STUDIES IN THE DYNAMICS OF DISINFECTION

IX. THE EFFECT OF LETHAL TEMPERATURES ON STANDARD CULTURES OF *BACT. COLI*. I. THE NATURE OF THE PROBIT-LOG SURVIVAL-TIME RELATIONSHIP AT *p*H 7·0

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(With 2 Figures in the Text)

When the end-point method of testing the activity of a germicide is replaced by one in which the actual course of the disinfection is followed, it becomes possible to obtain estimates of temperature coefficients and concentration exponents based on the times taken to reach any particular degree of mortality amongst the cells of the population being studied. These times may be read from freehand curves drawn through the experimental points, but it is preferable to use, if possible, times calculated from a mathematical relationship between survivors and time, as these will be free from the personal errors to which times read from freehand curves must be subject. The calculation of these mortality times is facilitated if some form of linear relationship between survivors and time can be shown to exist, and for this purpose it has often been claimed that the logarithm of the number of survivors is a linear function of time. However, the results of experiments on the disinfection of standard cultures of *Bact. coli* by phenol (Jordan & Jacobs, 1945*a*) and by heat (Jordan, Jacobs & Davies, 1947) under carefully controlled conditions did not support this view, as it was only when the reaction was very rapid that the logarithmic death-rate could be regarded as constant. Portions only of the logarithmic survivor curves towards the ends of the disinfections were sufficiently straight to be treated as linear for the purpose of calculating mortality times, and the range of mortality so covered was different in the case of disinfection by heat from that by phenol.

As an alternative, Withell (1942) suggested that there might be a linear relationship between the probit values of the observed percentage mortalities and the logarithms of the times at which they occurred, and he produced evidence based on the data of other workers as well as on his own to support this hypothesis. Gaddum (1945) has used the term 'lognormal' for the distribution of resistance in cell populations where this relationship exists. However, in the experiments with phenol referred to above a very extensive range of mortalities was covered, and an analysis of the probit-log survival-time graphs showed clearly that they were curves of a very asymmetric sigmoid shape which could only be regarded as linear over short ranges (Jordan & Jacobs, 1945*b*). The nature of this relationship in the case of heat disinfection of similar standard cultures of *Bact. coli* forms the subject of the present paper.

RESULTS AND DISCUSSION

The details of the experiments whose results are here considered have already been published (Jordan *et al.* 1947), and it is only necessary to emphasize the fact that whole cultures of *Bact. coli*, grown under standard controlled conditions, were subjected to the action of heat in such a way that the only alteration in the environment of the cells was the elevation of the temperature. In Table 1 are presented the results of ten experiments at eight temperatures ranging from 47 to 55° C., the observed numbers of survivors at various times being given together with the corresponding percentage mortalities and probit values. The latter were obtained either from the table given by Bliss (1935) or, for mortalities greater than 99·99 %, from the tables of Pearson (1930). There are relatively few of these high mortalities, in contrast to the results of the experiments with phenol cited above, since in the heat disinfections permanent populations of survivors became established (Jordan *et al.* 1947). Since occasionally these populations amounted to nearly 0·01 % of the initial number of cells, it was decided to curtail the data at about 99·99 % mortality in order to avoid the inclusion of points which really belonged to the phase of permanent survivors. The exact point at which the data were curtailed varied a little from

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Table 1. *The effect of temperature on the relationship between probit and* log_{10} *survival time for the disinfection of standard* Bact. coli *cultures by heat*

Temp. ° C.	Time min.	Log_{10} time	Survivors per ml.	Percentage mortality	Probit
47	0	—	358,300,000	0·0	—
	95	1·9777	313,500,000	12·50	3·8497
	425	2·6284	261,000,000	27·16	4·3920
	1100	3·0414	96,770,000	72·99	5·6125
	1280	3·1072	91,160,000	74·56	5·6607
	1400	3·1461	77,960,000	78·24	5·7804
	1550	3·1903	79,220,000	77·89	5·7685
	2105	3·3232	51,910,000	85·51	6·0585
	2555	3·4074	33,740,000	90·58	6·3153
	2735	3·4370	19,630,000	94·52	6·6000
	2990	3·4757	13,700,000	96·18	6·7720
	3995	3·6015	470,900	99·869	8·0092
	4160	3·6191	251,500	99·930	8·1947
	4385	3·6420	161,600	99·955	8·3127
	5435	3·7352	16,390	99·99543	8·9122
48	0	—	364,700,000	0·0	—
	50	1·6990	310,700,000	14·81	3·9554
	200	2·3010	287,000,000	21·31	4·2043
	305	2·4843	246,100,000	32·52	4·5468
	500	2·6990	159,000,000	56·40	5·1611
	620	2·7924	136,300,000	62·63	5·3221
	1370	3·1367	18,770,000	94·85	6·6305
	1520	3·1818	13,520,000	96·29	6·7854
	1640	3·2148	6,731,000	98·15	7·0867
	1775	3·2492	2,853,000	99·218	7·4172
	2825	3·4510	11,570	99·99683	9·0000
49	0	—	330,600,000	0·0	—
	60	1·7782	296,200,000	10·41	3·7415
	120	2·0792	265,500,000	19·69	4·1472
	240	2·3802	205,300,000	37·90	4·6919
	365	2·5623	149,000,000	54·93	5·1254
	485	2·6857	117,100,000	64·58	5·3740
	615	2·7889	52,210,000	84·21	6·0031
	720	2·8573	33,000,000	90·02	6·2827
	855	2·9320	15,160,000	95·41	6·6860
	1030	3·0128	2,019,000	99·389	7·5057
	1140	3·0569	1,404,000	99·575	7·6316
	1260	3·1004	658,000	99·801	7·8798
	1380	3·1399	348,000	99·895	8·0760
	1565	3·1945	98,490	99·970	8·4316
	1740	3·2405	26,380	99·99202	8·7756
50	0	—	297,600,000	0·0	—
	45	1·6532	263,200,000	11·56	3·8027
	90	1·9542	206,000,000	30·78	4·4979
	135	2·1303	135,300,000	54·54	5·1140
	180	2·2553	88,440,000	70·28	5·5324
	240	2·3802	43,730,000	85·31	6·0498
	300	2·4771	29,580,000	90·06	6·2855
	360	2·5563	12,230,000	95·89	6·7380
	450	2·6532	3,538,000	98·81	7·2603
	545	2·7364	495,300	99·834	7·9358
	630	2·7993	158,100	99·947	8·2835
	720	2·8573	13,620	99·99542	8·9117
51 (*a*)	0	—	293,200,000	0·0	—
	15	1·1761	289,300,000	1·33	2·7825
	35	1·5441	187,500,000	36·05	4·6429
	55	1·7404	81,610,000	72·17	5·5879
	85	1·9294	43,840,000	85·05	6·0386
	115	2·0607	33,080,000	88·72	6·2118
	145	2·1614	17,600,000	94·00	6·5548
	175	2·2430	9,318,000	96·83	6·8564
	205	2·3118	2,424,000	99·173	7·3969
	235	2·3711	1,271,000	99·567	7·6252
	265	2·4232	398,400	99·864	7·9979
	295	2·4698	187,900	99·936	8·2212
	355	2·5502	35,100	99·988	8·6832
	385	2·5855	12,020	99·99590	8·9483
51 (*b*)	0	—	355,900,000	0·0	—
	60	1·7782	112,800,000	68·31	5·4764
	130	2·1139	25,040,000	92·96	6·4728
	180	2·2553	6,090,000	98·29	7·1175
	310	2·4914	163,200	99·954	8·3154
	410	2·6128	28,850	99·99189	8·7716
52	0	—	325,100,000	0·0	—
	15	1·1761	171,300,000	47·31	4·9326
	30	1·4771	62,760,000	80·70	5·8669
	45	1·6532	28,840,000	91·13	6·3488
	60	1·7782	22,030,000	93·22	6·4924
	80	1·9031	10,530,000	96·76	6·8467
	100	2·0000	2,502,000	99·230	7·4228
	120	2·0792	1,122,000	99·655	7·7017
	150	2·1761	218,900	99·933	8·2080
53 (*a*)	0	—	386,700,000	0·0	—
	30	1·4771	64,870,000	83·22	5·9629
	60	1·7782	5,098,000	98·68	7·2203
	105	2·0212	14,710	99·9962	8·9569
53 (*b*)	0	—	367,900,000	0·0	—
	10	1·0000	178,600,000	51·45	5·0364
	20	1·3010	83,640,000	77·26	5·7474
	30	1·4771	49,200,000	86·63	6·1091
	55	1·7404	8,078,000	97·80	7·0141
	65	1·8129	3,308,000	99·101	7·3660
	80	1·9031	1,014,000	99·724	7·7755
	100	2·0000	132,800	99·964	8·3835
	120	2·0792	14,180	99·99615	8·9538
55	0	—	334,600,000	0·0	—
	10	1·0000	4,838,000	98·56	7·1849
	20	1·3010	12,240	99·99634	8·9656

one experiment to another for reasons which will appear later.

When the probit values are plotted against time, curves are obtained which are concave downwards and to the right. This departure from linearity shows that amongst the cells of these cultures the distribution of survival times was not normal, which is in accordance with the general experience with bacterial cultures exposed to lethal agencies. It has, therefore, not been considered necessary to present graphs to illustrate this point.

Fig. 1 shows the curves obtained when the probit values are plotted against the logarithms of the survival times. The data for the two temperatures (51 and 53° C.) at which duplicate experiments were made agree so well that separate curves for the two experiments at each temperature cannot be shown clearly, and consequently a single curve only is given in each case. It is evident that the distribution of resistance to heat amongst the cells of these cultures was not lognormal, since the graphs are all curves concave upwards and to the left, while in four of the eight curves there is a fairly well-defined 'hump'. One of the four remaining graphs is not sufficiently extensive to show this feature, which evidently corresponds with a similar phenomenon in the logarithmic survivor curves of these experiments (Jordan *et al.* 1947). The hump occurs at between two-thirds and three-quarters along the logarithmic time scale when the latter is regarded as extending from zero to the point corresponding to probit 9·0, but it does not always occur at the same probit level. However, in their general shape the curves are all similar in type and, moreover, have roughly equal slopes at corresponding stages in the disinfections. This is especially well seen in the parallelism of the upper portions of the graphs between the probit values of 7 and 9, and the situation thus resembles that in the phenol disinfections of similar standard cultures (Jordan & Jacobs, 1945*b*). Therefore, it should be possible to combine the heat disinfection data as was done with the phenol data into a composite curve whose slope would reflect the manner in which the reaction would proceed under all temperature conditions.

The method adopted was first to adjust the time scale of each experiment to a standard length, i.e. that of the experiment at 50° C., using the 99·99 % mortality times as the basis for comparison. These times were calculated from the regressions of log survivors on time published previously (Jordan *et al.* 1947). Then the values of the slopes of the probit-log survival-time graphs between successive observations were calculated and plotted on a scatter diagram against the mid-points of the corresponding intervals of standardized time. This diagram was terminated at 750 min. which was a convenient value close to the 99·99 % mortality time of the standard experiment. It is this arbitrary limit which has determined the highest level of mortality in any experiment to be included in the diagram, since the mid-point of the final interval of standardized time must have fallen at or below 750 min. for the corresponding value of the slope of the probit-log survival-time graph to be included. The scatter diagram was then divided up into intervals of two units of

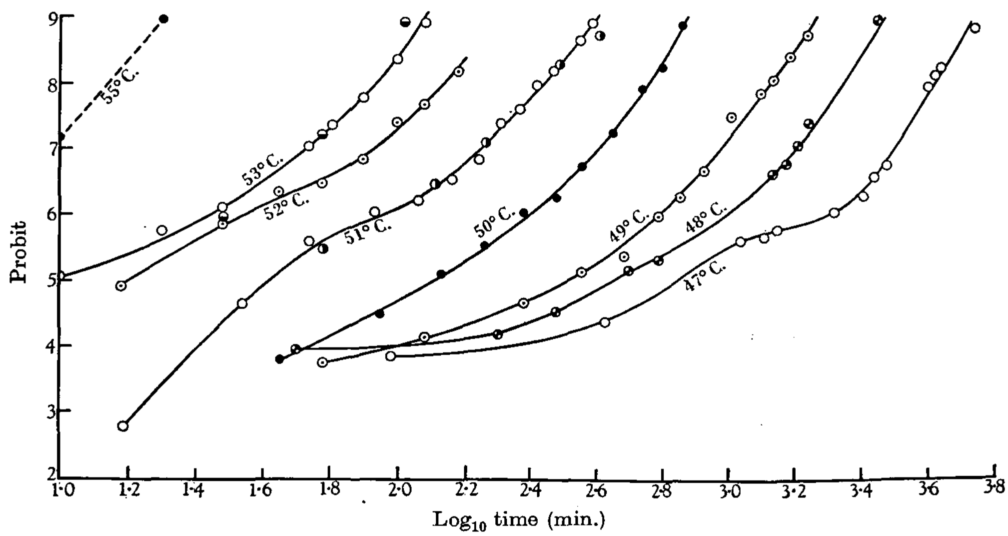

Fig. 1. Relation between probit and $\log_{10}$ survival time for *Bact. coli* at pH 7·0 when exposed to various lethal temperatures.

Table 2. *Frequency diagram of slopes of probit-log survival-time lines for all experiments*

Standardized time scale (min.)

Slope of probit-log survival-time line	0–50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750
10–12	—	—	—	—	—	—	—	—	1	—	—	1	—	1	—
8–10	—	—	—	—	—	—	—	1	1	2	1	0	—	0	—
6–8	—	—	—	—	1	—	—	1	0	0	2	1	2	1	4
4–6	—	2	—	—	1	3	3	1	2	2	2	4	1	1	0
2–4	—	3	5	5	0	5	2	0	3	1	0	0	0	1	0
0–2	3	2	1	3	1	1	0	0	0	0	0	0	0	0	0
Total	3	7	6	8	3	9	5	3	7	5	5	6	3	4	4
Mean	1·00	3·00	2·67	2·25	4·33	3·44	4·20	7·00	5·56	6·20	6·60	6·33	6·33	6·50	7·00
Standard deviation	0·00	0·67	0·33	0·37	1·76	0·67	0·49	1·15	1·21	1·20	0·75	0·99	0·67	1·71	0·00

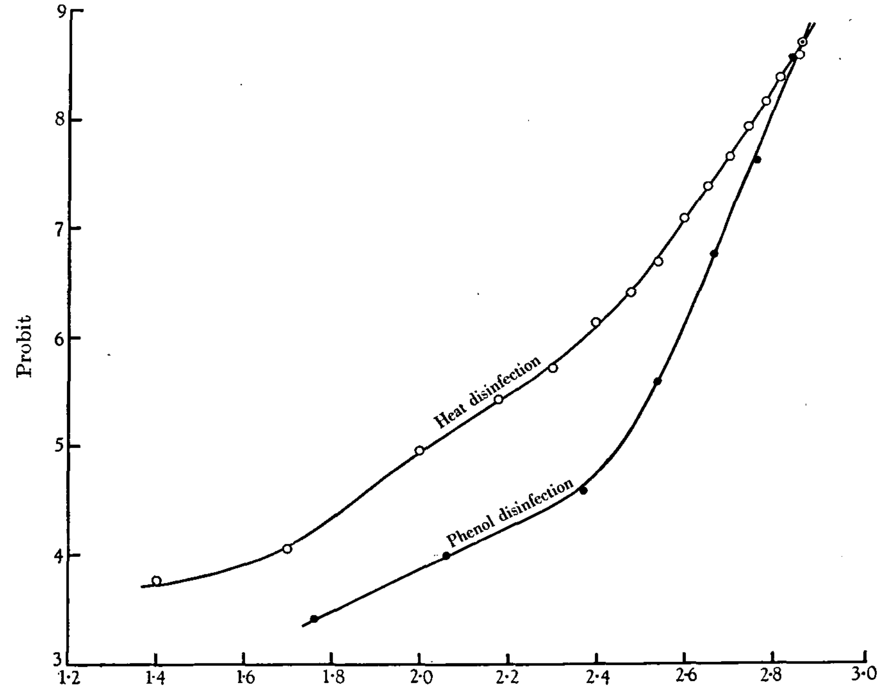

Fig. 2. Idealized probit-log$_{10}$ survival-time curve constructed from mean data given in Table 2.

slope and 50 min. on the standardized time scale, and thus a frequency diagram as shown in Table 2 was obtained by counting the points falling within the various ranges. For each 50 min. interval of standardized time a mean slope with its standard error has been calculated on the assumption that all the cases in a group had slopes equal to the mid-value of its range. It appears that the mean slope, after being quite small at first, tends on the whole to rise slowly until, about half-way through the disinfection, there appears at first sight to be a low peak. This is probably not a real peak, having regard to the magnitudes of the standard errors of this particular value and the succeeding one, but more likely represents the first of a series of approximately equal values extending over the latter half of the disinfection time. This picture is very different from that obtained when phenol was the lethal agent, in which case there was a very well-marked peak (Jordan & Jacobs, 1945*b*).

From the mean values of the slopes given in Table 2 an idealized probit-log survival-time graph can be constructed. The probit decrement for any interval was found by multiplying the mean slope by the difference between the logarithms of the final and initial values of the standardized time, and the actual probit values were obtained by deducting successively the various decrements, beginning from an initial value of 8·719 which represents 99·99 % mortality. This value was deemed to have occurred at 725 min. as it actually did in the standard experiment. The graph obtained is shown in Fig. 2, and it should be explained that it starts at a value of 1·4 for the logarithm of the survival time only because the first interval of standardized time was begun at 25 min. instead of at zero time. This was done to avoid an undue extension of the graph which would have been inconvenient for presentation. The idealized curve shows a marked concavity upwards and to the left, thus corresponding in general type to the graphs of the individual experiments. There is, in addition, some evidence of the hump which was noted in several experiments, but the fact that, although the individual humps all occurred at roughly the same stage in time in the various disinfections, they did not occur at the same probit values, has doubtless caused some cancelling out of this effect. The shape of the idealized curve suggests that there is a close approximation to linearity between probit and log survival time over the probit range of about 6·7–8·7, i.e. between 95 and 99·99 % mortality. It may be recalled that in the previous paper of this series it was shown that a linear relationship exists between log survivors and time between 90 and 99·99 % mortality, and it is noteworthy, therefore, that the same mortality range gives a linear relationship by both methods of treatment. This illustrates the point made by Irwin (1942) that very good data indeed would be needed to distinguish between a lognormal distribution and an exponential relationship between survivors and time.

From the idealized curve it appears that there may also be a roughly linear relationship between probit and log survival time over the probit range of 4–6, although the slope of this line is much smaller. This, however, must be regarded with suspicion, since the 4–6 probit range covers the zone in which the humps mentioned above occurred, and as these did not coincide in probit value there may have been a general smoothing out of the curve and an artificial appearance of linearity induced. Certainly the graphs of the individual experiments show marked evidence of systematic deviations from linearity. For comparison, in Fig. 2 is also given the idealized curve for the phenol disinfection of similar standard cultures of *Bact. coli*, the curve illustrated being the less sharply bent of the two types found in that case (Jordan & Jacobs, 1945*b*). The difference in shape caused by the two lethal agencies is quite apparent and the curve for heat disinfection, although actually possessing considerable variation in slope along its length, is relatively straighter than that for phenol disinfection.

SUMMARY

1. A study has been made of the probit-log survival-time relationship in a number of experiments in which whole standard cultures of *Bact. coli* were subjected to heat at temperatures ranging from 47 to 55° C.

2. It is concluded that the whole mortality up to 99·99 % could not be covered by a single straight-line relationship since the graphs are curves concave upwards and to the left.

3. Between 95 and 99·99 % mortality the relationship approximated closely to linearity.

4. Several individual experiments showed 'humps' in their curves. These, although always occurring at the same stage in time in the disinfections, varied in their probit levels.

5. By combining the data for all experiments, an idealized probit-log survival-time curve was obtained in which the changes of slope were less marked than in the curve representing the disinfection of similar cultures by phenol.

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