STUDIES IN THE DYNAMICS OF DISINFECTION

XIII. THE NATURE OF THE PROBIT-LOG SURVIVAL-TIME RELATIONSHIP IN DISINFECTIONS OF STANDARD CULTURES OF *BACT. COLI* AT 51° C. UNDER VARIOUS CONDITIONS OF pH

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(With 5 Figures in the Text)

The frequency of the occurrence in nature of 'log-normal' distributions was recently emphasized by Gaddum (1945). Withell (1942) showed that the distribution of resistance of bacteria to disinfectants was also apparently of that type, since when the probit values of the percentage mortalities were plotted against the logarithms of the times of exposure, straight-line graphs were often obtained. The importance of this relationship is very great, since the transformation of curved survivors-time or log survivors-time graphs into a completely linear form is of immense assistance in the calculation of the times required to reach particular percentage mortalities, these times being needed for the determination of other characteristics of disinfectants, namely the temperature coefficient and the concentration exponent. However, Jordan & Jacobs (1945) showed that when as much as possible of the full range of mortality was covered, the probit-log survival-time graphs given by the results of the exposure of standard cultures of *Bact. coli* to phenol under carefully controlled conditions were not of a simple linear form, but could be described with reasonable accuracy by two straight lines of widely different slopes intersecting in the region of probit 4·6. The conclusion was finally drawn that the true shape of the graph was that of a very asymmetrical sigmoid curve, the exact shape of which depended on the speed of the reaction.

When similar standard cultures were exposed to moderate temperatures (47–55° C.) at pH 7·0, Jordan, Jacobs & Davies (1947*b*) obtained probit-log time graphs which were curves, concave upwards and to the left, though between 95 and 99·99 % mortality the relationship was approximately linear. Higher mortalities could not be investigated owing to the establishment of a residual surviving population (Jordan *et al.* 1947*a, c*). Berry & Michaels (1948), who examined the effect of ethylene glycol and its mono-alkyl ethers on *Bact. coli*, found that although there was visually an apparently linear relationship between probits and log time over the probit range 4–6, an analysis of variance showed that this was not firmly founded. The linear relationship could usefully be assumed over that range for the purpose of comparing the activities of different concentrations of the same substance, but the graph was really a curve, concave upwards and to the left, which was adequately described by a quadratic equation. Perhaps if it had been possible to cover a wider range of probit values, the curvilinear nature of the relationship would have been more apparent.

Data provided by experiments on the disinfection of standard cultures of *Bact. coli* exposed at 51° C. to different concentrations of hydrogen and hydroxyl ions (Jordan & Jacobs, 1948) are used below to show that in these circumstances also single straight lines do not suffice to describe the probit-log survival-time relationship.

RESULTS AND DISCUSSION

The technique used has already been fully described by Jordan & Jacobs (1948) and in earlier papers of this series. The results obtained by exposing standard cultures of *Bact. coli* to pH values ranging from 2·8 to 8·8 at 51° C. are given in Table 1. Owing to the fact that permanently surviving populations became established it was impossible to attain as high a degree of mortality as in the earlier experiments with phenol referred to above, and in order that only the phase of active disinfection should be included it was decided to establish an arbitrary upper probit limit of 9·0, which corresponds to a mortality of 99·997 % approx. Only in the case of the experiment at pH 2·8 did it appear that this might be too high a limit (see Fig. 3), but that experiment yielded very scanty data and has not been included in the mathematical treatment.

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Table 1. *The relationship between probit and* $\log_{10}$ *survival time in the disinfection of standard cultures of* Bact. coli *at* 51° C. *under various conditions of* pH

pH	Time (min.)	$\log_{10}$ time	Survivors per ml.	Percentage mortality	Probit
8·8	0	—	338,100,000	0·0	—
	7	0·8451	178,300,000	47·26	4·9313
	17	1·2304	44,690,000	86·78	6·1161
	28	1·4472	12,160,000	96·40	6·7991
	39	1·5911	1,489,000	99·560	7·6197
	50	1·6990	199,700	99·941	8·2441
	63	1·7993	24,540	99·99274	8·7990
	73	1·8633	16,650	99·99508	8·8945
8·2	0	—	318,200,000	0·0	—
	15	1·1761	161,200,000	49·34	4·9835
	45	1·6532	23,470,000	92·62	6·4480
	75	1·8751	996,100	99·687	7·7339
	100	2·0000	17,280	99·99457	8·8705
7·7	0	—	332,400,000	0·0	—
	15	1·1761	205,000,000	38·33	4·7032
	50	1·6990	120,100,000	63·87	5·3550
	85	1·9294	30,170,000	90·92	6·3358
	125	2·0969	3,296,000	99·008	7·3293
	165	2·2175	280,700	99·916	8·1421
	205	2·3118	42,870	99·98710	8·6542
	245	2·3892	14,250	99·99571	8·9278
7·3	0	—	321,700,000	0·0	—
	15	1·1761	272,100,000	15·42	3·9814
	40	1·6021	204,600,000	36·40	4·6522
	70	1·8451	133,900,000	58·38	5·2116
	100	2·0000	104,500,000	67·52	5·4543
	130	2·1139	83,660,000	73·99	5·6430
	170	2·2304	14,140,000	95·61	6·7071
	195	2·2900	5,417,000	98·316	7·1238
	230	2·3617	2,045,000	99·371	7·4955
	265	2·4232	662,300	99·794	7·8644
	305	2·4843	25,670	99·99202	8·7756
7·0 (*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
7·0 (*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	98·289	7·1175
	310	2·4914	163,200	99·954	8·3154
	410	2·6128	28,850	99·99189	8·7716
6·8	0	—	320,000,000	0·0	—
	15	1·1761	302,700,000	5·41	3·3937
	35	1·5441	260,900,000	18·47	4·1024
	55	1·7404	240,800,000	24·75	4·3176
	75	1·8751	213,300,000	33·34	4·5695
	100	2·0000	166,600,000	47·94	4·9483
	125	2·0969	140,600,000	56·06	5·1525
	150	2·1761	132,400,000	58·62	5·2178
	180	2·2553	107,600,000	66·37	5·4226
	210	2·3222	70,200,000	78·06	5·7742
	240	2·3802	51,860,000	83·79	5·9859
	270	2·4314	32,990,000	89·69	6·2641
	300	2·4771	25,020,000	92·18	6·4173
	330	2·5185	14,860,000	95·36	6·6808
	360	2·5563	8,650,000	97·30	6·9268
	390	2·5911	5,870,000	98·166	7·0893
	420	2·6232	3,136,000	99·020	7·3339
	450	2·6532	925,100	99·711	7·7600
	480	2·6812	828,700	99·741	7·7947
	510	2·7076	391,800	99·878	8·0308
	540	2·7324	49,170	99·985	8·6296
	575	2·7597	13,070	99·99592	8·9400
6·65	0	—	306,800,000	0·0	—
	15	1·1761	302,000,000	1·56	2·8453
	35	1·5441	276,300,000	9·94	3·7150
	55	1·7404	241,000,000	21·45	4·2091
	75	1·8751	202,800,000	33·90	4·5848
	100	2·0000	192,500,000	37·26	4·6750
	125	2·0969	178,000,000	41·98	4·7976
	150	2·1761	158,200,000	48·44	4·9609
	180	2·2553	116,000,000	62·19	5·3104
	210	2·3222	103,500,000	66·26	5·4196
	270	2·4314	74,500,000	75·72	5·6973
	300	2·4771	55,860,000	81·79	5·9074
	330	2·5185	45,740,000	85·09	6·0403
	360	2·5563	32,820,000	89·30	6·2426
	390	2·5911	19,890,000	93·52	6·5157
	420	2·6232	13,910,000	95·47	6·6923
	450	2·6532	8,753,000	97·15	6·9187
	480	2·6812	1,466,000	99·522	7·5914
	525	2·7202	347,800	99·887	8·0540
	570	2·7559	94,770	99·969	8·4237
	615	2·7889	36,140	99·988	8·6832
6·4	0	—	319,900,000	0·0	—
	15	1·1761	279,000,000	12·79	3·8636
	45	1·6532	223,800,000	30·04	4·4768
	75	1·8751	174,800,000	45·36	4·8834
	105	2·0212	115,200,000	63·99	5·3582
	135	2·1303	79,020,000	75·30	5·6840
	165	2·2175	19,090,000	94·03	6·5573
	195	2·2900	15,850,000	95·04	6·6488
	225	2·3522	9,011,000	97·18	6·9079
	255	2·4065	5,086,000	99·410	7·1469
	285	2·4548	2,605,000	99·186	7·4027
	330	2·5185	2,012,000	99·371	7·4955
	375	2·5740	921,600	99·712	7·7612
	420	2·6232	414,900	99·870	8·0114
	525	2·7202	93,460	99·971	8·4425
	560	2·7482	72,960	99·977	8·5076
	595	2·7745	42,700	99·98665	8·6454
	630	2·7993	23,910	99·99253	8·8020
6·25	0	—	326,600,000	0·0	—
	20	1·3010	296,000,000	9·37	3·6817
	40	1·6021	265,600,000	18·68	4·1103
	60	1·7782	245,400,000	24·86	4·3211
	80	1·9031	209,300,000	35·92	4·6394
	100	2·0000	192,500,000	41·06	4·7727
	125	2·0969	166,100,000	49·14	4·9784
	150	2·1761	154,700,000	52·63	5·0660
	180	2·2553	115,800,000	64·54	5·3729
	210	2·3222	111,800,000	65·77	5·4062
	240	2·3802	74,060,000	77·32	5·7495
	270	2·4314	56,920,000	82·57	5·9373
	300	2·4771	41,660,000	87·24	6·1378
	330	2·5185	18,600,000	94·31	6·5814
	360	2·5563	4,135,000	98·734	7·2365
	390	2·5911	1,447,000	99·557	7·6174
	450	2·6532	171,500	99·947	8·2750
	480	2·6812	18,780	99·99425	8·8567
	510	2·7076	10,800	99·99669	8·9893
6·05	0	—	333,300,000	0·0	—
	10	1·0000	291,600,000	12·51	3·8502
	40	1·6021	247,100,000	25·86	4·3524
	70	1·8451	225,600,000	32·31	4·5410
	100	2·0000	166,100,000	50·17	5·0043
	130	2·1139	154,900,000	53·53	5·0886
	160	2·2041	140,200,000	57·94	5·2003
	190	2·2788	101,900,000	69·43	5·5081
	220	2·3424	105,200,000	68·44	5·4800
	250	2·3979	74,290,000	77·71	5·7624
	280	2·4472	45,590,000	86·32	6·0948
	310	2·4914	39,150,000	88·25	6·1876
	340	2·5315	19,020,000	94·29	6·5796
	370	2·5682	6,949,000	97·92	7·0375
	400	2·6021	1,562,000	99·531	7·5979
	430	2·6335	477,500	99·857	7·9825
	475	2·6767	80,130	99·976	8·4967
	520	2·7160	17,100	99·99487	8·8843
5·7	0	—	337,300,000	0·0	—
	30	1·4771	253,800,000	24·76	4·3179
	65	1·8129	209,600,000	37·86	4·6909
	105	2·0212	142,700,000	57·69	5·1940
	145	2·1614	81,630,000	75·80	5·6999
	225	2·3522	12,070,000	96·42	6·8017
	265	2·4232	2,500,000	99·259	7·4367
	305	2·4843	1,695,000	99·497	7·5738
	345	2·5378	57,320	99·98301	8·5829
	385	2·5855	19,410	99·99425	8·8567
4·8	0	—	356,400,000	0·0	—
	15	1·1761	255,000,000	28·45	4·4305
	45	1·6532	69,230,000	80·58	5·8626
	75	1·8751	11,870,000	96·67	6·8344
	105	2·0212	4,066,000	98·859	7·2765
	135	2·1303	1,268,000	99·644	7·6912
	165	2·2175	269,300	99·924	8·1714
	195	2·2900	136,500	99·962	8·3686
	225	2·3522	28,580	99·99198	8·7744
	255	2·4065	18,430	99·99483	8·8824
3·9	0	—	340,500,000	0·0	—
	10	1·0000	124,500,000	63·43	5·3433
	25	1·3979	9,669,000	97·16	6·9049
	40	1·6021	4,383,000	98·713	7·2301
	55	1·7404	2,591,000	99·239	7·4271
	70	1·8451	1,413,000	99·585	7·6397
	85	1·9294	770,300	99·774	7·8395
	100	2·0000	579,100	99·830	7·9291
	115	2·0607	161,400	99·953	8·3092
	130	2·1139	97,160	99·971	8·4425
	150	2·1761	18,810	99·99448	8·8665
2·8	0	—	340,500,000	0·0	—
	5	0·6990	18,780,000	94·48	6·5964
	15	1·1761	48,080	99·986	8·6474
	25	1·3979	14,770	99·99566	8·9250
	35	1·5441	17,020	99·99500	8·8905

The analysis previously made of the log survivors-time curves for these experiments (Jordan & Jacobs, 1948) showed that they could be placed in groups according to their general shape. These related curves ought also to give related probit-log time graphs, and it has proved convenient to adopt the same grouping here. The first of these groups comprised the curves for pH 6·8, 7·3, 7·7, 8·2 and 8·8, and the probit-log time graphs for these are shown in Fig. 1. There appeared to be little doubt that the data could be treated reasonably satisfactorily by assuming a bilinear form for each graph. This was slope of the lower portion of the graph with rising pH, and a decrease in slope of the upper portion, so that the whole graph tends to approach a single straight line at the highest pH tested. Indeed, it may be questioned whether the assumption of bi-linearity is justified for the experiments at pH 8·2 and 8·8, on the ground that the normal and inevitable experimental error involved in sampling might account for the divergences shown. This aspect will be referred to again later. Here it is appropriate to point out that the tendency for the slopes of the two branches of the graphs to become more nearly equal

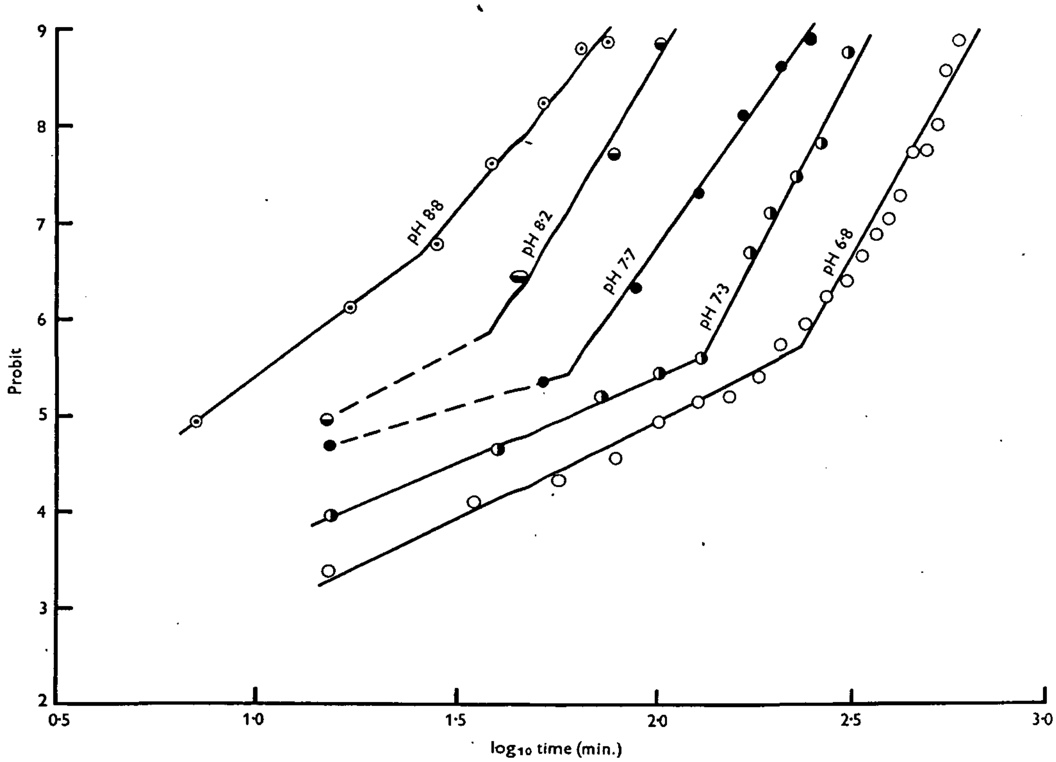

Fig. 1. Showing the relationship between probit and $\log_{10}$ survival time at pH 6·8, 7·3, 7·7, 8·2 and 8·8.

tested by calculating the equations of the lines of regression of probits on log time, and as shown in Table 2 a very satisfactory fit was obtained in all cases. The ratio of the slope of a line to its standard error was never less than 8·0 and in seven of the eight cases the ratio was over 13. The lines drawn in Fig. 1 correspond to the equations given in Table 2. For the purpose of calculating specific mortality times this bilinear method of treatment may, therefore, be adequate. The picture presented is incomplete, because of the difficulty of obtaining sufficient data for low mortalities when the disinfection is rapid, as at pH 8·2 and 8·8. The results available are, on the whole, consistent with a gradual increase in as the pH was raised was also evident with rising concentration in experiments with phenol at 35° C. (Jordan & Jacobs, 1944), though it was less marked or absent at other temperatures (Jordan & Jacobs, 1945).

Within this group the probit-log time relationship has given curves of a far greater constancy of shape than those obtained by plotting log survivors against time (Jordan & Jacobs, 1948). For example, the slopes of the upper portions of the former graphs vary only from 5 to 8 approx., the range being only about 60 % of the smaller value, while in the latter case the variation was from 0·0660 to 0·0108, which is a more than six-fold increase on the smaller value. How-

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ever, although the bilinear treatment appears adequate, it does not follow that the graphs are truly of that form. Indeed, by analogy with the results given by phenol, and heat at pH 7·0, it might be anticipated that a curve, concave upwards and to the left, would prove to be the real shape. As the results of the separate experiments do not lend themselves to combination into a single composite curve by the method of standardizing the abscissa scale previously found useful, this point cannot be pursued further, but there is distinct evidence of the curvilinear nature of the graph for pH 6·8, while in most of the other cases curved lines would fit quite well.

The second group of related curves comprises those for pH 6·65, 6·25, 6·05 and 5·7. These have also been treated on the basis of assumed bilinearity, and the regression equations are given in Table 2. Clearly, the two straight lines provide a satisfactory fit, the ratios of the slopes of the lines to their standard errors being over 15 in five of the eight cases and never less than 5·3. Two only of the four graphs are shown in Fig. 2, because the lines representing the lower portions for pH 6·05 and 6·25 intersect those for pH 5·7 and 6·65 and confuse the diagram. Also, the upper portions of the two former curves practically coincide, though they run midway between those for the two latter pH values. The whole group is evidently quite a compact one, the curves being very similar to those for pH 6·8 and 7·3, though differing in having steeper upper portions. The case of pH 5·7 forms a link between the two groups, having an intermediate value for the slope of its upper portion. This group is just as compact whether the probit-log time or the log survivors-time relationship is used (see Jordan & Jacobs, 1948), though whereas by the former comparison the curve for pH 5·7 is somewhat apart from the rest, by the latter pH 6·65 is the one to differ slightly. In this second group of curves, also, bilinearity may be only an approximation, in spite of the generally good fit,

Table 2. *Details of the regressions of probits on $\log_{10}$ survival-time, assuming the relationship to be bilinear*

pH	Regression equation* $Y=\bar{y}+b\,(x-\bar{x})$	Standard error of $\bar{y}$	Standard error of b	Ratio of b to its S.E.
	Lower line			
8·8	$Y=5{\cdot}9488+3{\cdot}0990\,(x-1{\cdot}1742)$	±0·0049	±0·0196	158·4
8·2	—	—	—	—
7·7	—	—	—	—
7·3	$4{\cdot}9885+1{\cdot}8077\,(x-1{\cdot}7474)$	0·0236	0·0710	25·5
6·8	$4{\cdot}8885+2{\cdot}0697\,(x-1{\cdot}9566)$	0·0428	0·1185	17·5
6·65	$4{\cdot}8469+2{\cdot}2801\,(x-2{\cdot}0511)$	0·0299	0·0767	29·7
6·25	$4{\cdot}8098+1{\cdot}8431\,(x-1{\cdot}9815)$	0·0342	0·1052	17·5
6·05	$4{\cdot}9764+1{\cdot}3462\,(x-1{\cdot}9760)$	0·0553	0·1317	10·2
5·7	$4{\cdot}9757+1{\cdot}9546\,(x-1{\cdot}8682)$	0·0948	0·3682	5·3
4·8	—	—	—	—
3·9	$7{\cdot}4951+1{\cdot}7293\,(x-1{\cdot}7525)$	0·0137	0·0673	25·7
7·0 (*a*)	$6{\cdot}0983+2{\cdot}1906\,(x-1{\cdot}9730)$	0·0342	0·2175	10·1
7·0 (*b*)	—	—	—	—
6·4	$4{\cdot}4079+1{\cdot}4313\,(x-1{\cdot}5681)$	0·0382	0·1311	10·9
	Upper line			
8·8	$Y=8{\cdot}0713+5{\cdot}2344\,(x-1{\cdot}6800)$	±0·0501	±0·3374	15·5
8·2	$7{\cdot}6841+6{\cdot}8469\,(x-1{\cdot}8428)$	0·1229	0·8571	8·0
7·7	$7{\cdot}8778+5{\cdot}7939\,(x-2{\cdot}1890)$	0·0430	0·2651	21·9
7·3	$7{\cdot}2682+7{\cdot}8048\,(x-2{\cdot}3173)$	0·0732	0·5956	13·1
6·8	$7{\cdot}4425+7{\cdot}9462\,(x-2{\cdot}6120)$	0·0615	0·6009	13·2
6·65	$7{\cdot}3902+11{\cdot}3198\,(x-2{\cdot}6713)$	0·0553	0·7311	15·5
6·25	$7{\cdot}4539+11{\cdot}8842\,(x-2{\cdot}5771)$	0·0518	0·5584	21·3
6·05	$7{\cdot}3576+11{\cdot}3291\,(x-2{\cdot}5833)$	0·0585	0·6807	16·6
5·7	$7{\cdot}8504+8{\cdot}9681\,(x-2{\cdot}4766)$	0·1042	1·2637	7·1
4·8	$7{\cdot}7327+4{\cdot}0243\,(x-2{\cdot}1183)$	0·0265	0·1101	36·6
3·9	$8{\cdot}3868+5{\cdot}0883\,(x-2{\cdot}0877)$	0·0364	0·5603	9·1
7·0 (*a*)	$7{\cdot}7855+5{\cdot}6977\,(x-2{\cdot}3895)$	0·0219	0·1590	35·8
7·0 (*b*)	$7{\cdot}6693+4{\cdot}6961\,(x-2{\cdot}3684)$	0·0300	0·1535	30·6
6·4	$7{\cdot}2170+4{\cdot}2557\,(x-2{\cdot}4337)$	0·0292	0·1059	40·2

* Y = calculated probit value; $\bar{y}$ = mean observed probit value; $x=\log_{10}$ time in min.; $\bar{x}$ = mean $\log_{10}$ time in min. b = slope of line.

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since there is strong evidence in all cases of a gradual rather than an abrupt transition between the two straight lines, and it is not unlikely that continuous curves would prove even better than the straight lines for describing the data.

Turning now to the third group, i.e. pH 2·8–4·8, the graphs for which are shown in Fig. 3, the position is less simple. The experiment at pH 2·8 has too few values for it to be satisfactory, and it seems doubtful whether two of the four points really belong to the active disinfection phase at all. The graph for the experiment at pH 4·8 could be regarded as linear over its whole course, but in common with the others it has been preferred to regard this too as fundamentally bilinear, the upper portion extending from probit 5·86 upwards and the lower having only one point on it. The equation of the upper portion is given in Table 2. This arbitrary treatment may or may not be justified, but it has been adopted because of the strongly bilinear nature of the graph for pH 5·7, which suggests that at pH 4·8 the shape should be similar, though perhaps with a smaller difference between the slopes of the two branches. The first real difficulty comes with the experiment at pH 3·9, where the placing of the points strongly suggests a sinuous curve. However, to preserve the uniformity of treatment, two portions have been selected and treated as if they were truly linear. As can be seen from Table 2 the fit is quite good. Nevertheless, the objection may be raised that the whole range of values could well be fitted to a single straight line, the apparent divergences being due to experimental error. This single line has been calculated and its position is shown by the broken line in Fig. 3. It has a slope of 2·6393 ± 0·1768 and the ratio of the slope to its standard error is satisfactorily high at 14·9. This raises the question of how closely the probit values ought to lie to the straight line for that relationship to be permissible as an adequate expression of the data. This aspect is dealt with below,

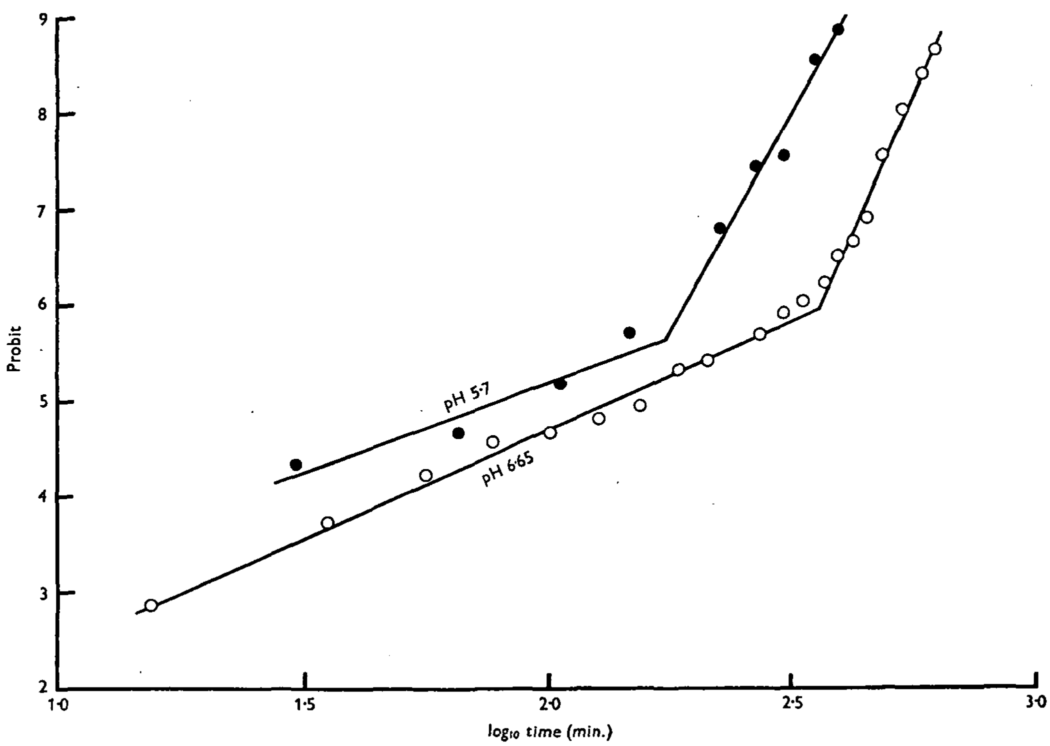

Fig. 2. Showing the relationship between probit and $\log_{10}$ survival time at pH 5·7 and 6·65.

but first it is necessary to examine the two remaining experiments, i.e. those at pH 7·0 and 6·4.

These are shown in Fig. 4, and evidently the experiment at pH 6·4 gives a curve of the bilinear type, though possibly a continuous curve would make an even better fit. The calculated regression lines, whose equations are given in Table 2, fit quite satisfactorily. The graph for pH 7·0, on the other hand, is of the same sinuous type as that for pH 3·9. Again, however, for uniformity, it has been treated as if consisting of linear portions. Reference to Table 2 will reveal that the upper two of the three linear portions into which the graph for the experiment at pH 7·0 (*a*) can be divided, are satisfactorily described

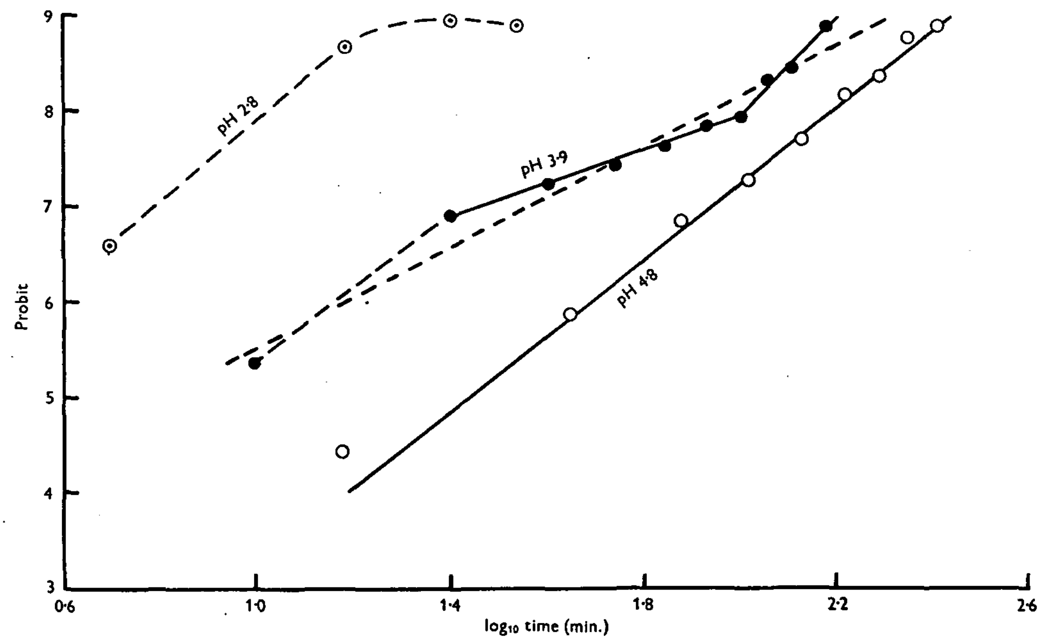

Fig. 3. Showing the relationship between probit and $\log_{10}$ survival time at pH 2·8, 3·9 and 4·8.

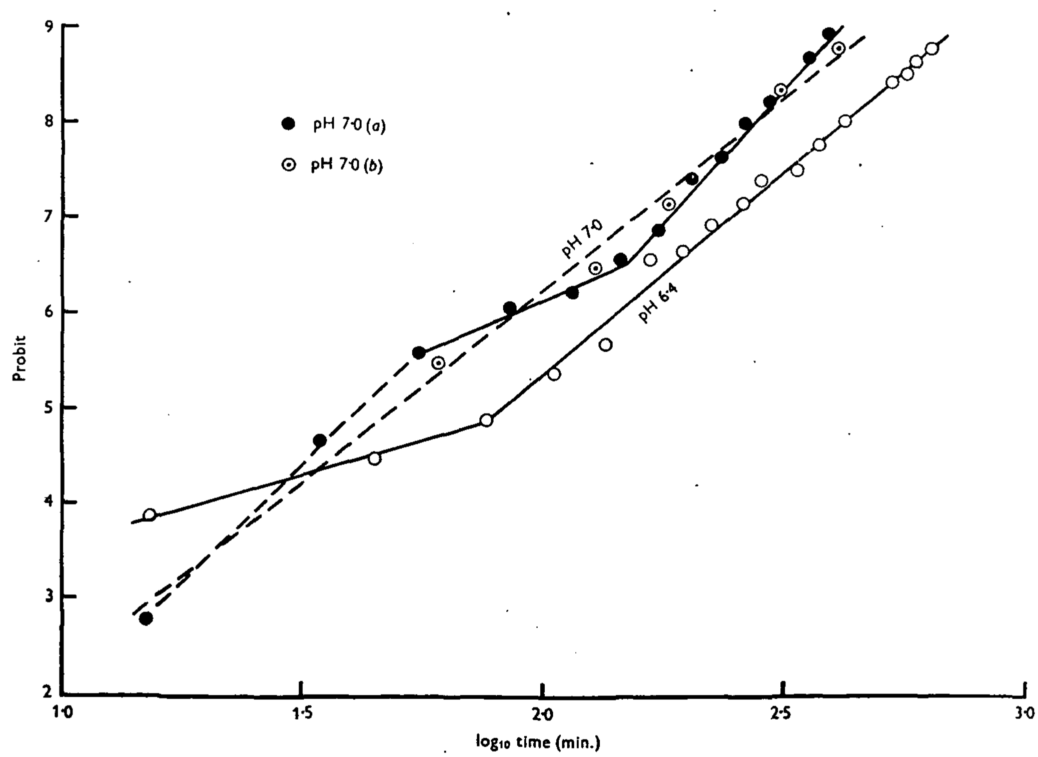

Fig. 4. Showing the relationship between probit and $\log_{10}$ survival time at pH 6·4 and 7·0.

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by straight lines. The lowest portion is similarly well described, the slope of the line being 4·9822 ± 0·0613 and the ratio 81·2. The lines shown in Fig. 4 are for the (*a*) experiment only, since the data are more extensive than in the (*b*) experiment, but the values obtained in the latter have also been plotted to show how nearly the two experiments coincide. The (*b*) experiment also shows evidence of the existence of the central flatter portion.

As in the case of pH 3·9, it may be objected that here also the divergences from complete linearity may be due solely to experimental error. Indeed, if the results from both the experiments at pH 7·0 are combined and treated as if a single straight line relationship applied, a line is obtained with a slope of 4·0468 ± 0·1434, the ratio of slope to standard error being 28·2. This line is shown dotted in Fig. 4. There are then five cases, i.e. pH 3·9, 4·8, 7·0, 8·2 and 8·8, where it might be better to adopt a single straight line treatment rather than cut the graphs into two or more short linear portions, on the ground that experimental error might account for the apparent divergences from linearity, and that if this treatment is adopted lines whose slopes have quite small standard errors are obtained. There are, however, good reasons against adopting this course. Apart from the question of analogy with the results of other experiments, the deviations from linearity are in all cases systematic, several successive points lying on one or other side of the calculated line instead of being irregularly distributed, as expected if experimental error were the sole cause. Again, the values of the logarithms of the times at which certain mortalities are attained may differ by as much as 0·1 according to whether the single straight line or the several short linear portions are used. This means a difference of about 25 % in the two estimates of the mortality time, which may amount to a considerable period. This situation would, of course, have to be tolerated if it could be shown that the observed divergences of the points from the single straight line were within the range covered by the inherent experimental error, but in fact it appears that this is unlikely to be so, at least in the case of the higher probit values.

Evidence for this contention may be adduced by determining the extent to which the probit of a given count of survivors may vary, purely through the inevitable error involved in the bacterial count. It may be assumed that the standard error of a count may amount to ± 5 %, including errors involved in making successive dilutions as well as those of sampling from the final dilution. In addition, there will be an error involved when the sample is withdrawn from the culture. To cover this as well, a standard error of ± 10 % may be assumed, though such an estimate may be generous. It is hard to say, in the absence of systematic investigation, whether this can be regarded as a constant figure applicable to all counts, no matter whether the mortality be high or low. With untreated organisms and at low mortalities, the experience in this now considerable series of experiments on disinfection has been that the counts were very reliable, but at high mortalities the χ^2 index tends to become unsatisfactory and the error therefore larger, though this may to some extent be offset because fewer dilutions have to be made. However, assuming a standard error of ± 10 %, then the limits of ± 20 % for all counts may be expected to cover the normal experimental error, since twice the standard error is exceeded only about once in twenty-two trials. On this basis, the limits between which the experimentally determined percentage mortalities corresponding to different 'true' survivor levels may be expected to lie can readily be calculated, and the corresponding probit limits obtained. These, together with the 'true' probit values, are given in Table 3. Clearly, at low mortalities the limits are widely separated, but as the mortality increases the difference between the upper and lower limits becomes progressively less. In other words, the higher the mortality the less effect will experimental error have in causing the observations to deviate from the 'true' readings, whether these fall on a straight line or a curve. It is also evident that if the assumptions regarding the magnitude of the standard error are true, little significance can be attached to estimates of mortalities below 20 %. This figure corresponds to probit 4·1584, so little weight should be given to probit values below 4·0. In the present data only eight such low values have been recorded out of a total of 171 observations.

The probit limits set out in Table 3 may be applied to any particular set of experimental data. At present it is desired to know whether sinuous probit-log time curves such as those obtained at pH 3·9 and 7·0 represent merely chance deviations from a single straight line or not. In Fig. 5 the data for experiment (*a*) at pH 7·0 are shown plotted around the straight line calculated from all the data available for that pH value. The curved lines above and below the straight line represent the probit limits set out in Table 3 and corresponding to the 'true' probit values which are assumed to lie on the straight line. Evidently, six of the plotted points are well outside the limits and several others are only just inside them, so it is concluded that the sinuosity of the curve which follows the points is probably not an artefact due to experimental error. Further, if the point at probit 2·7825 be disregarded as unreliable, then the remaining points would fit in very satisfactorily with a bilinear treatment, where the lower portion embraces the probit range 4·6429–6·8564 and the upper portion the range from 6·8564 upwards. In agreement with the limits shown in Table 3, the experimental points lie very closely to

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this upper line but less closely to the lower, though all still within the assumed limits of deviation. A separate diagram to illustrate this aspect is not included, because the treatment suggested is not very different from that actually carried out and shown in Table 2 and Fig. 4. The change involves practically no alteration to the upper line, but the lower one (the middle portion of Fig. 4) is made steeper. Rejection of the single point at probit 2·7825 brings the results of this experiment more closely into alinement with those of the other experiments performed at the same pH value but at different temperatures (Jordan *et al.* 1947*b*).

Table 3. *The limits to be attached to various percentage mortalities and the corresponding probit values, assuming the standard error of the bacterial count to be* ± 10 % *and the significance level* 0·05

True percentage mortality	True probit	Limits of percentage mortality	Probit limits	Probit difference
10	3·7184	0–28	– ∞–4·4172	—
20	4·1584	4–36	3·2493–4·6415	1·3922
30	4·4756	16–44	4·0055–4·8490	0·8435
40	4·7467	28–52	4·4172–5·0502	0·6330
50	5·0000	40–60	4·7467–5·2533	0·5066
60	5·2533	52–68	5·0502–5·4677	0·4175
70	5·5244	64–76	5·3585–5·7063	0·3478
80	5·8416	76–84	5·7063–5·9945	0·2882
90	6·2816	88–92	6·1750–6·4051	0·2301
99	7·3263	98·8–99·2	7·2571–7·4089	0·1518
99·9	8·0902	99·88–99·92	8·0357–8·1559	0·1202
99·99	8·7190	99·988–99·992	8·6728–8·7750	0·1022

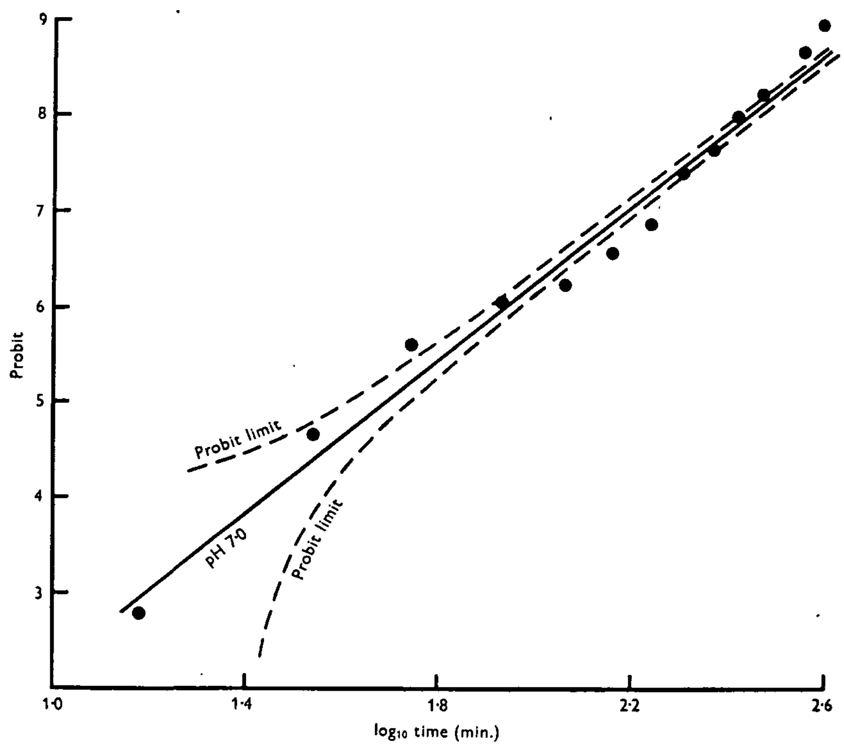

Fig. 5. Showing the best straight line to fit all the data for the experiment at pH 7·0, together with the probit limits, assuming a standard error of a bacterial count to be ± 10% and the level of significance to be 0·05 (see text).

Similarly, application of the probit limits to the results of the experiments at pH 8·8, 8·2 and 3·9 shows that here also the divergences from complete linearity are probably significant. Only at pH 4·8 is there no indication of anything other than a single straight line. It is clear that the impression of linearity or otherwise given by a set of results must depend not only on the adequacy of the technique, but also on where the points secured happen to fall on the time scale. Evidently, the aim should always be to secure as many observations as possible. When that has been done, a linear treatment of limited sections of the graph obtained may be applied in order to obtain calculated estimates of various mortality times, and it is suggested that the use of the probit limits corresponding to the expected experimental error would prove helpful in deciding the extent of the range over which linearity may be assumed in any given case.

SUMMARY

1. The nature of the probit-log survival-time relationship in the disinfection of standard cultures of *Bact. coli* at 51° C. at pH values ranging from 2·8 to 8·8 has been studied.

2. It is concluded that there is a very close approximation to a bilinear form in all cases, though there was evidence that a continuous curve concave upwards and to the left would provide a better fit.

3. Probit limits, corresponding to the range of percentage mortality within which, having regard to the experimental error involved, an observation might lie, have been worked out. The range covered by these probit limits decreases as the percentage mortality rises.

4. These limits have been used to decide whether a bilinear or a single straight line treatment should be applied to certain sets of data. It is suggested that they would often materially assist in deciding the range over which linearity may be assumed in any given case.

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