## 296. Physicochemical Aspects of Bacterial Growth. Part VII. Influence of Phenol on the Death Rate of Bact. lactis ærogenes.

By (MISS) E. A. POOLE and C. N. HINSHELWOOD.

The influence of phenol on the death rate of cells of Bact. lactis ærogenes is quantitatively smaller than the influence on the division rate. It depends upon the stage of growth at which the phenol is added. Viable counts confirm that the stationary phase is not due to a simple balancing of death rate and division rate, but depends upon an actual cessation of division.

The death of bacteria is known to follow the law  $n = n_0 e^{-\lambda t}$ , where  $n_0$  is the number of living organisms initially and n the number at time t. According to this law, the life of

individual cells must vary, the mean survival time, S, being  $1/\lambda$ , and the chance that a given cell lives for a time greater than tbeing  $e^{-t/S}$ .

In continuation of the studies in Part VI (preceding paper), the influence of phenol on S has been determined for Bact. lactis ærogenes.

Viable counts were determined by the method of Penfold (J. Hyg., 1914, 14, 215). Two series of experiments were made. In the first the organism was grown without phenol in the glucose-phosphate medium till the total count just became constant. Phenol was then added, and viable counts made at intervals. From the slope of the curve of log (count) against time,  $\lambda$ , and hence S, can be calculated.

The figure shows the total and viable counts as a function of time in the absence of phenol. The horizontal portion of the curves is important. The fact that the total count, as well as the viable count, remains constant for a considerable time indicates that there is no mere balancing of division rate and death rate in this region, but that Growth curve for glucose-phosphate medium containthe phase of division ceases entirely and is succeeded by a stationary phase. Only after this has lasted some time does death occur at the rate given by the logarithmic law followed in the final phase.



Phenol, %	0·0	0·048	0·0 <b>96</b>	0·144	0·193
S, mins	85	75	72	72	56·5
Relative value of S	1·00	0·88	0·85	0·85	0·66
	1.00	0.99	0.90	0.99	0.00

Under these conditions the influence of the phenol on S is much smaller relatively than its influence on the mean generation time or on  $n_s$ .

In the second series the death rate of inocula taken from a 12-hour culture was measured. When introduced into a new medium the inocula began to die off at a rate which could be measured before division set in. With no phenol present the magnesium had to be omitted from the medium or division began too soon for this to be possible (cf. Part V; J., 1939, 1692). In these circumstances, S, although initially greater than in the presence of the metabolic products (series 1) is very much more sensitive to the action of the poison



ing no phenol, showing total and viable counts. Key : • Total count. O Viable count.

(following table), a fact probably connected with the greater age of the culture in the second series.

Phenol, %	0.00	0.039	0.079	0.118	0.158
S, mins	(725)	76	40	28	20

(In this series the initial viable counts were about 500 times smaller than those of series 1, viz.,  $10^6$  bacteria per c.c. compared with  $5 \times 10^8$ .)

A quantity of phenol which is just sufficient entirely to inhibit growth (Part VI, Fig. 1b) lowers the mean survival time in series 1 to about 70 mins. and in series 2 to about 22 mins. The mean generation time in the glucose-phosphate medium without phenol is 46 mins. If the effect of the phenol were principally on the death rate, then, with S = 22, the proportion of each generation which could survive until fresh division occurred is easily shown to be  $e^{-46/22}$ , which corresponds to about  $12\cdot4\%$ . With S = 70, the proportion of each generation surviving for a new mean generation time would be 52%. At the concentration of phenol to which these numbers correspond division, in fact, is completely inhibited.

Oxford University.

[Received, August 8th, 1940.]