SALT EFFECTS IN BACTERIAL GROWTH[1]

II. THE GROWTH OF BACT. COLI IN RELATION TO H-ION CONCENTRATION

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It has been shown in a previous publication (Holm and Sherman, 1921) that sodium chloride and various other neutral salts in 0.20M concentration affected the rate of growth of Bact. coli. Using neutral salts with a common cation (sodium) but with various anions a marked difference was observed between the action of the various salts. The effect of the chlorides of sodium, potassium and ammonium seemed to be approximately the same while the calcium and iron salts tested retarded greatly or inhibited growth. These experiments were carried out at a pH of approximately 7.0 and with a salt concentration of 0.20M in 1 per cent pepton.

In as much as we know that there are limiting pH values for bacterial growth, varying with different organisms, it would be of interest and value to know just to what extent this neutral salt action is affected by various H-ion concentrations In the following experiments, as in our former communication, the rate of growth was determined by the time that expired between inoculation and the first sign of turbidity. The medium used was 1 per cent pepton to which had been added various amounts of salts, and the H-ion concentration adjusted by the use of concentrated HCl and NaOH solutions.

The effects of various concentrations of NaCl at various H-ion concentrations were first tried. The H-ion and salt concentra-

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tions, and the time for growth in each series, are shown in table 1. This table shows a decided accelerating effect upon growth with added NaCl in low concentrations. Although there was little difference between the effects of 0.10, 0.20 and 0.30M NaCl media upon growth, there seems to be an optimum effect at about 0.20M. The optimum H-ion concentration for growth either in controls or in pepton containing NaCl at various concentrations seems to be about the same, approximately pH 7.8. At optimum salt concentrations there is very little difference in the rate of growth over a wide range of H-ion concentration,

TABLE 1

The rate of growth of Bact. coli in various concentrations of NaCl in 1 per cent peptone and at various H-ion concentrations

NaCl CONCENTRATION	SERIES I		SERIES II		SERIES III		SERIES IV		SERIES V	
	pH	Hours	pH	Hours	pH	Hours	pH	Hours	pH	Hours
Control	5.3	36	6.3	10¾	7.0	7	7.7	6	8.3	7
0.05M	5.3	6¾	6.3	8½	7.1	5¾	7.7	3¾	8.3	4
0.10M	5.3	4	6.4	4	7.1	4	7.8	3¼	8.3	3½
0.20M	5.3	4	6.5	3½	7.2	3½	7.8	3¼	8.3	3½
0.30M	5.3	4½	6.5	3¾	7.3	3¾	7.9	3¾	8.3	3½
0.40M	5.3	5¼	6.5	4½	7.3	4	7.9	4	8.3	4

varying from 5.3 to 8.3 on the pH scale, while in the pepton solution alone the range is somewhat narrower. Beyond the range for optimum growth there seems to be a decided retardation for each small change of H-ion concentration. These results are brought out more clearly in figure 1 which shows a pronounced widening of the limiting pH values for growth with added NaCl, especially in optimum salt concentrations, and a retardation of growth for each small change in pH near the limiting values.

In order to ascertain if there was actually a shifting of the limits of growth, or merely a widening of the optimum range for growth, the effect of NaCl was tried at pH values representing the approximate limit of growth in the acid region. At a pH of 4.8 it was found that only rarely would Bact. coli grow in 1 per cent pepton at 37°C., but that it did grow quite readily in the

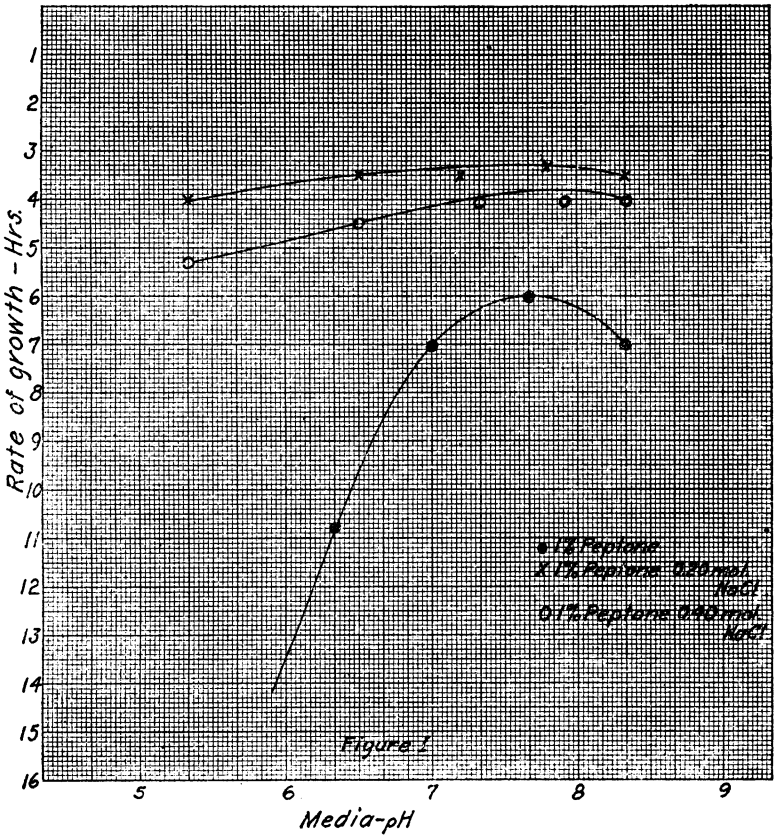


TABLE 2

The shifting of the limit of growth of Bact. coli at 37°C. in the acid region by the addition of NaCl

TEST NUMBER	TIME REQUIRED TO SHOW TURBIDITY AT A pH VALUE OF 4.8 IN	
	1 per cent pepton	1 per cent pepton 0.20 M NaCl
	hours	*hours*
1	No growth	18
2	No growth	22
3	No growth	18
4	No growth	18
5	26	21

same medium to which had been added NaCl to make a 0.20M solution. These media were adjusted colorimetrically, the end point being determined with methyl red, and it is of course recognized that there may be a slight error in the measurements. The significant fact is not whether the point established is exactly pH 4.8 but that the limiting H-ion zone of growth may be

TABLE 3

The shifting of the limit of growth of Bact. alkaligenes at 37°C. in the acid region by the addition of NaCl

MEDIUM	TEST NUMBER	TIME REQUIRED TO SHOW TURBIDITY AT pH VALUES OF		
		5.6	5.4	5.2
		hours	*hours*	*hours*
1 per cent pepton	1	90	No growth	No growth
1 per cent pepton	2	No growth	No growth	
1 per cent pepton 0.20M NaCl	1	24	24	120
1 per cent pepton 0.20M NaCl	2	24	24	

TABLE 4

The effect of NaCl and Na citrate upon Bact. coli at various H-ion concentrations

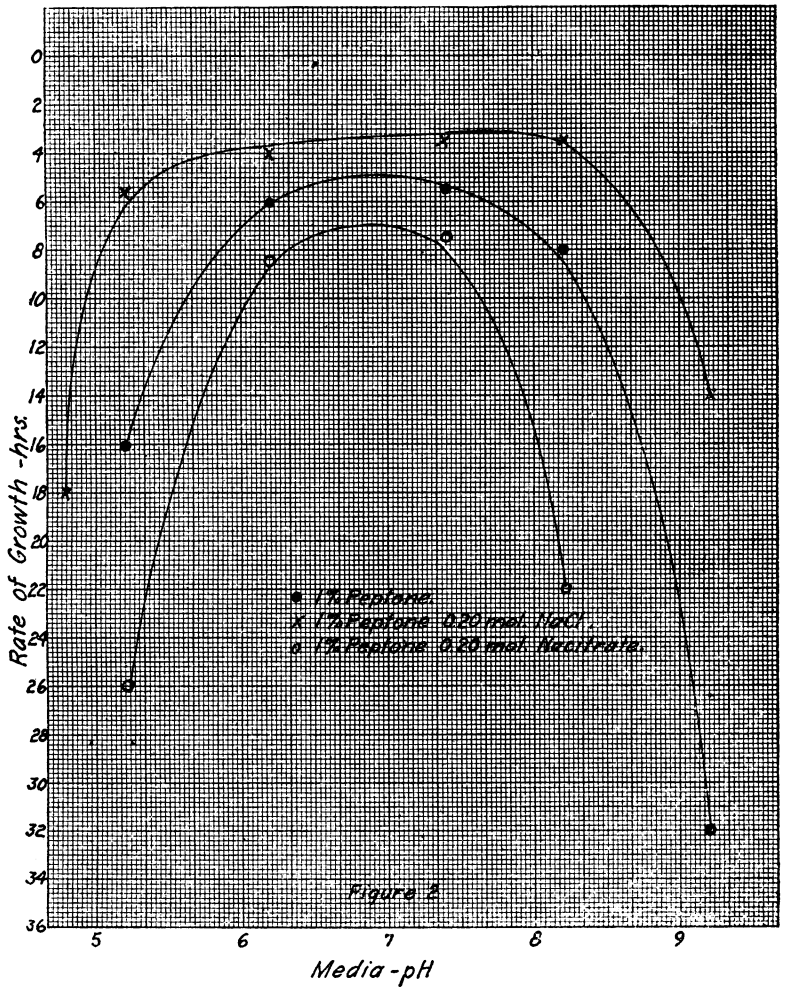
MEDIA	TIME REQUIRED TO SHOW TURBIDITY AT pH VALUE OF					
	4.8	5.2	6.2	7.6	8.2	9.2
	hours	*hours*	*hours*	*hours*	*hours*	*hours*
1 per cent pepton	No growth	16	6	5½	8	32
1 per cent pepton 0.20M NaCl	18	5½	4	3½	3½	14
1 per cent pepton 0.20M Na citrate	No growth	26	8½	7½	22	No growth

modified by the addition of NaCl to the medium. The results of five different tests made under these conditions are recorded in table 2.

Although the widening effect upon the pH limit of growth is not general for all bacteria which we have tried, we have found the effect upon Bact. alkaligenes to be even more pronounced than the effect upon Bact. coli. This is shown in table 3.

Tables 2 and 3 show that there is actually an extension of the zone in which Bact. coli and Bact. alkaligenes will grow in the

acid region by the addition of 0.20M NaCl. It is possible that this effect might be increased by using a more dilute salt solution, and perhaps, in the same way, this effect might be produced



with organisms which thus far have failed to show any such modification in their limits of growth.

The same results which we have noted with NaCl may be produced with other neutral salts, but the degree of the widening

effect varies with the nature of the salt. On the other hand, a salt (e.g., Na citrate) which lowers the rate of growth also narrows the limits of H-ion concentration at which Bact. coli will grow. Table 4 shows the results obtained with NaCl and Na citrate, as compared with pepton alone, at different H-ion concentrations. These results are shown to better advantage in figure 2 which brings out clearly the widening effect on the limits of growth with NaCl, while Na citrate shows a decided narrowing of the range.

REFERENCE

HOLM, G. E., AND SHERMAN, J. M. 1921 Jour. Bact., **6**, 511.