STUDIES IN THE DYNAMICS OF DISINFECTION

III. THE REACTION BETWEEN PHENOL AND *BACT. COLI*: THE EFFECT OF TEMPERATURE AND CONCENTRATION: WITH A DETAILED ANALYSIS OF THE REACTION VELOCITY

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(With 6 Figures in the Text)

In a previous paper (Jordan & Jacobs, 1944*a*) the results of experiments on the death of cultures of *Bact. coli* in the presence of phenol, using a special apparatus and standardized technique, were presented, and the reasons for adopting the particular methods were given. Those experiments, at various phenol concentrations, were all conducted at the same temperature, 35° C., but a complete analysis of the reaction between phenol and *Bact. coli* requires that the temperature should also be varied. Such experiments have now been carried out and the results reported below have proved extremely fruitful. They throw considerable light on a number of aspects of the disinfection process, namely, the true shape of the logarithm of survivors-time curve, the distribution of resistance amongst the cells of the bacterial population, and the effect of both temperature and phenol concentration on the time taken to produce various degrees of mortality. It is impossible to deal adequately with the data from all these points of view in a single paper, and it is proposed, therefore, to discuss the various aspects in separate papers. Obviously, the true shape of the logarithm of survivors-time curve is of prime importance, since from such curves the times required to reach different degrees of mortality are obtained. Accordingly, the present paper is devoted to that aspect of the problem.

METHODS AND RESULTS

The apparatus and methods used were the same as those previously described (Jordan & Jacobs, 1944*a*) except for a slight modification in technique necessitated by the employment of temperatures other than 35° C. As before, the cultures were grown at that temperature to obtain the standard bacterial population, but immediately before the addition of the phenol solution the temperature of the thermostatically controlled water-bath was rapidly adjusted to that at which the experiment was to be carried out. Water was siphoned from the bath, hot or cold water added as required and the thermostat readjusted. The temperature of the culture within the flask rapidly attained the desired value, but in order to ensure complete equilibrium at the new temperature a period of 1 hr. was allowed before further operations were carried out. The required amount of phenol solution was then added and samples removed at intervals and plated out as previously described.

Experiments were performed at several temperatures at each of five phenol concentrations which were chosen so that the data previously obtained at 35° C. could be included in a study of the effect of varying temperature with phenol concentration constant. The results are given in Table 1 and the graphs of logarithms of survivors against time appear in Figs. 1–5. It is evident that the death-rate varied widely during the course of each experiment. At first the rate was low but increasing, and after rising sharply to a peak value it usually appeared finally to decline. Further, in many cases there were signs of an initial rush in the disinfection process, a feature which is apparent in Table 1 but not in the graphs. A table of the distribution of the index of dispersion (χ^2) derived from the numbers of colonies on the replicate plates in each count has not been presented since this distribution closely resembled that observed in the experiments at 35° C. already reported (Jordan & Jacobs, 1944*a*). As before, the values of χ^2 were distributed in accordance with expectation until a mortality of about 95% was reached, after which there was an abnormally high incidence of large values.

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Table 1. *Numbers of survivors and death-rates in* Bact. coli *cultures exposed to phenol at various concentrations and temperatures*

Phenol conc. g./l.	Temp. °C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$	Death-rate $K \times 1000$*
7·95	29	0	348,400,000	8·5421	—
		5	213,500,000	8·3294	42·54
		25	76,010,000	7·8809	22·43
		45	50,890	4·7067	158·71
		65	3,837	3·5840	56·14
		80	300	2·4771	73·79
		95	63	1·7993	45·19
		110	15	1·1761	41·55
	24	0	336,800,000	8·5273	—
		10	236,000,000	8·3729	15·44
		45	152,000,000	8·1818	5·46
		80	23,940,000	7·3791	22·93
		115	863,700	5·9364	41·22
		160	2,958	3·4710	54·79
		195	248	2·3945	30·76
		230	165	2·2175	5·06
		265	43	1·6335	16·69
	20	0	321,600,000	8·5073	—
		10	256,400,000	8·4089	9·84
		45	211,200,000	8·3247	2·41
		85	190,800,000	8·2806	1·10
		125	60,950,000	7·7850	12·39
		175	12,290,000	7·0895	13·73
		220	1,645,000	6·2161	19·60
		260	106,000	5·0253	29·78
		305	20,830	4·3187	15·70
		350	671	2·8267	33·16
		395	119	2·0755	16·69
		440	29	1·4624	13·62
6·98	30·5	0	323,800,000	8·5103	—
		5	223,600,000	8·3495	32·16
		35	37,330,000	7·5720	25·91
		65	54,520	4·7366	94·52
		110	965	2·9845	38·94
		120	299	2·4757	50·88
		130	134	2·1271	34·86
		140	17	1·2304	89·67
	28	0	301,600,000	8·4795	—
		10	249,900,000	8·3978	8·17
		40	148,700,000	8·1724	7·51
		80	1,423,000	6·1532	50·48
		115	10,820	4·0342	60·54
		150	2,900	3·4624	16·34
		185	358	2·5539	25·96
		220	43	1·6335	26·30
	25	0	314,700,000	8·4979	—
		10	231,300,000	8·3642	13·37
		65	168,600,000	8·2269	2·50
		120	34,590,000	7·5389	12·51
		185	258,200	5·4119	32·72
		240	11,970	4·0781	24·25
		295	6,461	3·8103	4·87
		345	2,303	3·3623	8·96
		405	154	2·1875	19·58
		450	15	1·1761	22·48
		485	6	0·7782	11·37
	22	0	325,900,000	8·5131	—
		5	287,700,000	8·4590	10·82
		65	208,300,000	8·3187	2·34
		555	1,092	3·0382	10·78
		625	70	1·8451	17·04
		695	28	1·4472	5·68
		755	6	0·7782	11·15
		830	2	0·3010	6·36
6·01	31	0	330,000,000	8·5185	—
		15	226,900,000	8·3558	10·85
		45	156,800,000	8·1953	5·35
		75	35,180,000	7·5463	21·63
		105	1,959,000	6·2920	41·81
		135	10,310	4·0132	75·96
		165	892	2·9504	35·43
		200	252	2·4014	21·40
		230	11	1·0414	45·33
		265	2	0·3010	21·15
	28	0	310,000,000	8·4914	—
		5	281,400,000	8·4493	8·42
		60	244,100,000	8·3876	1·12
		90	233,200,000	8·3678	0·66
		125	113,700,000	8·0557	8·92
		195	2,173,000	6·3371	24·55
		240	78,600	4·8954	32·04
		275	9,239	3·9656	26·57
		305	5,868	3·7685	6·57
		345	4,042	3·6066	4·05
		375	1,437	3·1574	14·97
	26	0	310,200,000	8·4917	—
		5	295,600,000	8·4707	4·20
		90	231,100,000	8·3638	1·26
		185	129,200,000	8·1113	2·73
		270	6,050,000	6·7818	15·64
		360	164,000	5·2148	17·41
		535	1,278	3·1066	12·05
		615	69	1·8388	15·85
		690	13	1·1139	14·85
	23	0	329,400,000	8·5177	—
		1040	752	2·8762	5·43
		1115	480	2·6812	2·60
		1190	96	1·9823	9·32
		1270	33	1·5185	5·80
		1340	22	1·3424	2·52
		1400	8	0·9031	7·32
		1460	8	0·9031	0·00
		1655	1	0·0000	4·63
5·20	39	0	321,600,000	8·5073	—
		5	208,300,000	8·3187	37·72
		35	7,366,000	6·8673	48·38
		60	1,731	3·2382	145·16
		85	14	1·1461	83·68
		105	3	0·4771	33·45
	31	0	319,800,000	8·5049	—
		30	301,700,000	8·4796	0·84
		90	258,600,000	8·4126	1·12
		160	239,500,000	8·3793	0·48
		330	22,460,000	7·3514	6·05
		375	4,763,000	6·6779	14·97
		415	590,200	5·7710	22·67
		465	32,560	4·5127	25·17
		515	9,381	3·9722	10·81
		565	5,197	3·7158	5·13
	27	0	337,300,000	8·5280	—
		60	310,200,000	8·4917	0·61
		115	296,900,000	8·4726	0·35
		310	274,100,000	8·4380	0·18
		355	226,000,000	8·3541	1·86
		415	218,500,000	8·3395	0·24
		475	181,400,000	8·2586	1·35
		535	138,100,000	8·1402	1·97
		915	1,149,000	6·0603	5·47
		1120	5,123	3·7096	11·47

* $K = \frac{1}{t}\log_{10}\frac{B}{b}$, where B and b are the numbers of survivors per ml. at the beginning and end of the time interval t min.

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Table 1 (*continued*)

Phenol conc. g./l.	Temp. °C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$	Death-rate $K \times 1000$*	Phenol conc. g./l.	Temp. °C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$	Death-rate $K \times 1000$*
5·20	27	1195	1,320	3·1206	7·85	4·62	38	155	274,800	5·4391	54·74
		1285	206	2·3139	8·96			185	13,380	4·1265	43·75
		1355	48	1·6812	9·04			215	551	2·7412	46·18
		1420	32	1·5051	2·71			245	49	1·6902	35·03
		1480	4	0·6021	15·05			275	13	1·1139	19·21
4·62	42	0	339,500,000	8·5308	—		32·5	0	311,300,000	8·4932	—
		5	282,700,000	8·4513	15·90			90	278,000,000	8·4440	0·55
		25	149,800,000	8·1756	13·79			220	246,000,000	8·3909	0·41
		70	1,301	3·1142	112·48			335	204,700,000	8·3111	0·69
		95	152	2·1818	37·30			390	169,100,000	8·2282	1·51
		115	4	0·6021	78·99			580	13,220,000	7·1212	5·83
	39·5	0	330,000,000	8·5185	—			635	4,390,000	6·6425	8·70
		5	294,200,000	8·4686	9·98			685	896,300	5·9524	13·80
		40	215,900,000	8·3342	3·84			760	153,000	5·1847	10·24
		80	5,152,000	6·7120	40·56			840	10,900	4·0374	14·34
		165	816	2·9117	44·71						
		200	156	2·1931	20·53		30	0	341,100,000	8·5329	—
		240	49	1·6902	12·57			800	148,300,000	8·1712	0·45
		280	2	0·3010	34·73			930	49,540,000	7·6950	3·66
								1050	32,560,000	7·5127	1·52
	38	0	333,200,000	8·5227	—			1160	9,979,000	6·9991	4·67
		5	291,100,000	8·4640	11·74			1235	6,860,000	6·8363	2·17
		35	245,500,000	8·3901	2·46			1440	408,700	5·6115	6·46
		65	224,000,000	8·3502	1·33			1530	110,700	5·0440	6·31
		95	111,100,000	8·0457	10·15			1670	29,890	4·4755	4·06
		125	12,060,000	7·0813	32·15			2040	14	1·1461	9·00

* $K = \frac{1}{t}\log_{10}\frac{B}{b}$, where B and b are the numbers of survivors per ml. at the beginning and end of the time interval t min.

DISCUSSION

In the two previous papers of this series (Jordan & Jacobs, 1944 *a*, *b*) the results of experiments on the destruction of *Bact. coli* cultures by phenol at 35° C. were treated as though the death-rates had risen from initially very low values to maxima and thereafter remained constant. A straight line was fitted to the data after the maximum rate had been reached in each experiment and, the standard errors of the slopes of these lines being small, this method of treatment was considered to give a good approximation to the actual course of the disinfection process. From these regression lines the times taken to reach various degrees of mortality could readily be calculated. The same method of treatment can be applied to the results reported here with equally satisfactory results. Table 2 shows that the standard errors of the slopes of the regression lines obtained as above are small, and clearly reveals that increasing either the temperature or the phenol concentration leads to a rise in the maximum death-rate. However, despite the generally satisfactory nature of this method of treating the data, which also has the merit of simplicity, it is not now considered the best for yielding the most accurate estimates of various mortality times. In the two publications cited above reference was made to the fact that in all except the longest experiments the death-rates showed a distinct tendency to decline after having reached their maximum values. At that time it was decided that the evidence for the decline was not very strong, but in the experiments carried out since then the same tendency has repeatedly been observed so that it is felt that the weight of evidence can no longer be neglected. The principal reason for regarding the evidence for the peak and decline with some suspicion was that the counts on which the death-rates concerned were based had an excessive amount of variation between the replicate plates, which is shown by an abnormally high incidence of large values of χ^2 (Jordan & Jacobs, 1944*a*) and the counts may, therefore, be unreliable. It was hoped to find means for overcoming this difficulty and of obtaining counts which were not subject to this uncertainty, but it is now considered that this ideal may not be completely realizable when dealing with bacteria which have been exposed to phenol for a considerable time. In order that a set of χ^2 values should be distributed in accordance with expectation, it is essential that not only should the sampling technique be perfect but also that the bacterial cells should be identical in the sense that they are all equally capable of giving rise to colonies on the growth medium after passing through the plating-out procedure. These two conditions were evidently fulfilled in counts made before and shortly after the addition of the phenol since the χ^2 values were then properly distributed but later in each experiment high values of χ^2 became excessively frequent. As it is unlikely

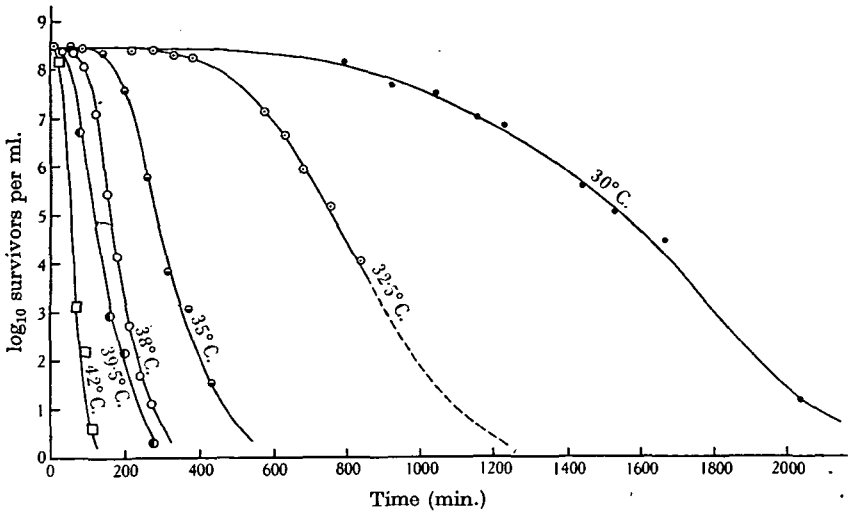

Fig. 1. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 4·62 g. phenol per litre at various temperatures.

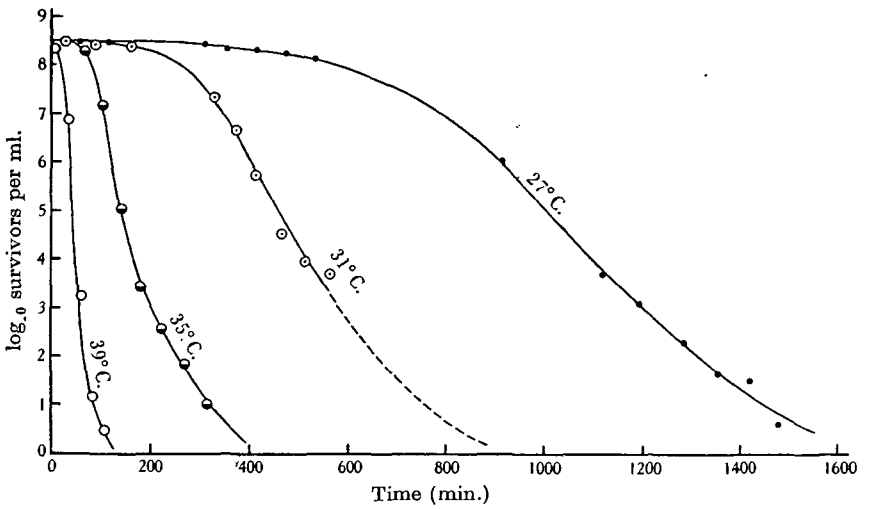

Fig. 2. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 5·20 g. phenol per litre at various temperatures.

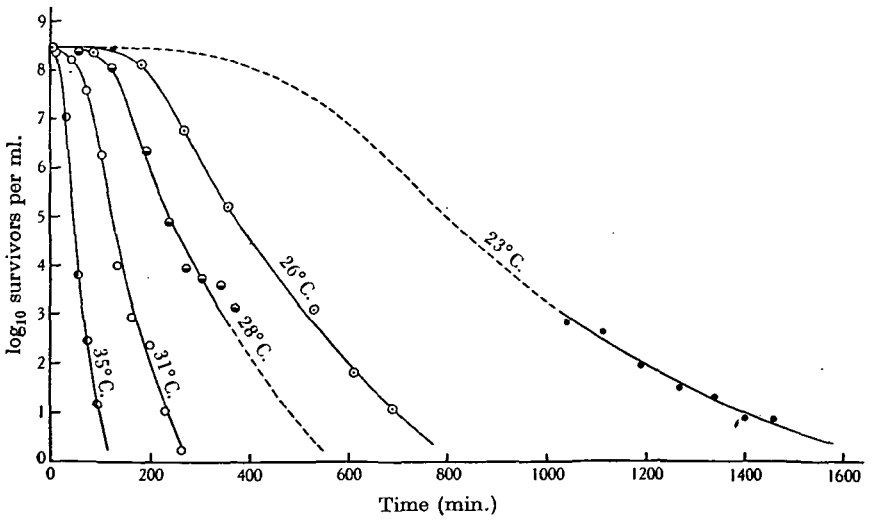

Fig. 3. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 6·01 g. phenol per litre at various temperatures.

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that the sampling technique changed radically, it may be concluded that the bacterial cells, after considerable exposure to phenol, were no longer uniform in their ability to form colonies. Evidence in support of this conclusion is not lacking. It is known that bacterial cells and spores which have survived drastic lethal agencies such as heat, ultra-violet radiation and mercuric chloride (Curran & Evans, 1937; Nelson, 1943), are more exacting in their nutrient requirements than the untreated bacteria. The former authors showed that the addition of growth-promoting substances to the medium for testing viability increased the number of cells surviving the treatment, and also that the effectiveness of the enrichment increased as the percentage mortality rose. Curran & Evans (1938) showed that bacterial spores which survived ultra-violet irradiation were more sensitive to heat than the non-irradiated spores. Accordingly, it may be suggested that in the present work each replicate sample in a count made towards the end of an experiment contained a proportion of sensitive cells that were no longer capable of developing on the plating medium but needed nutrient enrichment, or that succumbed to the mild degree of heating involved in the addition of the melted agar. In practice, the proportion of sensitive cells in the replicate samples will not be constant because of sampling variation, and in

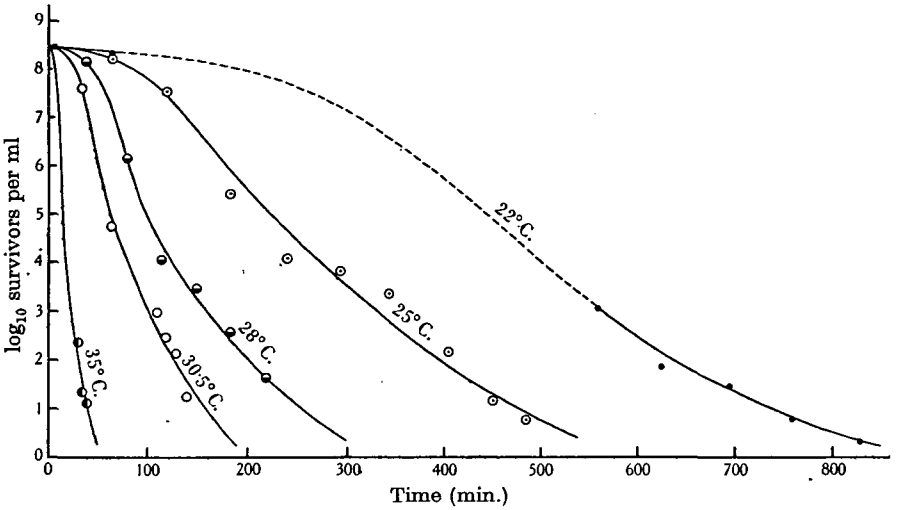

Fig. 4. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 6·98g. phenol per litre at various temperatures.

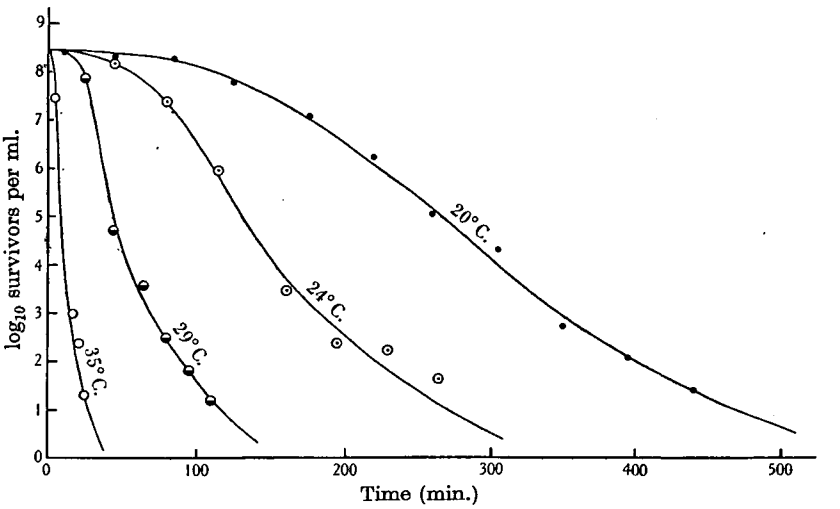

Fig. 5. Showing relationship between logarithm of survivors and time for *Bact. coli* when exposed to 7·95g. phenol per litre at various temperatures.

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extreme cases all or none of the cells in the sample could be sensitive. The number of colonies on a plate could never exceed the 'true' value but could be lower according to the proportion of sensitive cells present. Any such heterogeneity in the viability of the organisms would increase χ^2 because it is superimposed on the sampling variation. The effect of this would be expected to show most prominently towards the end of a disinfection when many of the cells might be sensitive. It is not contended that as to lead to the belief that the observed counts are not very inaccurate. Also, it may be argued that if almost dead cells are the cause of the discrepancy they would in any case have been quite dead very shortly and the effect of the low counts may be regarded as equivalent to small errors in the timing of the counts.

If the straight lines whose formulae are given in Table 2 are drawn through the points plotted in Figs. 1–5 (these lines are not shown in the figures in

Table 2. *The calculated relationship between log survivors and time assuming a constant maximum death-rate*

Phenol conc. g./l.	Temp. °C.	Regression formula $\log_{10} S = \overline{\log_{10} S} + b\,(t-\bar{t})$*	Standard error of $\overline{\log_{10} S}$	Standard error of b	Ratio of b to its standard error	No. of experimental points used in calculation of line	Maximum value of $\log_{10} S$
4·62	30	$\log_{10} S = 4{\cdot}6227 - 0{\cdot}007028\ (t - 1583{\cdot}00)$	±0·1510	±0·0005623	13	5	6·8363
	32·5	$5{\cdot}7876 - 0{\cdot}01187\ (t - 700{\cdot}00)$	0·0484	0·0005274	23	5	7·1212
	35	$4{\cdot}3710 - 0{\cdot}02540\ (t - 317{\cdot}00)$	0·1723	0·002083	12	5	7·5759
	38	$4{\cdot}3197 - 0{\cdot}04080\ (t - 185{\cdot}00)$	0·1225	0·002042	20	7	8·0457
	39·5	$3{\cdot}6904 - 0{\cdot}03333\ (t - 167{\cdot}50)$	0·2401	0·002840	12	6	8·3342
	42	$3{\cdot}5184 - 0{\cdot}08282\ (t - 76{\cdot}25)$	0·3796	0·01129	7	4	8·1756
5·20	27	$2{\cdot}7133 - 0{\cdot}009228\ (t - 1252{\cdot}86)$	0·0844	0·0004695	20	7	6·0603
	31	$5{\cdot}3335 - 0{\cdot}01666\ (t - 444{\cdot}17)$	0·1393	0·001735	10	6	7·3514
	35	$4{\cdot}1824 - 0{\cdot}03008\ (t - 186{\cdot}43)$	0·2656	0·003227	9	7	8·2650
	39	$4{\cdot}0095 - 0{\cdot}08578\ (t - 58{\cdot}00)$	0·3466	0·009779	9	5	8·3187
6·01	23	$1{\cdot}5259 - 0{\cdot}004779\ (t - 1308{\cdot}75)$	0·0594	0·0003187	15	8	2·8762
	26	$4{\cdot}3612 - 0{\cdot}01385\ (t - 442{\cdot}50)$	0·1000	0·0005443	25	6	8·1113
	28	$5{\cdot}2693 - 0{\cdot}01985\ (t - 243{\cdot}75)$	0·1652	0·001736	11	8	8·3678
	31	$4{\cdot}0903 - 0{\cdot}03815\ (t - 152{\cdot}50)$	0·1848	0·002575	15	8	8·1953
	35	$4{\cdot}5844 - 0{\cdot}08470\ (t - 57{\cdot}00)$	0·2162	0·006782	12	5	8·4267
6·98	22	$2{\cdot}6214 - 0{\cdot}01074\ (t - 587{\cdot}50)$	0·0988	0·0003960	27	6	8·3187
	25	$4{\cdot}0634 - 0{\cdot}01751\ (t - 287{\cdot}78)$	0·1493	0·001077	16	9	8·2269
	28	$4{\cdot}3349 - 0{\cdot}03539\ (t - 131{\cdot}67)$	0·2401	0·003936	9	6	8·1724
	30·5	$3{\cdot}5211 - 0{\cdot}05510\ (t - 100{\cdot}00)$	0·1833	0·001886	29	6	7·5721
	35	$3{\cdot}3298 - 0{\cdot}1941\ (t - 26{\cdot}25)$	0·2083	0·01338	15	4	8·5211
7·95	20	$4{\cdot}5999 - 0{\cdot}02142\ (t - 283{\cdot}75)$	0·0868	0·0008504	25	8	7·7850
	24	$4{\cdot}4591 - 0{\cdot}03243\ (t - 155{\cdot}71)$	0·2478	0·003333	10	7	8·1818
	29	$3{\cdot}6040 - 0{\cdot}07469\ (t - 70{\cdot}00)$	0·2915	0·01010	7	6	7·8809
	35	$3{\cdot}5381 - 0{\cdot}3111\ (t - 17{\cdot}00)$	0·2220	0·02967	10	4	7·4664

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

these considerations afford a complete explanation for the abnormal distribution of χ^2, but it is satisfactory to know that a reasonable explanation of the apparent deterioration of a sound technique can be advanced. In the absence of any knowledge of the true form of the distribution of the numbers of colonies on the plates of a count made in such conditions, the arithmetic mean has been used as before as the basis for calculation. If the explanation advanced is correct, then the observed counts must be lower than the 'true' values, and by an unknown amount, but the nature of the explanation is such order to avoid confusion), it becomes evident that the deviations of the points from the straight lines tend to be systematic. The points in the upper regions are mainly above, those in the middle regions below, and those in the lower regions again above the lines. This implies that the death-rates in the cultures must have risen above, and finally fallen below, the values given by the slopes of these regression lines, which have hitherto been regarded as fixed maxima. If the death-rates calculated between successive points in each experiment (Table 1) are examined, it will be seen that after rising to a

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high value they appear to decline but often fluctuate considerably, so that the general tendency to decline is sometimes obscured. This fluctuation is to be expected on account of the error to which any plate count, however satisfactory, is subject. The purpose of the analysis which follows is to group the data so as to compensate for these fluctuations and to reveal more clearly the sequence of changes in the death-rates throughout the disinfections.

It is clear that the general course of the disinfection was the same in each experiment. There was always a period of low and a period of high death-rate. When each experiment is expanded in its time scale to a constant length and the data replotted, the new curves lie, very roughly, over one another. The correspondence is far from exact but sufficiently close for the present purpose of grouping the data in order to reveal changes in the death-rates. Naturally, any marked lack of correspondence would tend to obscure such changes. It has not been considered advisable to present a figure to illustrate this point, as it would necessitate a complicated diagram which would not reproduce well. Comparison can be made, however, of the groups of curves in Figs. 1–5 in which the time of the longest experiment at each phenol concentration has been expanded approximately to the same length in the process of drawing the diagrams. For the purpose of grouping the data the procedure actually adopted was to take the longest experiment of all as the standard. This has a lowest value for log survivors of 1·1461 at 2040 min. In all other experiments the time corresponding to the value of log survivors nearest to 1·1461 was divided into 2040 and the resulting factor used to multiply all the times in that experiment. In the three cases in which there was no suitable value near 1·1461 a different point of comparison was used. From these standardized times the adjusted death-rates between successive observations were calculated. To facilitate subsequent grouping, the adjusted death-rates (× 1000 for convenience) were then plotted as a scatter-diagram against the mid-points of the corresponding standardized time intervals. On this diagram data derived from the previously published experiments at 35° C. were included, making a total of 226 points. Groups were then made at intervals of two units of adjusted death-rate and 200 min. of standardized time up to 2000 min., the remaining points being placed in one group extending from 2000 to 2400 min. on account of the small number of cases involved. The frequencies obtained from this scatter-diagram are shown in Table 3, which includes the mean values of the adjusted death-rates in each interval of standardized time with their standard errors. These mean values were calculated on the assumption that all cases in a group had an adjusted death-rate equal to the mid-value for the group. It is clear that the mean adjusted death-rate rises from a very low value to a maximum and then declines, and it appears that the decline is rapid at first and then slow. Fig. 6, in which the mean adjusted death-rates are plotted against the corresponding mid-points on the standardized time scale, illustrates this fact. The upper and lower interrupted lines in the graph have been obtained by plotting the mean adjusted death-rates plus and minus twice their standard errors in order to give a visual representation of the accuracy with which each mean has been fixed. Great accuracy cannot obviously be expected from this method, in view of the fact that the standardized curves do not exactly match. The minor peak in Fig. 6 at 700 min. is probably due to this cause, since the highest death-rate tends to occur relatively more early in the disinfection when the whole process is rapid. Also, the decline after the peak is much less marked in the longer experiments, and this must have tended to obscure the final decline in the adjusted death-rate. Reference has been made above to the fact that in some experiments there appeared to be an initial rush in the disinfection. With the coarse grouping used in Table 3 and Fig. 6 the evidence for this is slight, but if a finer grouping in single units of adjusted death-rate and 100 min. of the standardized time scale be employed the evidence becomes more convincing. From 0 to 100 min. the mean adjusted death-rate is 1·45, while from 100 to 200 min. it is only 0·5. It is not proposed to stress this point further at the present time, since the possibility exists that the initial rush may be due to local accumulations of phenol at high concentrations. The higher the desired final concentration the more rapidly the 5% phenol solution has to be run in, since a standard time of 5 min. is allowed for this operation. However, the possibility that all the initial rush could be accounted for in this way is regarded as remote. This initial high death-rate, being followed by a period of lower death-rate, differs from that recorded by Chick (1930) where the death-rate decreased continuously until the end of the disinfection.

A general picture of the changes in the death-rate during the disinfection of these cultures thus emerges. After a short period in which there may be a relatively high death-rate, the latter is low for a time and then increases rapidly to a peak from which it declines, sharply at first and then more slowly. The curves in Figs. 1–5 have been drawn in conformity with this pattern except that the initial rush cannot be shown. Chick (1930) cites several instances of this type of curve and, more recently, Hobbs & Wilson (1942) have again observed the final decline in death-rate.

Evidently, in view of these findings, the straight lines of regression shown in Table 2 and based on an assumed constant maximum death-rate will not

Table 3. *Showing the grouping of the adjusted death-rates in relation to the standardized time scale (see text)*

Adjusted death-rate (× 1000)	Standardized time scale (min.) 0	200	400	600	800	1000	1200	1400	1600	1800	2000	2400
14	—	—	—	—	—	—	—	—	—	—	—	
12	—	—	—	—	—	—	—	1	—	—	1	
10	—	—	—	2	—	2	2	1	—	1	0	
8	—	—	—	3	2	7	3	4·5	5·5	4	0	
6	1	—	3	5	7	5	5	1·5	8·5	9	1	
4	5	1	7	2	8	2·5	9·5	2	8	10	4	
2	29	17·5	12·5	6	2	0	·3	5	3	2	2	
0												
Totals	35	18·5	22·5	18	19	16·5	22·5	15	25	26	8	
Mean adjusted death-rate	1·40	1·11	2·16	4·22	3·95	6·03	4·24	4·67	4·32	4·38	3·75	
Standard error of mean	0·16	0·11	0·31	0·67	0·37	0·45	0·49	0·86	0·39	0·38	1·13	

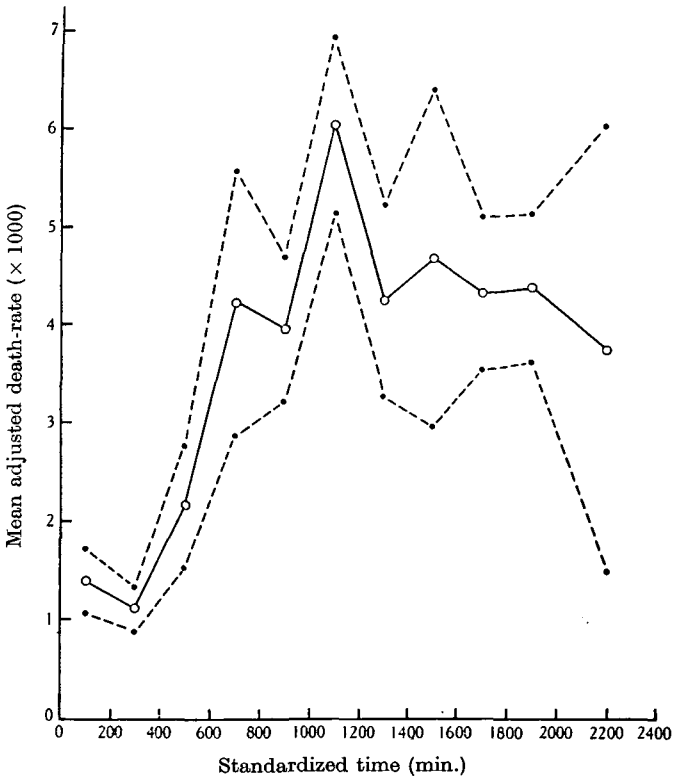

Fig. 6. Showing relationship between mean adjusted death-rate and standardized time for *Bact. coli* when exposed to phenol (see text).

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lead to the best estimates of the times required to attain various degrees of mortality. In particular, the virtual sterilization times (*v.s.t.*'s, corresponding to a mortality of 99·999999 %, the use of which was earlier advocated by the present authors) calculated from them will tend to be low. It is hoped that eventually a formula will be found which will fit accurately these mortality curves, but pending the elucidation of such a formula a better approximation than the method of Table 2 may be used to give an improved calculated estimate of the *v.s.t.* It has been shown above that after the rapid decline in the death-rate following the peak a lower and nearly constant value is attained. Little error from the true value of the *v.s.t.* will be introduced, therefore, if the data are treated as if the death-rate had become constant at this lower value, and the regression lines whose formulae are given in Table 4 have been calculated on this basis. Naturally, the number of points available for the calculation of each of these new lines is less than were available for calculation of the regressions when the death-rate was assumed constant at a maximum value, and the standard errors are, on the whole, relatively larger. But the effect of the reduction in the number of points available has been offset to some degree by an improvement in fit so that the ratios of the regression coefficients to their standard errors are still in most cases reasonably high. There are four experiments in which the *v.s.t.* is greater than 1000 min. In three of these the regression line is the same when calculated by either method and in the fourth the slope has decreased only very slightly.

Table 4. *The calculated relationship between log survivors and time assuming a final low constant death-rate*

Phenol conc. g./l.	T °C.	Regression formula $\log_{10} S = \overline{\log_{10} S} + b\,(t-\bar{t})$*	Standard error of $\overline{\log_{10} S}$	Standard error of b	Ratio of b to its standard error	No. of experimental points used in calculation of line	Maximum value of $\log_{10} S$
4·62	30	$\log_{\ldots}$ 028 $(t-1583{\cdot}00)$	±0·1510	±0·0005623	13	5	6·8363
	32·5	5·7876 − 0·01187 $(t-700{\cdot}00)$	0·0484	0·0005274	23	5	7·1212
	35	2·8026 − 0·01921 $(t-375{\cdot}00)$	0·1675	0·003420	6	3	3·8367
	38	1·8484 − 0·02712 $(t-245{\cdot}00)$	0·1119	0·004568	6	3	2·7412
	39·5	1·7740 − 0·02170 $(t-221{\cdot}25)$	0·1380	0·003203	7	4	2·9117
	42	1·9660 − 0·05507 $(t-93{\cdot}33)$	0·2325	0·01263	4·4	3	3·1142
5·20	27	2·1554 − 0·008252 $(t-1309{\cdot}17)$	0·0652	0·0005236	16	6	3·7096
	31	4·4929 − 0·01341 $(t-490{\cdot}00)$	0·1804	0·003227	4	4	5·7710
	35	2·2080 − 0·01797 $(t-247{\cdot}50)$	0·0259	0·0005143	35	4	3·4544
	39	1·6205 − 0·06227 $(t-83{\cdot}33)$	0·2626	0·01426	4·4	3	3·2382
6·01	23	1·5259 − 0·004779 $(t-1308{\cdot}75)$	0·0594	0·0003187	15	8	2·8762
	26	2·8185 − 0·01267 $(t-550{\cdot}00)$	0·0697	0·0005687	22	4	5·2148
	28	3·8787 − 0·01123 $(t-308{\cdot}00)$	0·1116	0·002319	5	5	4·8954
	31	1·6736 − 0·02805 $(t-215{\cdot}00)$	0·1395	0·003779	7·5	4	2·9504
	35	2·4984 − 0·06489 $(t-78{\cdot}00)$	0·0049	0·0003031	214	3	3·7997
6·98	22	1·0929 − 0·007820 $(t-726{\cdot}25)$	0·0526	0·0006960	11	4	1·8451
	25	2·5654 − 0·01454 $(t-370{\cdot}00)$	0·1347	0·001573	9	6	4·0781
	28	2·9210 − 0·02317 $(t-167{\cdot}50)$	0·0668	0·001706	14	4	4·0342
	30·5	2·7109 − 0·04426 $(t-113{\cdot}00)$	0·0983	0·003783	12	5	4·7366
	35	1·6024 − 0·1257 $(t-35{\cdot}00)$	0·1981	0·04853	2·6	3	2·3711
7·95	20	2·1215 − 0·01516 $(t-395{\cdot}00)$	0·0326	0·0008859	17	3	2·8267
	24	2·4291 − 0·01626 $(t-212{\cdot}50)$	0·1351	0·003452	5	4	3·4710
	29	1·8175 − 0·04337 $(t-95{\cdot}00)$	0·0129	0·001051	41	3	2·4771
	35	2·2304 − 0·2127 $(t-21{\cdot}00)$	0·1110	0·03399	6	3	3·0029

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

In four other experiments of similar length previously reported (Jordan & Jacobs, 1944*a*), there was similarly no possible alteration of the regression line. In the remaining twenty experiments in Tables 2 and 4 the new regression line is markedly less steep than the old, usually to the extent of about 30 %. On the new basis, as on the old, the slopes of the regression lines increase with rising temperature at constant phenol concentration and also with rising concentration at constant temperature. In Table 5 the *v.s.t.*'s calculated by both methods are compared and, clearly, where a change has occurred the new method has resulted in an

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increase. The new values are preferred to the old because they have been obtained by a method which takes some account of the changes in the death-rate towards the end of a disinfection. The *v.s.t.*'s have not all been altered to the same degree. Long experiments are practically unaffected. Amongst the others the increases are least at the lowest phenol concentration. At all other concentrations the experiments of moderate length have been affected most. In Table 5 there are also given the *v.s.t.*'s read from the freehand curves of Figs. 1–5 which *v.s.t.*'s calculated by the older regression method have been used (Jordan & Jacobs, 1944*b*) for the calculation of a concentration exponent for phenol at 35°C. The use of the *v.s.t.*'s calculated by the new method gives a value of $5{\cdot}7602 \pm 0{\cdot}1916$, which does not differ significantly from that of $5{\cdot}8421 \pm 0{\cdot}1876$ obtained previously. The slight decrease is due to the fact that *v.s.t.*'s below 1000 min. (4·25 g. phenol/l. and over) have been increased, while those above 1000 min. are unaltered, but as a result of the decrease the experimental value for the *v.s.t.* at

Table 5. *Comparison of virutal sterilization times obtained by three methods of assessment*

Phenol conc. g./l.	Temp. °C.	*v.s.t.* assuming constant maximum death-rate (*A*)	*v.s.t.* assuming constant final low death-rate (*B*)	Difference *B* − *A*	*v.s.t.* from free-hand curve (*C*)	Difference *C* − *B*
4·62	30	2165 ± 51·27	2165 ± 51·27	0	2230	+65
	32·5	1146 ± 20·22	1146 ± 20·22	0	1190	+44
	35	470·0 ± 14·10	494·3 ± 22·96	+ 14·3	525	+31
	38	278·1 ± 5·54	293·9 ± 9·21	+ 15·8	310	+16
	39·5	264·2 ± 10·09	279·1 ± 10·65	+ 14·9	275	− 4
	42	112·3 ± 6·72	119·4 ± 7·32	+ 7·1	120	+ 1
5·20	27	1490 ± 15·13	1506 ± 14·80	+ 16·0	1540	+34
	31	734·0 ± 28·99	787·3 ± 72·80	+ 53·3	820	+33
	35	308·0 ± 15·80	341·1 ± 3·04	+ 33·1	360	+19
	39	98·8 ± 6·16	101·2 ± 5·88	+ 2·4	105	+ 4
6·01	23	1520 ± 18·77	1520 ± 18·77	0	1550	+30
	26	721·9 ± 13·15	733·7 ± 9·92	+ 11·8	760	+26
	28	484·4 ± 22·64	609·6 ± 63·04	+125·2	520	−90
	31	246·1 ± 7·96	256·2 ± 7·45	+ 10·1	260	+ 4
	35	102·0 ± 4·60	107·8 ± 0·15	+ 5·8	110	+ 2
6·98	22	783·7 ± 11·70	800·4 ± 9·42	+ 16·7	800	0
	25	491·4 ± 15·15	512·2 ± 17·96	+ 20·8	524	+12
	28	240·6 ± 13·89	272·9 ± 8·27	+ 32·3	290	+17
	30·5	154·6 ± 5·88	162·7 ± 4·80	+ 8·1	180	+17
	35	41·0 ± 1·50	43·6 ± 3·68	+ 2·6	45	+ 1
7·95	20	474·8 ± 8·60	501·5 ± 6·58	+ 26·7	510	+ 8
	24	277·0 ± 14·62	329·5 ± 26·20	+ 52·5	300	−30
	29	109·5 ± 6·78	124·4 ± 0·77	+ 14·9	135	+11
	35	27·0 ± 1·20	28·9 ± 1·37	+ 1·9	34	+ 5

are presumably nearer to the true *v.s.t.*'s but lack the objective precision of calculated values. These curves have been drawn on the assumption that their slopes should continually decrease instead of falling to constant levels after the peaks although the decrease is very slow towards the end of the disinfection. The result has been to give *v.s.t.*'s which are, in all cases except three, even larger than those obtained by the modified regression method. Since, however, the actual rate at which the death-rate declines at the end of the disinfection cannot be determined with certainty from the data available, the new regression method is regarded at the present as the method of choice for giving an estimate of the true *v.s.t.*

3·48 g./l. is even more in excess of the calculated value than before. This condition was attributed to the approach of this concentration to the threshold value for phenol. If this determination be omitted, the value for the concentration exponent at 35°C. based on the new estimates of the *v.s.t.*'s becomes $5{\cdot}5877 \pm 0{\cdot}1599$ compared with $5{\cdot}6588 \pm 0{\cdot}1422$, and again the difference is not significant.

SUMMARY

1. Details are given of further experiments on the disinfection of *Bact. coli* cultures by phenol using an improved standardized technique. These

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were carried out at several temperatures at each of five phenol concentrations.

2. An excess of high values of χ^2 was again observed at high mortalities. Reasons for the occurrence of this phenomenon are discussed.

3. The logarithmic death-rates calculated between successive determinations of the surviving cells have been combined and analysed. Strong evidence was obtained that the death-rate rises to a maximum and then falls, sharply at first and then more slowly. There was some evidence for an initial rush in the death-rate.

4. The method hitherto adopted for treating these data, based on the assumption that the death-rate rises to a maximum at which it remains constant, has been shown to be a fair approximation but one which leads to low values for the *v.s.t.*'s.

5. Calculated estimates nearer to the true values for the *v.s.t.*'s have been obtained by treating the last phase of slow decline as one of constant death-rate. All values except those exceeding 1000 min. are increased by this change in method of calculation.

6. The new *v.s.t.*'s do not alter significantly the value previously obtained for the concentration exponent for phenol at 35°C.

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