	960	10,100	390	19,890,000	390	1,447,000	665	14,860	1390	0
155	8,204	195	5,417,000	1150	196	420	13,910,000	450	171,500	1385	4,518	1450	0
180	2,082	230	2,045,000	1320	228	450	8,753,000	480	18,780	1505	3,882		pH 2·8
205	1,066	265	662,300	1570	1,426	480	1,466,000	510*	10,800		pH 4·8	0	340,500,000
230	574	305*	25,670	1930	478	525	347,800	540	969	0	356,400,000	5	18,780,000
255	4,045	350	3,452		pH 6·8	570	94,770	570	630	15	255,000,000	15*	48,080
280	3,560	390	2,501	0	320,000,000	615*	36,140	1435	5,817	45	69,230,000	25	14,770
305	34	450	517	15	302,700,000	1365	2,322		pH 6·05	75	11,870,000	35	17,020
335	3,198	495	8,143	35	260,900,000	1485	5,205	0	333,300,000	105	4,066,000	45	2,690
365	21	540	1,870	55	240,800,000		pH 6·4	10	291,600,000	135	1,268,000	65	7,372
395	7,060	585	7,995	75	213,300,000	0	319,900,000	40	247,100,000	165	269,300	75	3,567
1435	5,297	625	11,450	100	166,600,000	15	279,000,000	70	225,600,000	195	136,500	90	180
1465	21,870	660	12,460	125	140,600,000	45	223,800,000	100	166,100,000	225	28,580	105	5,125
	pH 7·7†		pH 7·0 (*a*)	150	132,400,000	75	174,800,000	130	154,900,000	255*	18,430	120	2,903
0	332,400,000	0	293,200,000	180	107,600,000	105	115,200,000	160	140,200,000	285	6,790	135	804
15	205,000,000	15	289,300,000	210	70,200,000	135	79,020,000	190	101,900,000	315	2,362	150	1,050
50	120,100,000	35	187,500,000	240	51,860,000	165	19,090,000	220	105,200,000	345	369	165	894
85	30,170,000	55	81,610,000	270	32,990,000	195	15,850,000	250	74,290,000	470	4,073	180	303
125	3,296,000	85	43,840,000	300	25,020,000	225	9,011,000	280	45,590,000	495	1,450	195	451
165	280,700	115	33,080,000	330	14,860,000	255	5,086,000	310	39,150,000	520	41	210	746
205*	42,870	145	17,600,000	360	8,650,000	285	2,605,000	340	19,020,000	1360	1,497	225	41
245	14,250	175	9,318,000	390	5,870,000	330	2,012,000	370	6,949,000	1440	1,174	240	115
265	6,245	205	2,424,000	420	3,136,000	375	921,600	400	1,562,000	1640	4,886		

* End of phase of active disinfection, arbitrarily defined as approximately 99·99 % mortality.

† A number of values have been omitted from the permanent population to make the table more uniform.

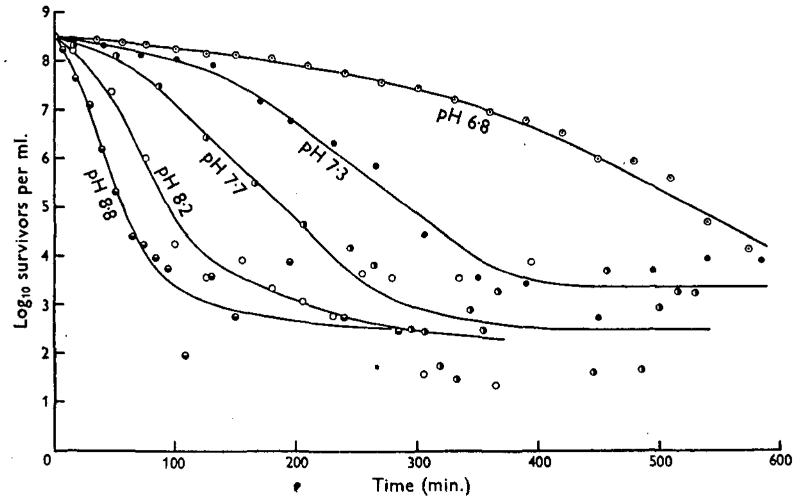

Fig. 1

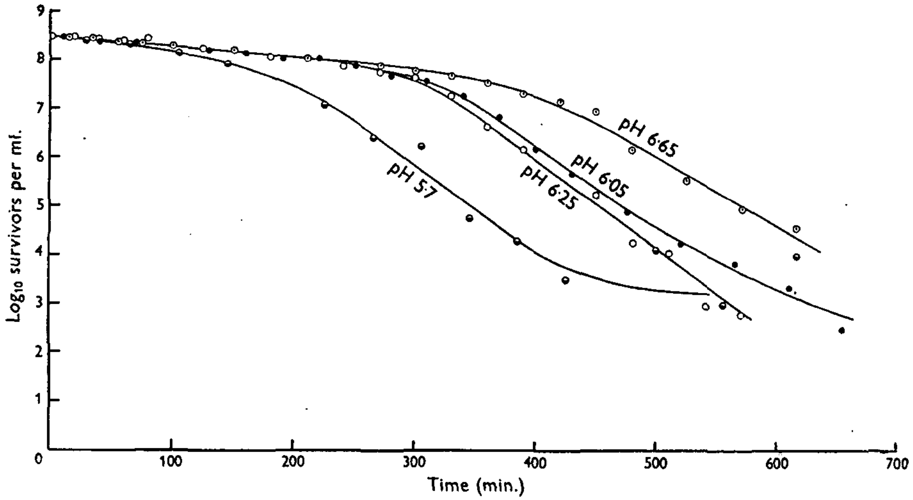

Fig. 2

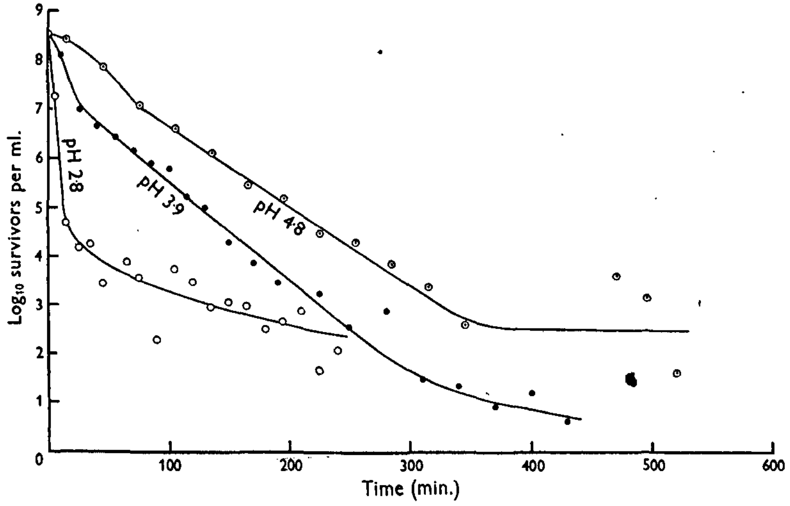

Fig. 3

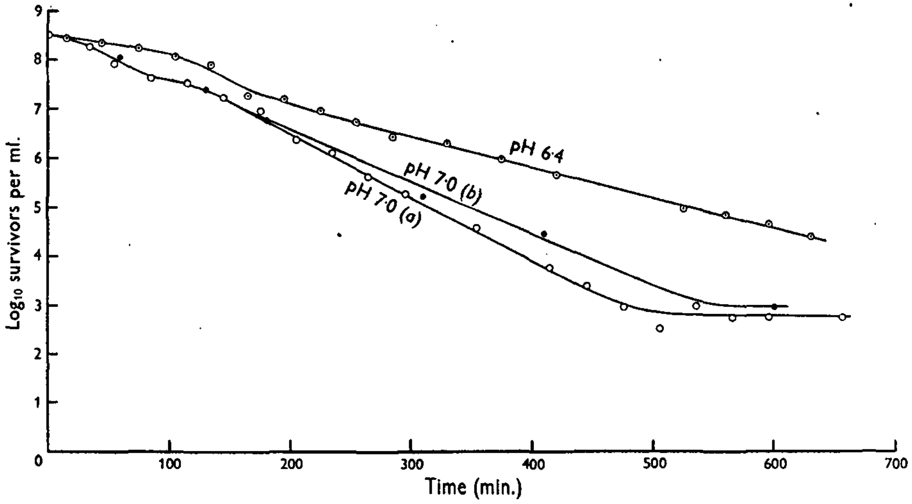

Fig. 4

Figs. 1–4. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 51° C. at various pH values

Table 2. *Distribution of χ^2 values for 462 sets of replicate plates in experiments on the disinfection of standard cultures of* Bact. coli *at* 51° C. *and various* pH *values*

Range of P	No. observed $(m+x)$	Numbers expected excluding the first group (m)	Difference (x)	x^2/m
< 0·01	64	—	—	—
0·01–0·02	9	4·02	+ 4·98	6·17
0·02–0·05	13	12·06	+ 0·94	0·07
0·05–0·10	39	20·10	+ 18·90	17·77
0·10–0·20	54	40·20	+ 13·80	4·74
0·20–0·30	29	40·20	− 11·20	3·12
0·30–0·50	84	80·40	+ 3·60	0·16
0·50–0·70	75	80·40	− 5·40	0·36
0·70–0·80	30	40·20	− 10·20	2·59
0·80–0·90	31	40·20	− 9·20	2·11
0·90–0·95	18	20·10	− 2·10	0·22
0·95–0·98	9	12·06	− 3·06	0·78
0·98–0·99	3	4·02	− 1·02	0·26
> 0·99	4	4·02	− 0·02	0·00
Totals	462			38·35

$n' = 13$; $P = 0{\cdot}0005$ approx. for $\chi^2 = 38{\cdot}35$.

was made, the counts of the phase of continuous decline being grouped according as they fell in the mortality ranges 0–95, 95–99 and 99–99·999 %. All other counts were grouped together, no matter what the actual percentage mortality, under the heading 'remainder'. The incidence of the cases of excessively high values in these four groups is given in Table 4, and there is no doubt about the definite association of high values with the mortality range 99–99·999 %. Actually, 60·9 % of the cases fell in that range and 67·2 % in the range 95–99·999 % mortality. Thus there appears to be no essential difference in this respect between disinfections by warm acid or alkali, and by phenol, where 75 % of all cases of unduly high χ^2 value occurred when the mortality was greater than 95 %.

There is a further resemblance between the two sets of experimental data, since when the 64 cases of excessively high χ^2 are excluded and the agreement of the rest with the expected numbers is tested, the fit is found to be unsatisfactory, as the value of P is only 0·0005 (approx.) as shown in Table 2. This is similar to the situation with phenol and, moreover, the bulk of the divergence from expectation occurs in the same range of the distribution. The resemblance actually is very striking since in the range of $P = 0{\cdot}2 - 0{\cdot}3$ there is in both sets of experiments a marked deficit, which is almost exactly balanced by an excess in the range $P = 0{\cdot}1$–0·2. Also, a marked excess of values occurs in the range 0·05–0·1 in the present series, while with phenol a similar excess occurred in the range 0·02–0·05. When the counts are grouped into two classes on the basis of the arbitrarily fixed end of the phase of active disinfection, and the agreement with expectation of the two distributions of χ^2 is tested (see Table 3), excluding in each case the excessively high values, it is found that the counts in the active disinfection phase do agree with expectation, though not very strikingly, since $P = 0{\cdot}087$ (approx.). The poor agreement is due almost wholly to a large excess of values in the 0·05–0·1 range, while the ranges 0·1–0·2 and

Table 3. *Distributions of χ^2 values obtained from counts made in the active disinfection and permanent population phases*

	Active disinfection phase				Permanent population phase			
Range of P	Numbers observed $(m+x)$	Numbers expected excluding the first group (m)	Difference (x)	x^2/m	Numbers observed $(m+x)$	Numbers expected excluding the first group (m)	Difference (x)	x^2/m
< 0·01	46	—	—	—	18	—	—	—
0·01–0·02	4	1·86	+ 2·14	2·46	5	2·16	+ 2·84	3·73
0·02–0·05	8	5·58	+ 2·42	1·05	5	6·48	− 1·48	0·34
0·05–0·10	19	9·30	+ 9·70	10·12	20	10·81	+ 9·19	7·81
0·10–0·20	21	18·59	+ 2·41	0·31	33	21·62	+ 11·38	5·99
0·20–0·30	17	18·59	− 1·59	0·14	12	21·62	− 9·62	4·28
0·30–0·50	38	37·17	+ 0·83	0·02	46	43·23	+ 2·77	0·18
0·50–0·70	37	37·17	− 0·17	0·00	38	43·23	− 5·23	0·63
0·70–0·80	13	18·59	− 5·59	1·68	17	21·62	− 4·62	0·99
0·80–0·90	14	18·59	− 4·59	1·13	17	21·62	− 4·62	0·99
0·90–0·95	8	9·30	− 1·30	0·18	10	10·81	− 0·81	0·06
0·95–0·98	3	5·58	− 2·58	1·19	6	6·48	− 0·48	0·04
0·98–0·99	1	1·86	− 0·86	0·40	2	2·16	− 0·16	0·01
> 0·99	1	1·86	− 0·86	0·40	3	2·16	+ 0·84	0·33
Totals	230			19·08	232			25·38

$n' = 13$; $P = 0{\cdot}087$ for $\chi^2 = 19{\cdot}08$

$n' = 13$; $P = 0{\cdot}013$ for $\chi^2 = 25{\cdot}38$

0·2–0·3 do not possess the respective excess and deficit shown by the complete series of counts. In fact, the excess and deficit in these ranges are confined to the counts belonging to the phase of permanent population, and in this set there is also a marked excess in the range 0·05–0·1. Notwithstanding the good agreement in the ranges above $P=0{\cdot}3$, this set as a whole is unsatisfactory in its agreement with expectation, P having the value 0·013. When the incidence of the values of χ^2 falling in the range of $P=0{\cdot}05$–$0{\cdot}1$ is examined, no definite association with a particular mortality range or culture phase can be detected. The cases are evenly divided between the active disinfection and permanent population phases while 28 % of the cases fall in each of the mortality ranges 0–95 and 95–99·999 %, with 44 % in the 'remainder'.

Table 4. *The incidence of cases of excessive* χ^2 *values in various mortality ranges*

	Mortality group (%)			
	0–95	95–99	99–99·999	Remainder
Number of counts	113	24	115	210
Cases of excessive χ^2	7	4	39	14
Percentage of counts showing excessive χ^2	6·2	16·7	33·9	6·7
Percentage of all cases of excessive χ^2	10·9	6·3	60·9	21·9

The influence of the period of incubation of the plates on the colony numbers was tested in four experiments, two at acid reactions (pH 4·8 and 5·7) and two at alkaline reactions (pH 7·7 and 8·8), the colonies being counted twice, after 2 and 10 days' incubation. The results obtained are summarized in Table 5, where the changes in viable count are expressed as percentages of the value obtained after 2 days' incubation. Naturally, when the plates are recounted small discrepancies are sometimes found due to the inherent errors in counting, but these should affect the more crowded plates most and must tend to cancel out in a series of replicates. When the recounting is done after a further period of incubation, moreover, there is occasionally another source of divergence, since a colony included in the first count may be rejected in the second if the extended incubation has allowed its characteristics to appear more prominently and reveal it as an airborne contaminant. This situation was, however, rare. In view of these inevitable sources of error it was hardly surprising to find that in many cases the counts after 10 days' incubation were slightly different from the 2-day values but, in order to present as clear a picture as possible, differences both positive and negative not exceeding 1 % have been ignored and reported as zero. Table 5 reveals clearly a distinct tendency for the count to increase with an extended period of incubation of the plates, but only in the phase of active disinfection and then chiefly when the mortality was over 95 %. This is similar to the result obtained by Eijkman (1908), who heated *Bact. coli* in saline at 52° C., but that worker obtained relatively enormous increases in count amounting to several hundreds per cent, and the mortality range where the phenomenon appeared was more extensive. In two of the present series of experiments there was no change at all during the succeeding phase of permanent population, and in the others the changes were relatively small and often of doubtful significance owing to the individual plate counts being low, when the influence of chance contaminants on the numbers is high. An increase in count after prolonged incubation means that organisms having an extended lag phase were present, and these are to be regarded as extensively, though not irreparably, damaged individuals. The absence of these cells from the culture when it is in the phase of permanent population supports the contention raised in a previous paper (Jordan *et al.* 1947*a*) that the cells present in that phase are not damaged.

It is interesting to note that the same mortality range, i.e. 95–99·999 %, is concerned both with the high incidence of excessively large values of χ^2 and with the presence of organisms having an extended lag phase. In view of the possibility that the high χ^2 values might have been connected with inadequate incubation of the plates, the influence of extended incubation on the χ^2 values was examined, but there was no indication of any material alteration in the distribution. Often χ^2 did not change, and when changes did occur they were usually small, though very high values were sometimes substantially increased. Of the total of 125 counts made, 52 % showed an increase and 48 % a decrease in the magnitude of χ^2, these figures being arrived at by scoring ½ to each class when the value did not change. Within the mortality range 95–99·999 %, 38 counts were made, of which 63 % showed an increase and 37 % a decrease. The difference between these percentages is probably not significant, in view of the small numbers involved, but the indication actually is that extended incubation had an unfavourable effect on the size of χ^2 within this mortality range.

The effect of change in pH on the course of the disinfection is best judged from the graphs of the logarithms of the numbers of survivors plotted against time. These are shown in Figs. 1–4, where the results after 2 days' incubation only are used, partly because such data are available for all the experiments and partly because the increases recorded after 10 days' incubation do not greatly affect the positions of the curves. The grouping of the curves in the figures has been made in such a way as to reveal as far as possible the similarities that exist. Fig. 1 shows the curves for pH 6·8 and pH 7·3–8·8. They are all generally similar in type, having slopes which increase as the reaction proceeds until at about 90 % mortality (log survivors = 7·5 approx.) they reach maximum values which are maintained to the end of the active disinfection at about 99·99 % mortality (log survivors = 4·5 or less). The tendency for the curves to approach the straight line form as the speed of the reaction increases is clearly marked. The standardization technique devised by Jordan & Jacobs (1945) was applied to these five curves to see how closely they resembled one another, and it was found that, although they were evidently closely related, they were not made superimposable by expanding the abscissa scales to make the 99·99 % mortality times coincide. These standardized curves are not presented here as they yield confused diagrams. It did appear, however, that they were less convex than the phenol disinfection type but more convex than the heat disinfection type (Jordan, *et al.* 1947*a*). The latter point becomes clear when the curves in Fig. 1 are compared with that for pH 7·0 in Fig. 4, the latter curve being evidently much flatter than that for pH 7·3. As the 99·99 %

Table 5. *The effect of prolonged incubation of plates in raising the apparent number of survivors, and the association of the increase with the level of mortality*

Time (min.)	Mortality after 2 days' incubation (%)	Increase in count after 10 days' incubation (%)	Time (min.)	Mortality after 2 days' incubation (%)	Increase in count after 10 days' incubation (%)
	pH 4·8			pH 7·7	
0	0·00	−4	0	0·00	—
15	28·45	0	50	63·87	0
45	80·58	10	85	90·92	2
75	96·67	0	125	99·01	20
105	98·86	0	165	99·92	35
135	99·64	0	205	99·99	51
165	99·92	9	245	99·996	6
195	99·96	0	295	99·99991	92
225	99·992	14	307	99·99992	0
255	99·995	10	331*	99·99998	0
285	99·998	13	367	—	0
315	99·999	6	458	—	0
345*	99·9999	6	500	—	0
470	—	4	1385	—	0
495	—	7	1411	—	0
1360	—	10	1450	—	0
1440	—	0			
1640	—	0		pH 8·8	
	pH 5·7		0	0·00	0
0	0·00	0	7	47·26	0
30	24·76	0	28	96·40	18
65	37·86	0	39	99·56	21
105	57·69	0	50	99·94	16
145	75·80	4	63	99·993	3
225	96·42	13	73	99·995	0
265	99·26	0	83	99·997	7
305	99·50	8	93	99·998	0
345	99·98	16	108*	99·99997	9
385	99·994	18	130	—	−7
425*	99·999	30	150	—	5
495	—	0	195	—	0
555	—	0	240	—	0
615	—	0	285	—	−7
665	—	0			
1385	—	0			
1505	—	0			

* End of phase of continuous decline in numbers of survivors.

mortality time for pH 7·0 is somewhat greater than that for pH 7·3 (Table 7) it is obvious that the two curves, when plotted on a standardized time scale, must differ markedly. The standardized curve for pH 6·8 was very similar to that for pH 7·3, making it clear that the results at pH 7·0 were anomalous in not fitting in with those for the rest of the group. Between the standardized curve for pH 7·0 and those for pH 7·7 and over there was, however, relatively little difference, and it must be emphasized that the curves in Fig. 1 do not form a type but a closely related group, in which the shape changes steadily with declining pH, apparently in the direction of increasing convexity.

Table 6. *The calculated relationships between log survivors and time at different* pH *values assuming a constant maximum death-rate*

pH	Regression equation* $\log_{10} S = \overline{\log_{10} S} + b\ (t-t)$	Standard error of $\overline{\log_{10} S}$	Standard error of b	Ratio of b to its standard error	Standardized slope of regression equation (× 1000)
8·8	$\log_{10} S = 5{\cdot}4339 - 0{\cdot}0660\ (t - 50{\cdot}6)$	±0·1088	±0·00675	9·8	8·5
8·2	$5{\cdot}8688 - 0{\cdot}0566\ (t - 73{\cdot}3)$	0·1585	0·00705	8·0	11·0
7·7	$6{\cdot}0195 - 0{\cdot}0240\ (t - 145{\cdot}0)$	0·0384	0·00086	28·0	10·0
7·3	$6{\cdot}0851 - 0{\cdot}0190\ (t - 233{\cdot}0)$	0·1327	0·00275	6·9	12·0
7·0 (*a*)	$5{\cdot}9691 - 0{\cdot}0129\ (t - 241{\cdot}7)$	0·0254	0·00029	44·2	9·3
7·0 (*b*)	$5{\cdot}9640 - 0{\cdot}0107\ (t - 257{\cdot}5)$	0·0846	0·00077	13·9	8·3
6·8	$6{\cdot}1929 - 0{\cdot}0108\ (t - 435{\cdot}0)$	0·0770	0·00112	9·6	12·8
6·65	$5{\cdot}8879 - 0{\cdot}0140\ (t - 510{\cdot}0)$	0·0675	0·00100	14·0	17·1
6·4	$5{\cdot}9406 - 0{\cdot}0063\ (t - 380{\cdot}0)$	0·0187	0·00012	52·8	7·7
6·25	$5{\cdot}5980 - 0{\cdot}0182\ (t - 420{\cdot}0)$	0·0643	0·00099	18·4	17·5
6·05	$5{\cdot}8551 - 0{\cdot}0173\ (t - 422{\cdot}5)$	0·0265	0·00043	39·7	17·2
5·7	$5{\cdot}7510 - 0{\cdot}0181\ (t - 305{\cdot}0)$	0·1462	0·00259	7·0	13·5
4·8	$5{\cdot}5820 - 0{\cdot}0163\ (t - 165{\cdot}0)$	0·0147	0·00076	21·4	7·4
3·9	$5{\cdot}8122 - 0{\cdot}0203\ (t - 85{\cdot}6)$	0·0463	0·00117	17·4	6·0
2·8	$6{\cdot}8292 - 0{\cdot}2570\ (t - 6{\cdot}7)$	0·0178	0·00285	90·1	8·0

* S = no. of survivors per ml. and t = time in min.

The next group of similar curves is shown in Fig. 2 and comprises those for pH 6·65, 6·25, 6·05 and 5·7. These are again all convex in shape, with the slope increasing with time until a maximum is reached at about 90 % mortality and thereafter remaining approximately constant. Again the application of the standardization technique revealed the curves to be similar but not identical in type. The convexity, which is much more pronounced than that of the curves in Fig. 1, increases with falling pH from 6·65 to 6·05, but the curve for pH 5·7 is anomalous. Although of the same general type as the group, its convexity is less than that for pH 6·65 instead of greater than that for pH 6·05. The continuous nature of the change in shape of these curves as the pH is altered is emphasized by the fact that those for pH 6·8 and 7·3 could well be placed in either group, the actual arrangement chosen having been influenced considerably by the necessity for producing clear figures. It is only possible to detect groups at all because in certain pH ranges the change in shape is relatively slight for a given pH interval. Even so, a sudden change in slope may occur within such a group. The cases of pH 7·0 and 5·7 have been instanced already, but pH 6·4 affords another striking example. This curve is altogether flatter than the others of the group comprising pH 5·7–6·65, and, in fact, shows some resemblance to that for pH 7·0. This is illustrated in Fig. 4, but by virtue of the sudden sharp increase in slope at about 90 % mortality, followed by a decrease, there is a closer resemblance to the curves for pH 4·8 and 3·9. These, together with that for pH 2·8, where the death-rates are so high that the graph appears straight, are shown in Fig. 3. These three graphs do not, however, form a closely knit group, as there is a marked difference in shape between the standardized curves for pH 4·8 and 3·9. That for pH 2·8 agrees with that for pH 4·8, but too few points are available for there to be any certainty about this.

The method of grouping the curves according to their apparent shape thus reveals the existence of three pH ranges, viz. 2·8–4·8, 5·7–6·65 and 6·8–8·8, each with its group of more or less similar curves, plus two aberrant cases at pH 6·4 and 7·0. Much of the evidence for this grouping has come from the inspection of the standardized curves but, as mentioned above, these cannot be adequately presented here nor can they be reduced to single representative curves for each group. Some indication that the arrangement is justified can be obtained by making use of a characteristic common to all the curves, namely, the maximum death-rate which holds over a part of each disinfection. The regressions of log survivors on time over the appropriate portions of the curves have been calculated, and the equations, to which the linear portions of the graphs in Figs. 1–4 correspond, are given in Table 6. Since the curves could not be made to conform to a single pattern it was not feasible to lay down rigid survivor limits for the linear relation between log survivors and time, so each case has been taken on its merits. The upper limit was, as a rule, clearly marked and occurred within the range of log survivors = 7·0–7·5 approx., with the exception of pH 2·8 where the initial observation was used to provide the third point necessary to enable a line to be calculated. The lower limit was arbitrarily set at log survivors = 4·0 in order to avoid including values which might properly belong to the phase of permanent population. Actually, this restriction has led in some cases to the exclusion of data which appeared unquestionably to belong to the linear regression, and hence to larger standard errors for the slopes of the lines. In spite of this the standard errors are all satisfactorily small, the ratio of the slope to its standard error (Table 6) being never less than 6·9 and as a rule much greater. This method of treatment, therefore, appears justified. From the regression equations the 99·99 % mortality times were calculated, with their standard errors. Each time value was then divided into 500 to give a factor which, in turn divided into the slope of the corresponding regression of log survivors on time, gave the slope of the linear portion of the standardized log survivors–time curve. These slopes (× 1000) are also given in Table 6 and are shown graphically in Fig. 5 where, however, the value for pH 2·8 is omitted because of its unreliability. These standardized slopes provide measures of the convexity of the standardized log survivors–time graphs, though not an equal measure in all cases because of the different shapes of the curves for the lower pH values tested. It appears that there is a general tendency for the slope to be small and to decrease relatively slowly with increasing acidity or alkalinity respectively, in the pH ranges 4·8–3·9 and 7·3–8·8. Between these ranges the slope is much steeper and almost constant at pH 6·05, 6·25 and 6·65. Links between the latter and the former are provided by the intermediate values obtained for pH 5·7 and 6·8, which are very similar. The anomalous behaviour at pH 6·4 and 7·0 is clearly revealed by the small values for these slopes, which cause sharp depressions in the graph. It might be though that these anomalous cases are due to errors in experimentation, but this is unlikely, since not only was the result at pH 7·0 closely duplicated, but the value for pH 6·8 falls nicely in place between those duplicate values and that for pH 6·65. Another reason why it cannot be accepted that these apparent anomalies are due solely to experimental error lies in the very large size and consistent direction (of abnormally low survivor numbers) of the errors which must have occurred to explain them.

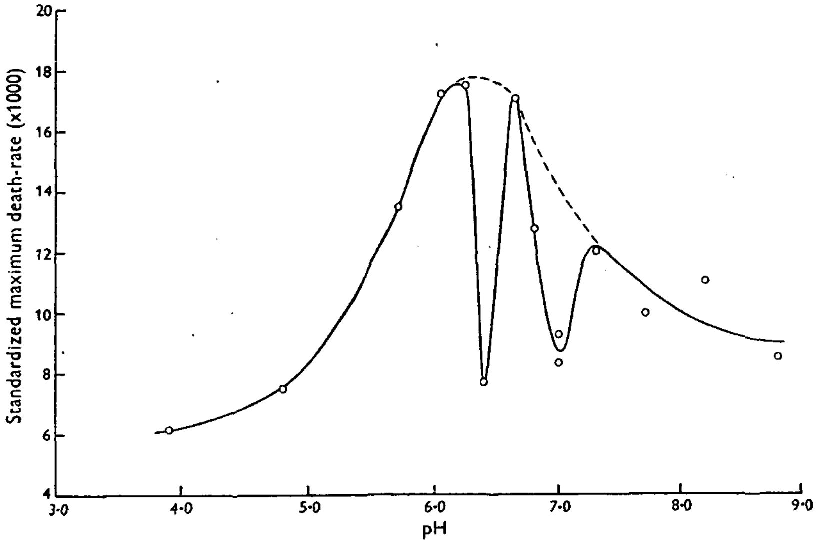

Fig. 5. Showing relationship between standardized maximum death-rate and pH for *Bact. coli* exposed to 51° C.

It is not to be supposed that the magnitude of the slope of the standardized log survivors–time curve is a direct measure of the general rate of any given disinfection. In fact, the reverse is true, for the greater the slope the more convex the curve and, with certain exceptions, the slower the general disinfection. An interesting picture is obtained by relating the times needed to reach different degrees of mortality to the pH. The 99, 99·9 and 99·99 % mortality times can be calculated from the

Table 7. *The effect of* pH *on the time taken to reach certain percentage mortalities in disinfections of* Bact. coli *cultures at* 51° C.

	Time in min. needed to produce mortalities of				
pH	50 %	90 %	99 %	99·9 %	99·99 %
8·8	7	18·9 ± 3·64	34·0 ± 2·36	49·2 ± 1·65	64·3 ± 2·16
8·2	16	42	62·1 ± 3·13	79·8 ± 2·91	97·5 ± 4·11
7·7	32	80	124·1 ± 1·77	165·7 ± 1·76	207·3 ± 2·74
7·3	57	156	210·8 ± 7·67	263·3 ± 8·23	315·8 ± 13·84
7·0 (*a*)	41	115	203·2 ± 2·15	280·4 ± 2·15	357·7 ± 3·28
7·0 (*b*)	37	115	202·5 ± 8·87	296·2 ± 8·41	389·9 ± 12·41
6·8	115	280	406·0 ± 7·75	498·8 ± 9·74	591·6 ± 17·76
6·65	150	365	467·1 ± 5·71	538·6 ± 5·24	610·0 ± 8·62
6·4	74	158	290·2 ± 3·43	449·3 ± 3·24	608·3 ± 5·25
6·25	135	310	369·8 ± 4·46	424·6 ± 3·54	479·4 ± 4·79
6·05	115	312	383·8 ± 1·82	441·8 ± 1·61	499·7 ± 2·48
5·7	80	193	262·0 ± 10·17	317·3 ± 8·28	372·7 ± 12·62
4·8	26	52	105·5 ± 3·95	166·9 ± 2·81	228·2 ± 4·07
3·9	8	16	50·1 ± 3·06	99·4 ± 2·42	148·6 ± 4·29
2·8	1	3·9 ± 0·08	7·8 ± 0·07	11·7 ± 0·09	15·6 ± 0·12

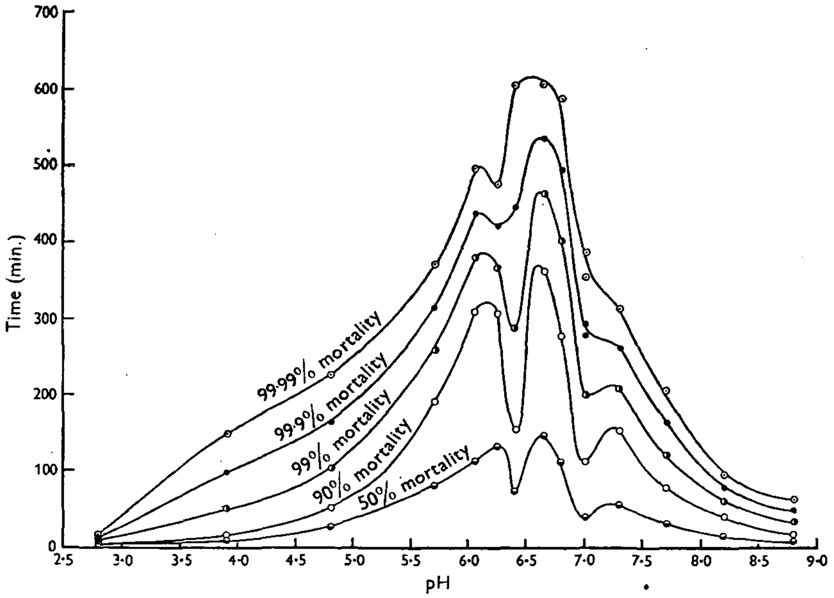

Fig. 6. Showing relationship between the times required to reach various degrees of mortality and pH for *Bact. coli* exposed to 51° C.

regression equations given in Table 6, and the 90 % mortality times can also be calculated for pH 8·8 and 2·8. The remainder of the 90 % and all the 50 % mortality times have been read from the freehand parts of the graphs in Figs. 1–4. All these times, with standard errors where appropriate, are given in Table 7, and their variation with pH is shown in Fig. 6. The 99·99 % mortality times follow a smooth curve which rises to a peak at about pH 6·5–6·6 except for an irregularity at pH 6·25 which might have been attributable to experimental error except for the evidence of the other curves. There is also a slight change of slope at about pH 7·0. The curves for the other mortality times also show peaks at about pH 6·5–6·6 which is, therefore, the pH of maximal survival for all mortality levels. In this connexion it is interesting to note that this pH is very close to that of pure water at 51° C. (the temperature at which these disinfections were carried out), i.e. it is the neutral point at that temperature, where the concentrations of H˙ and OH′ ions are equal. Winslow & Falk (1923) found that the pH of maximal survival of *Bact. coli* in distilled water at 37° C. was 6·0, but as at this temperature growth can occur, the two sets of experiments are not strictly comparable. Jordan & Jacobs (1948) found that pH 5·0 was optimal for survival at 35° C. of starved cultures of *Bact. coli* of the same strain as was employed in the present investigation.

On the acid side of this neutral point there is another but lower peak in the curves of Fig. 6 in the region of pH 6·1–6·2, whose existence is due to a well-defined minimum which is most strongly marked in the 90 % mortality curve. The position of the minimum shifts slightly from pH 6·4 to 6·25 as the mortality is raised. It is difficult to be certain of the exact course that these curves should follow, but there is no doubt that the position of the minimum must shift as indicated since, while the 99·99 % mortality time for pH 6·4 is above that for pH 6·25, the 50 % mortality time is less. On the alkaline side, the slight change in the slope at pH 7·0 in the curve for the 99·99 % mortality times is more definite for the 99·9 % times, and for the lower mortalities it becomes a well-marked minimum, which is most clearly defined in the 90 % mortality time curve. There is a strong suggestion that the basic curve for all mortality times is smooth, but slightly asymmetric, rising to a peak at pH 6·5 approx., but that some heightened susceptibility in the regions of pH 7·0 and 6·25–6·4 has complicated the picture. It is difficult to assign a cause to this heightened susceptibility, but possibly it may be associated with changes in membrane permeability or with the isoelectric points of the bacterial proteins. However, it is clear that above pH 7·3 and below pH 6·05 the effect of increasing acidity or alkalinity is consistent and regular at all mortality levels. Between these limits great variation may occur, and it is evident that according to the mortality level chosen there may be a different assessment of the effect of a given pH change. Thus, using 99·99 % mortality times, a change from pH 6·25 to 6·4 appears to slow the reaction, and this is true also, though to a lesser degree, when the 99·9 % mortality times are considered, but when lower mortalities are employed as the criterion the reaction is apparently accelerated. Also, between pH 7·0 and 7·3 the disinfection reaction, judged by the 99·99 and 99·9 % mortality times, has been quickened, but on the basis of lower mortalities it has been slowed down.

In order to determine how the results of these experiments can be used to explain the apparently enhanced resistance of a culture generated from the survivors of a disinfection at 51° C. and pH 7·0 (Jordan *et al.* 1947 *b*), and the subsequent changes in successive regenerated cultures, it is necessary to examine the effects of small changes in pH on both the maximum death-rate and on the disinfection time (99·99 % mortality time). In the regenerated cultures, the maximum death-rate after four regenerations, when the pH had risen to 7·3, was almost identical with that in the primary culture. In the present work a similar increase in pH produced a marked rise in death-rate (see Table 6), and it is clear that increasing alkalinity above pH 7·0 leads to a consistently rising maximum death-rate. Now in the disinfections of the regenerated cultures, previously reported, there was a decline in death-rate after one and two regenerations, but an increase after three and four regenerations brought the value back to that for the primary culture. Hence it is possible that the bulk of the pH rise in the regeneration experiments occurred after the third disinfection (when the buffering power of the medium was becoming exhausted) and caused the increase in the maximum death-rate, possibly despite an actual continued increase in the resistance of the organisms to the high temperature at pH 7·0. Unfortunately, no evidence is available of the manner in which the pH changed during the course of this long experiment, but in the unlikely event of there having been a temporary slight shift to a lower pH initially, there is no indication from the results in Table 6 that this would have led to a diminished value for the maximum death-rate. Rather, the reverse is true, except in the extreme event of the reaction having fallen to below pH 6·65. The cause for the temporarily reduced maximum death-rate in the once and twice regenerated cultures must, therefore, be sought in causes other than small pH changes.

As shown in Fig. 6, a change in pH from 7·0 to 7·3 decreased the disinfection time but increased the 50 and 90 % mortality times. Alkali production alone, therefore, was unlikely to have caused the marked increase in resistance shown by the once and twice regenerated cultures, but it could have contributed to the complex changes, including the reduction in the disinfection time, which occurred in the three and four times regenerated cultures. On the other hand, a slight reduction in pH caused a marked increase in all mortality times, and could have been the origin of much, if not all, of the enhanced resistance shown by the once and twice regenerated cultures. However, there is no evidence that such a pH change occurred, and it is considered unlikely to have done so. Elucidation of the cause of the enhanced resistance must, therefore, await further experimentation.

SUMMARY

1. Standard *Bact. coli* cultures have been heated at 51° C. in phosphate buffered media having pH values ranging from 2·8 to 8·8, and survivor curves obtained.

2. Except at pH 3·9, the first continuous decline in viable numbers was followed by the establishment of a small fluctuating residual population which was apparently permanent.

3. The colony counts showed a moderate excess of unduly high values of χ^2, the bulk of which were encountered when the mortality was between 95 and 99·999 %.

4. Prolonged incubation of the plates led to increased colony counts, but the rise was confined to the active disinfection phase. It occurred chiefly in the 95–99·999 % mortality range and was moderate in degree. The χ^2 values were little affected.

5. The log survivors–time graphs of the disinfection phase were convex in type but approached linearity in the faster reactions. The logarithmic death-rate became maximal at about 90 % mortality and thereafter remained constant, though under the more acid conditions a somewhat lower rate was maintained. At pH 7·0 and 6·4 the curves were exceptionally straight.

6. Standardization of all the curves revealed a continuous change in shape with pH but, nevertheless, they could be placed in three closely knit overlapping groups which were best revealed by the variation in maximum death-rate.

7. The 50–99·99 % mortality times were obtained either from the calculated regressions of log survivors on time over the linear portions of the curves or, outside that range, from the freehand curves. Maximum survival at all mortality levels occurred at about pH 6·5–6·6, i.e. close to the neutral point of pure water at 51° C. On both sides of this point sensitivity increased regularly but was abnormally high at pH 7·0 and 6·4.

8. Within these zones of abnormally high sensitivity the assessment of the effect of a given change in pH varied in amount and direction according to the mortality level chosen.

9. The enhanced resistance of a culture regenerated from the survivors of a disinfection at 51° C. and pH 7·0, as previously reported, could possibly have been largely due to a small increase in acidity but not to a slight shift towards alkalinity.

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