STUDIES IN THE DYNAMICS OF DISINFECTION

VIII. THE EFFECT OF LETHAL TEMPERATURES ON STANDARD CULTURES OF *BACT. COLI*. I. A DETAILED ANALYSIS OF THE VARIATIONS OF DEATH-RATE WITH TIME

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(With 4 Figures in the Text)

Previous papers in this series have dealt with the action of phenol on standard cultures of *Bact. coli* under rigidly controlled conditions (Jordan & Jacobs, 1944, 1945). It was shown that the logarithmic death-rate throughout each disinfection process was not constant but varied with time, rising to a maximum from which it fell at first sharply and then more slowly. When the reaction was accelerated by the use of higher temperatures or greater concentrations of phenol these variations from constancy became less conspicuous, largely owing to the technical difficulty of obtaining, in short experiments, sufficient data of the necessary accuracy relating to the first 50 % of the mortality. Much of the work which had previously led to the belief, commonly held, that bacteria exposed to lethal agencies of all kinds show constant logarithmic death-rates has consisted of short experiments with relatively high concentrations of germicides and small bacterial populations, and clear proof that the death-rate is constant in the early as well as in the later stages of a disinfection has not been forthcoming. In order to account for a supposedly constant death-rate it has been necessary to postulate that all the cells in a bacterial culture are essentially identical in their properties (Chick, 1930), and further that only a single vital molecule in each cell needs to be damaged or inactivated for the cell to be rendered incapable of division (Rahn, 1943). These postulates are unsatisfactory if only because they would make bacteria unique among living things if all the cells of a culture were exactly alike and exhibited no gradation in properties, e.g. in respect of the rate of penetration of germicides. But if the idea of a constant death-rate be abandoned, then the anticipated variations between individual cells no longer constitute a problem of interpretation. Where chemical disinfection is concerned variations in the rate of penetration of the lethal agent into the cell are to be expected, and these might play an important part in determining the shape of the survivor curve. In the case of a lethal agent such as heat, penetration may be assumed to be virtually instantaneous and, accordingly, the survivor curves in heat disinfection might be expected to differ from those obtained in a chemical disinfection. An investigation of the course of disinfection by heat of standard whole cultures of *Bact. coli* therefore promised to yield useful information. The results reported below enable a direct comparison to be made of the effects of heat with those of phenol, since similar standard populations of bacteria have been employed in both cases.

EXPERIMENTAL

The apparatus and methods used were the same as those employed previously for phenol disinfection (Jordan & Jacobs, 1944), except for modifications made necessary by the need to raise the 1450 ml. of standard culture rapidly from its growth temperature of 35° C. to the lethal temperature to be used, which ranged from 47 to 55° C. in different experiments. The temperature of the water-bath was raised by siphoning water rapidly from the bath and replacing it with hot water, and the thermostat readjusted. This procedure raised the temperature of the culture but not with sufficient rapidity for the purpose in view, which was, of course, to raise the temperature of the culture in the shortest possible time without involving the risk of any local overheating. At the same time as the bath temperature was being adjusted, therefore, the contents of the flask were further warmed by means of a stream of steam-heated air. Filtered air was admitted through the upper orifice of the side-neck *F* of the culture flask, a lettered diagram of which was given in the first paper of this series (Jordan & Jacobs, 1944). At the same time steam was conducted into the same

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neck by means of the side-tube *H* which was connected to a flask of water kept gently boiling on an electric hot-plate. Air and steam were made to mix thoroughly in the tube *E* before reaching the culture, so that only warmed moist air actually came into contact with it and not the steam. The danger of causing the death of a proportion of the cells through local overheating was thus minimized. A means of reading the actual temperature of the culture was provided by using a culture flask having an extra (fourth) side-neck into which was fitted a very thin glass tube sealed at its lower end where it dipped into the culture fluid. Into this tube was placed a thermometer, with its bulb surrounded by mercury, which was separated from the culture only by the very thin wall of the glass tube. Rapid conduction of heat between the culture and thermometer was thus assured without exposing the culture to the risk of contamination, and the rise in temperature of the culture could be followed closely. The steam flow was regulated to bring the culture to the desired temperature, when the steam was cut off and the temperature was thereafter kept constant by the thermostatic water-bath. The whole operation of raising the culture temperature occupied 5–10 min., and the disinfection was deemed to have begun at the moment that the desired temperature was attained.

All the experiments reported below were carried out at *p*H 7·0.

RESULTS AND DISCUSSION

The results obtained in ten experiments at eight temperatures ranging from 47 to 55° C. are given in Table 1. Inspection of the data reveals at once that temperatures of 53° C. or lower were insufficient to kill all the cells of the standard culture. This was possibly also true of 55° C., but in this case the experiment was not prolonged sufficiently to make certain. In Fig. 1 the data for two typical experiments (at 50 and 52° C.) are shown graphically, and it is obvious that, following the phase of rapid decrease in numbers of surviving cells, each culture passed into a condition where there was a small surviving population which persisted for a long time and, as far as could be seen, would have persisted indefinitely. In the experiments at 52 and 53° C., for example, observations were continued for a period ten times as long as the phase of declining numbers, but without any indication that these survivors would eventually disappear. Other workers have observed the occurrence in bacterial cultures of a proportion of cells with an abnormally high heat resistance (Rahn, 1945), and the importance of these resistant cells is obviously very great. In the present instance the bacteria were heated in a growth medium, and this may be the reason for the apparently indefinite survival of these cells. It is proposed to discuss the nature of this surviving population more fully in a later communication, but it may be pointed out here that the numbers of these permanent survivors apparently fluctuated considerably from one observation to another in the same experiment. It is not certain whether this is a real fluctuation or not, as the magnitudes of the surviving populations in these experiments cannot be stated with certainty, since the counts made on cultures in which so great a mortality had occurred showed a tendency to excessive variation between replicate plates. This situation recalls the experience previously recorded with phenol-treated cultures (Jordan & Jacobs,

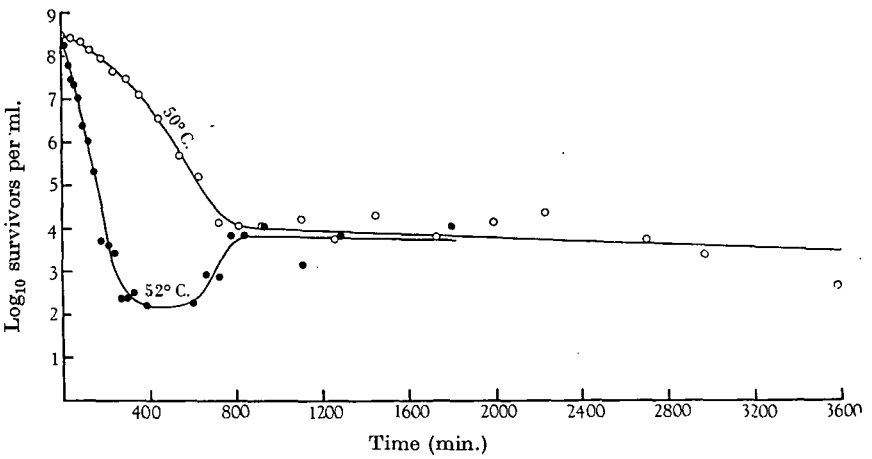

Fig. 1. Showing the relationship between logarithm of survivors and time for *Bact. coli* when exposed to lethal temperatures at *p*H 7·0.

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Table 1. *Numbers of survivors in* Bact. coli *cultures exposed to various degrees of heat*

Temp. ° C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$	Temp. ° C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$	Temp. ° C.	Time min.	Survivors per ml. No.	Survivors per ml. $\log_{10}$
47	0	358,300,000	8·5543	50	0	297,600,000	8·4737	52	0	325,100,000	8·5120
	95	313,500,000	8·4962		45	263,200,000	8·4203		15	171,300,000	8·2337
	425	261,000,000	8·4166		90	206,000,000	8·3139		30	62,760,000	7·7977
	1100	96,770,000	7·9857		135	135,300,000	8·1313		45	28,840,000	7·4600
	1280	91,160,000	7·9598		180	88,440,000	7·9467		60	22,030,000	7·3430
	1400	77,960,000	7·8918		240	43,730,000	7·6408		80	10,530,000	7·0224
	1550	79,220,000	7·8988		300	29,580,000	7·4710		100	2,502,000	6·3982
	2105	51,910,000	7·7153		360	12,230,000	7·0874		120	1,122,000	6·0500
	2555	33,740,000	7·5281		450	3,538,000	6·5488		150	218,900	5·3403
	2735	19,630,000	7·2930		545	495,300	5·6949		180	5,018	3·7005
	2990	13,700,000	7·1367		630	158,100	5·1990		210	3,981	3·6000
	3995	470,900	5·6729		720	13,620	4·1341		240	2,759	3·4407
	4160	251,500	5·4006		820	10,990	4·0411		270	246	2·3909
	4385	161,600	5·2084		925	10,850	4·0355		300	246	2·3909
	5435	16,390	4·2146		1105	16,270	4·2113		330	320	2·5051
	5600	5,581	3·7467		1260	5,661	3·7529		390	164	2·2148
	5870	10,620	4·0261		1450	19,830	4·2974		600	180	2·2553
	6860	246	2·3909		1725	6,225	3·7941		660	845	2·9269
	7070	25,100	4·3997		1995	13,700	4·1367		720	758	2·8797
	9755	13,160	4·1192		2235	22,950	4·3608		780	6,904	3·8390
	10025	1,620	3·2095		2700	5,529	3·7426		840	6,536	3·8153
	10145	1,150	3·0607		2965	2,508	3·3993		930	10,989	4·0411
					3585	4,731	2·6750		1110	1,415	3·1507
									1290	6,285	3·7983
48	0	364,700,000	8·5619						1800	10,650	4·0274
	50	310,700,000	8·4924	51 (*a*)	0	293,200,000	8·4672				
	200	287,000,000	8·4579		15	289,300,000	8·4614	53 (*a*)	0	386,700,000	8·5874
	305	246,100,000	8·3911		35	187,500,000	8·2730		30	64,870,000	7·8121
	500	159,000,000	8·2014		55	81,610,000	7·9118		60	5,098,000	6·7074
	620	136,300,000	8·1345		85	43,840,000	7·6375		105	14,710	4·1676
	1370	18,770,000	7·2734		115	33,080,000	7·5195		140	7,293	3·8629
	1520	13,520,000	7·1309		145	17,600,000	7·2455		300	1,647	3·2166
	1640	6,731,000	6·8281		175	9,318,000	6·9693		360	2,814	3·4493
	1775	2,853,000	6·4553		205	2,424,000	6·3845		420	1,361	3·1338
	2825	11,570	4·0633		235	1,271,000	6·1041		510	1,619	3·2092
	3080	1,250	3·0969		265	398,400	5·6003		695	732	2·8645
	4385	1,198	3·0785		295	187,900	5·2739		780	6,492	3·8123
	7140	1,080	3·0334		355	35,100	4·5453		870	2,162	3·3349
	7470	2,050	3·3118		385	12,020	4·0799		1010	6,416	3·8073
					415	5,486	3·7393		1110	217	2·3365
									1500	126	2·1004
49	0	330,600,000	8·5193						1740	704	2·8476
	60	296,200,000	8·4716	51 (*b*)	0	355,900,000	8·5513				
	120	265,500,000	8·4240		60	112,800,000	8·0520	53 (*b*)	0	367,900,000	8·5658
	240	205,300,000	8·3124		130	25,040,000	7·3986		10	178,600,000	8·2519
	365	149,000,000	8·1732		180	6,090,000	6·7846		20	83,640,000	7·9224
	485	117,100,000	8·0686		310	163,200	5·2127		30	49,200,000	7·6920
	615	52,210,000	7·7178		410	28,850	4·4602		55	8,078,000	6·9073
	720	33,000,000	7·5185		600	855	2·9320		65	3,308,000	6·5195
	855	15,160,000	7·1807		780	1,082	3·0342		80	1,014,000	6·0060
	1030	2,019,000	6·3051		960	10,100	4·0043		100	132,800	5·1232
	1140	1,404,000	6·1473		1150	196	2·2923		120	14,180	4·1516
	1260	658,000	5·8182		1320	228	2·3579		145	1,435	3·1568
	1380	348,000	5·5416		1570	1,426	3·1541		175	262	2·4183
	1565	98,490	4·9934		1930	478	2·6794				
	1740	26,380	4·4213					55	0	334,600,000	8·5245
	1980	6,184	3·7913						10	4,838,000	6·6846
	2280	17,070	4·2321						20	12,240	4·0878
									35	1,352	3·1309
									50	1,435	3·1568
									65	246	2·3909
									80	115	2·0607

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1944), but the excessive variation was much less prevalent in the present instance. Only 15 % of counts made when the mortality was greater than 95 % were subject to it, and 4·5 % of counts made when the mortality was less than 95 %, as compared with 72 and 16 % respectively when phenol was used. Actually only 8 % of counts made when the mortality was below 99·7 % (*c.* 1 million survivors per ml.) showed excessive variation, and only 14·5 % of the counts made at higher mortalities, so the situation may be regarded as fairly satisfactory. It was suggested earlier (Jordan & Jacobs, 1945) that excessive variation in counts of bacteria exposed to phenol would arise if replicate samples, taken towards the end of an experiment when the mortality was high, contained a proportion, varying by chance from sample to sample, of cells which had been so damaged by the phenol that they were no longer capable of growth on the plating medium, or succumbed to the slight exposure to heat involved in the plating procedure. In the case of heat disinfection the latter point would be of very minor significance, the cells having been exposed previously to higher temperatures for a considerable time. Again, since permanent populations of heat-resistant cells became established, such cells could hardly be considered to be damaged. Most of the counts made at high mortalities during the heat disinfections were, therefore, not in fact made on damaged bacteria, and if the above suggestions cover the main causes of excessive variation this should not have been so prominent as when phenol was used.

In the phase of each experiment during which rapid death amongst the cells was occurring, the death-rate, as may be seen from Figs. 2 and 3, was not constant throughout the disinfection, but at four out of the eight temperatures used (i.e. at 47, 48, 51 and 52° C.) there was a strong tendency for the process to be divided into three phases. These are: (*a*) a short period of fairly constant death-rate followed by (*b*) a temporary marked decrease in death-rate, and finally (*c*) a longer period of constant death-rate which was appreciably higher than the initial rate. In the experiments at 49 and 53° C. there was a hint of the same three phases but at 50° C. there was no evidence of phase (*b*), as the initial phase appeared to merge gradually into the final phase. At the remaining temperature (55° C.) the data were insufficient to give a clear indication of the true shape of the curve. The fact that the final death-rate was higher than the initial rate parallels the condition encountered in the phenol disinfection of similar standard cultures (Jordan & Jacobs, 1945), but the situation differs, apart from the absence of a permanent residual population in the latter case, in that the variation in death-rate during each experiment appears to be less marked in the heat disinfections. Another point of interest is that, as with phenol, the divergence from a constant death-rate lessened as the speed of disinfection increased, i.e. as the temperature was raised. For technical reasons it is exceedingly difficult to obtain adequate data covering the first half of the population to succumb during a rapid disinfection, but it is believed, since the slower disinfections do show a marked tendency for the final death-rate to be higher than the initial rate, that this condition probably exists in the faster ones also, and that the true over-all shape of the logarithmic survivor curve is always concave downwards.

For the calculation of temperature coefficients and concentration exponents it is desirable to know the times at which, under various conditions, certain percentages of mortality occurred. These times may be read from freehand curves drawn through the data for any experiment, but it is felt that this subjective method should be avoided wherever possible and replaced by an objective method, by means of which the desired mortality times can be calculated and standard errors attached to them. Such calculations are greatly facilitated if a linear relationship can be found connecting mortality with time, and in this lies much of the attraction of the theory of a constant logarithmic death-rate. But since it is clear that in the present case the whole disinfection (i.e. the phase of decreasing numbers of survivors preceding the establishment of the permanent population) often did not proceed at a constant rate, it is hardly satisfactory to adopt that treatment for some of the experiments only. Such a procedure would militate against the objectivity of the treatment and would still leave half of the experimental data to be dealt with in some other way. It is considered advisable to use an objective method which would apply equally well to all cases.

In the treatment of the results of the experiments using phenol, a method was devised for combining all the data so as to obtain the true shape of the disinfection curve (Jordan & Jacobs, 1945), and from this a uniform procedure for handling the results was worked out. A similar method has been used in the present case and has proved successful. A time for a mortality greater than 99·99 % could not be used as a basis for combining the experiments owing to the existence of the permanent population, the peak value of which was 25,100 cells per ml. occurring in the experiment at 47° C., but usually the values obtained were much lower than this figure. The logarithm of this number is nearly 4·4, and as the logarithm of the survivors at 99·99 % mortality is close to 4·5 in all cases, it is clear that this mortality was about the highest that could be used with safety. For convenience the time corresponding to this mortality is called the disinfection time. The procedure adopted was as follows. First, freehand curves were drawn through the data for

each experiment, and from these curves approximate values for the disinfection times were read off. The experiment at 50° C. was taken as the standard for comparison, and a factor was obtained for each experiment by dividing its disinfection time into that for the standard experiment. Then, all times in each experiment at which observations had been made were multiplied by the appropriate factor so as to obtain the standardized times. The modified data for all the experiments were then plotted on a single graph, when it became clear that all the experiments had followed a similar course, since the

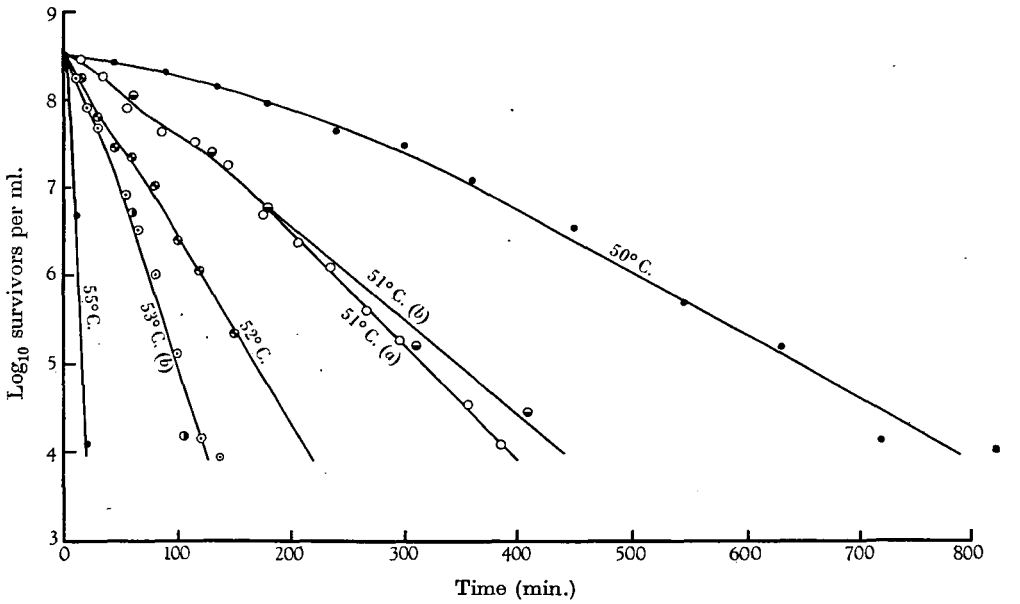

Fig. 2. Showing the relationship between the logarithm of survivors and time during the phase of active disinfection for *Bact. coli* when exposed to lethal temperatures at *p*H 7·0.

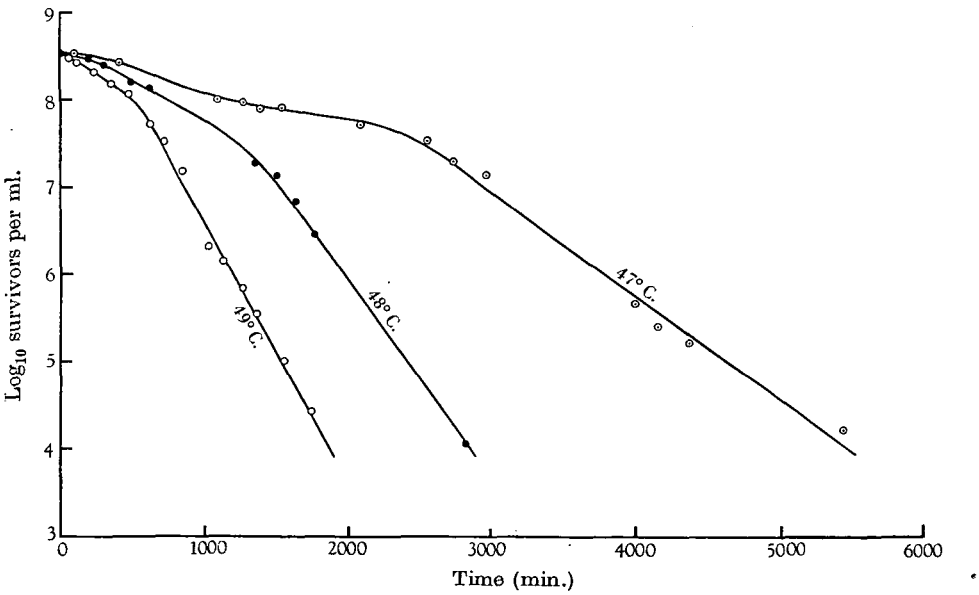

Fig. 3. Showing the relationship between the logarithm of survivors and time during the phase of active disinfection for *Bact. coli* when exposed to lethal temperatures at *p*H 7·0.

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new curves ran very close to or over one another through a narrow band. These standardized curves were then combined into a single one in the following way. For each experiment an adjusted death-rate between each pair of successive observations was calculated by dividing the decrement in the logarithm of the number of survivors by the interval on the standardized time scale. These adjusted death-rates ($\times 1000$ for convenience) were then plotted as a scatter diagram against the mid-points of the corresponding standardized time intervals. The diagram was then ruled off at intervals of two units of adjusted death-rate and 50 min. of standardized time, the latter scale being taken only as far as 750 min. in order to avoid including any data which really belonged to the stage of permanent population. The frequencies obtained by counting the points falling within the various ranges are shown in Table 2 together with the mean adjusted death-rate for each interval of standardized time, calculated by assuming that all cases in a group had an adjusted death-rate equal to the mid-value of the group. The attached standard errors have been obtained on the same basis. It appears that the mean adjusted death-rate was low at first but gradually increased until it reached a maximum value about half-way through the disinfection, and thereafter remained roughly constant until the 99·99% mortality time was reached. Subsequently the death-rate must have decreased again because of the establishment of the permanent population, but that feature is not at present under discussion. From the mean values in Table 2 a logarithmic survival curve applicable to all the experimental conditions can be constructed. This has been done by multiplying each mean adjusted death-rate by 50 to give the actual logarithmic decrements of survivors in the successive intervals of standardized time. The initial value of the logarithm of survivors has been taken as 8·533, which is the logarithm of the mean of the initial populations in all the experiments. The successive values of the logarithms of survivors were then plotted against the standardized time and the resulting curve is shown in Fig. 4. The variations in the mean adjusted death-rate from 300 min. onwards were relatively slight and irregular in occurrence, and the graph derived from the means may evidently be treated as linear between log survivor values of approximately 7·5 and 4·5 or possibly slightly lower. These figures correspond (approximately) to mortalities of 90 and 99·99% respectively.

Table 2. *Showing the grouping of the adjusted death-rates in relation to the standardized time scale (see text)*

Adjusted death-rate ($\times 1000$)	Standardized time scale (min.) 0	50	100	150	200	250	300	350	400	450	500	550	600	650	700	750
12	—	—	—	—	—	—	—	—	1	—	1	1	—	1	—	
10	—	1	—	—	—	—	1	2	1	2	2	0	1	0	—	
8	—	1	—	—	1	1	1	1	0	1	2	2	1	1	4	
6	4	3	4	3	1	6	2	0	4	1	0	3	1	2	0	
4	0	4	2	5	1	1	1	0	1	1	0	0	0	0	0	
2	6	0	0	1	0	1	0	0	0	0	0	0	0	0	0	
0																
Total	10	9	6	9	3	9	5	3	7	5	5	6	3	4	4	
Mean adjusted death-rate	2·60	4·78	4·33	3·44	5·00	4·56	5·80	8·33	6·14	6·60	8·60	6·67	7·00	7·00	7·00	
Standard error of mean	0·65	0·70	0·42	0·44	1·15	0·56	1·02	0·67	1·06	1·17	0·75	0·95	1·15	1·41	0·00	

In the light of this finding it was decided to treat each individual experiment as if it had conformed to the above pattern and to assume that between the values of 7·5 and 4·5 the regression of log survivors on time was linear. This provides an objective mathematical method for handling the data, the desirability of which was pointed out above. The formulae of the lines of regression were calculated in the usual way and are given in Table 3, together with the standard errors of their slopes. These errors are all quite small in relation to the magnitudes of the slopes, and thus the method of treatment appears to be justified. In Figs. 2 and 3, therefore, the straight lines drawn through the points within approximately the above log survivor limits are those whose regression values are given in Table 3. Only one line

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Table 3. *The calculated relationship between log survivors and time, assuming a constant maximum death-rate between approximately* 90 *and* 99·99 % *mortality*

Temp. ° C.	Regression formula* $\log_{10} S = \log_{10} S + b\,(t - \bar{t})$	Standard error of $\log_{10} S$	Standard error of b	Ratio of b to its standard error
47	$\log_{10} S = 6{\cdot}0649 - 0{\cdot}001205\,(t - 3750{\cdot}71)$	± 0·0547	± 0·00005660	21·3
48	$6{\cdot}3502 - 0{\cdot}002273\,(t - 1826{\cdot}00)$	± 0·0396	± 0·00007657	29·7
49	$5{\cdot}9908 - 0{\cdot}002981\,(t - 1211{\cdot}25)$	± 0·0414	± 0·0001277	23·3
50	$5{\cdot}7395 - 0{\cdot}007126\,(t - 546{\cdot}43)$	± 0·0792	± 0·0004503	15·8
51 (*a*)	$5{\cdot}9691 - 0{\cdot}012947\,(t - 241{\cdot}67)$	± 0·0254	± 0·0002928	44·2
51 (*b*)	$5{\cdot}9640 - 0{\cdot}010674\,(t - 257{\cdot}50)$	± 0·0846	± 0·0007703	13·9
52	$6{\cdot}6023 - 0{\cdot}021001\,(t - 92{\cdot}50)$	± 0·0472	± 0·0013280	15·8
53 (*a*)	$5{\cdot}6375 - 0{\cdot}038670\,(t - 83{\cdot}75)$	± 0·2768	± 0·0065835	5·9
53 (*b*)	$6{\cdot}0666 - 0{\cdot}039336\,(t - 75{\cdot}00)$	± 0·0534	± 0·0018148	21·7
55†	$6{\cdot}4323 - 0{\cdot}221835\,(t - 10{\cdot}00)$	± 0·1785	± 0·021849	10·2

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

† In this regression use is made of the logarithm of the initial population (see text).

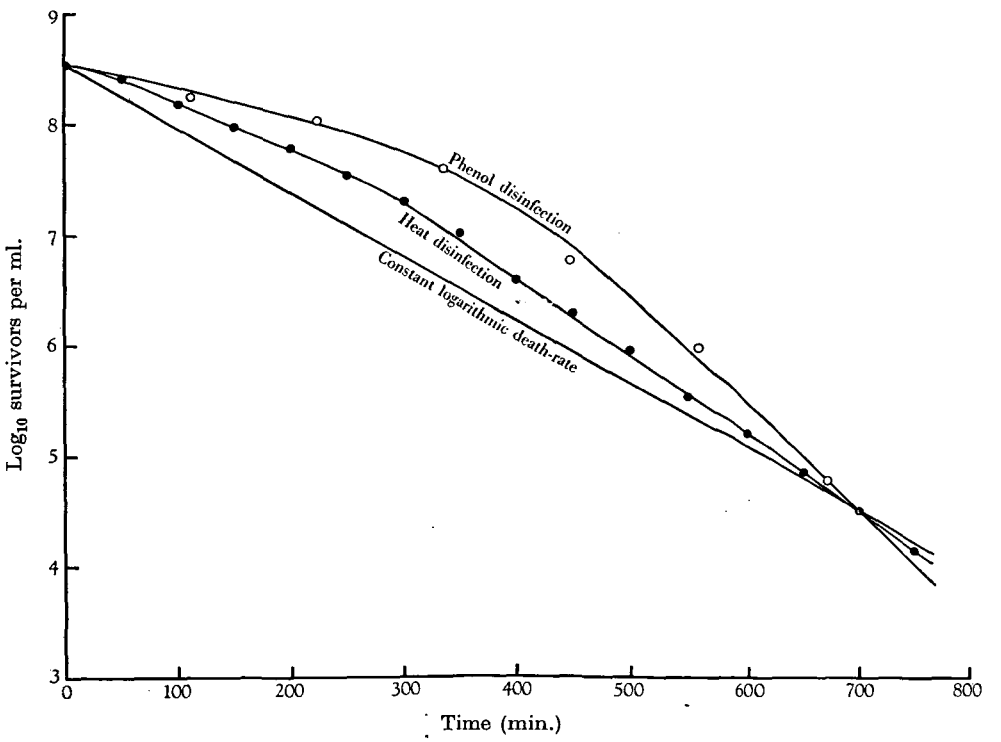

Fig. 4. Showing idealized log survivor-time curve for heat disinfection with the corresponding idealized curve for phenol disinfection and the theoretical line for a constant logarithmic death-rate. All curves adjusted to the length of the experiment at 50° C. (see text).

is shown for 53° C., that for experiment (*b*) in Tables 1 and 3, as the two lines for that temperature practically coincide. It is noteworthy that in some experiments, particularly at the higher temperatures, the linear regression of log survivors on time appears to have continued well below the limit of log 4·0–4·5 imposed for calculating the regressions, before the permanent populations appeared. However, only the points actually used in the calculations are shown in the figures.

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In the experiment at 55° C. there were only two points within the limits set for calculating the regressions, and in this case, therefore, the logarithm of the initial value of the population was included with the sole object of enabling a regression to be calculated. That the regression coefficient thus obtained has such a small error relative to its magnitude, although based on three values only, affords a good illustration of the point made above that the departure from a strictly constant logarithmic death-rate becomes impossible to detect when the disinfection is very rapid. This departure is also much more difficult to detect in disinfections by heat than with phenol, since the divergences from a strictly constant death-rate are so much less marked in the former than in the latter case. To emphasize this the composite logarithmic survivor curve for phenol and the corresponding line of constant logarithmic death-rate have also been drawn in Fig. 4. The difference in the curvatures in the two cases is easily apparent, and its existence may be considered to afford support for the hypothesis that in phenol disinfections variation in ease of penetration of the germicide into the cells of the bacterial population employed or variations of other physical properties among the cells may be of importance in determining the shape of the logarithmic survivor curve.

From the mean adjusted death-rates shown in Table 2 and the composite curve in Fig. 4 there is some evidence for the temporary decline in death-rate before the final constant rate is attained, i.e. phase (*b*) above, which had been noted as a feature of some of the individual experimental curves, but its effect on the composite curve is apparently partly obscured, possibly by the fact that it occurred at slightly different survivor levels in the separate experiments. More extensive data at the lower mortalities will be necessary before the detailed shape of the early part of the survivor curve can be finally established.

SUMMARY

1. Whole cultures of *Bact. coli* grown under carefully controlled conditions have been subjected to the action of heat at temperatures ranging from 47 to 55° C. and the survivor curves determined.

2. The occurrence of excessive variation between replicate plates of counts made when the mortality exceeds 95 % was very much less evident than when phenol was the lethal agent.

3. At temperatures of 35° C. and below, disinfection of the cultures was never complete, as a permanent population of cells became established. In some cases the numbers of heat-resistant cells reached nearly 0·01 % of the original population, but great fluctuations were observed.

4. The death-rate during the active part of the disinfection was not constant but, in general, increased with time. In the faster disinfections this increase was difficult to detect and the logarithmic death-rate appeared to be virtually constant, but the increase is nevertheless believed to have been present in all cases.

5. The data for all the experiments have been combined to give a composite disinfection curve from which it was concluded that the death-rate was low at first but rose to a maximum at which it remained constant until the mortality had reached at least 99·99 %.

6. This finding led to the decision to treat all the experiments as if the regression of log survivors on time had been linear between mortalities of 90 and 99·99 %. The standard errors of the calculated regression coefficients were small, so that this method of treating the experimental data appears to be justified.

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