SALT EFFECTS IN BACTERIAL GROWTH

III. SALT EFFECTS IN RELATION TO THE LAG PERIOD AND VELOCITY OF GROWTH[1]

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In a previous paper of this series (Holm and Sherman, 1921), it has been shown that certain neutral salts, in proper concentrations, accelerate the growth of *Bact. coli*, as measured by the time required to produce visible turbidity, the time required to reduce methylene blue, and the rate of acid production in the presence of a fermentable carbohydrate.

In the present work we have extended these experiments by the use of the plate count in an effort to throw more light on the mechanism of the salt action. Since our previous experiments have shown that the salt effect is magnified on the acid side of the region of optimum growth (Sherman and Holm, 1922), we have used in the present work media adjusted to a reaction of pH 5.4. All of the counts here reported represent the average of triplicate plates on extract-pepton agar incubated for three days at 33°C. Further details of the experiments are given in the appendix.

From figures 1 and 2, plotted from the data obtained in experiments 1 and 2 in which the growth of *Bact. coli* in 1 per cent pepton, 1 per cent pepton plus 0.2 M NaCl, and 1 per cent pepton plus 0.1 M Na_2SO_4 was measured, it is seen that the accelerating action of the salts is due to an increase in the velocity of growth of the organisms. In other words, the period of logarithmic growth is shortened since the number of bacteria present in the

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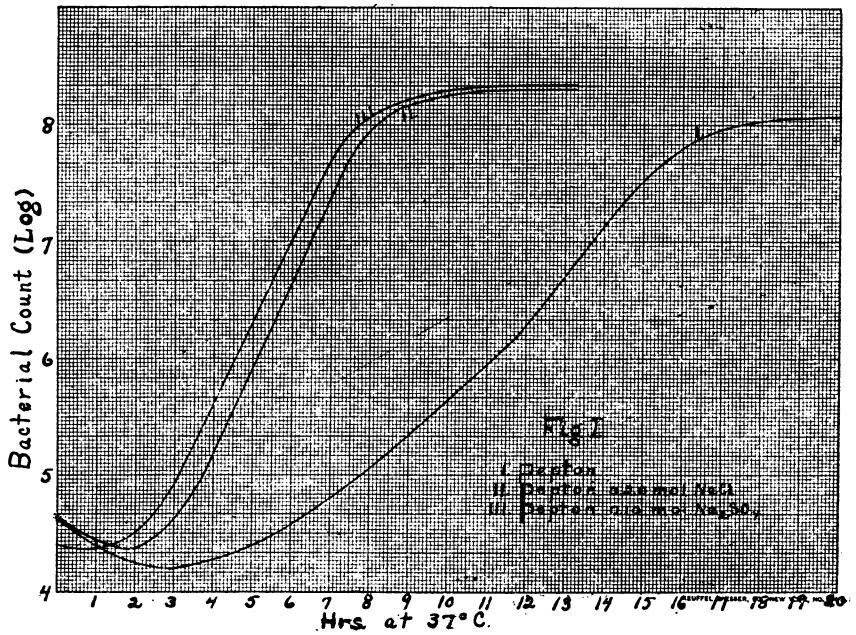
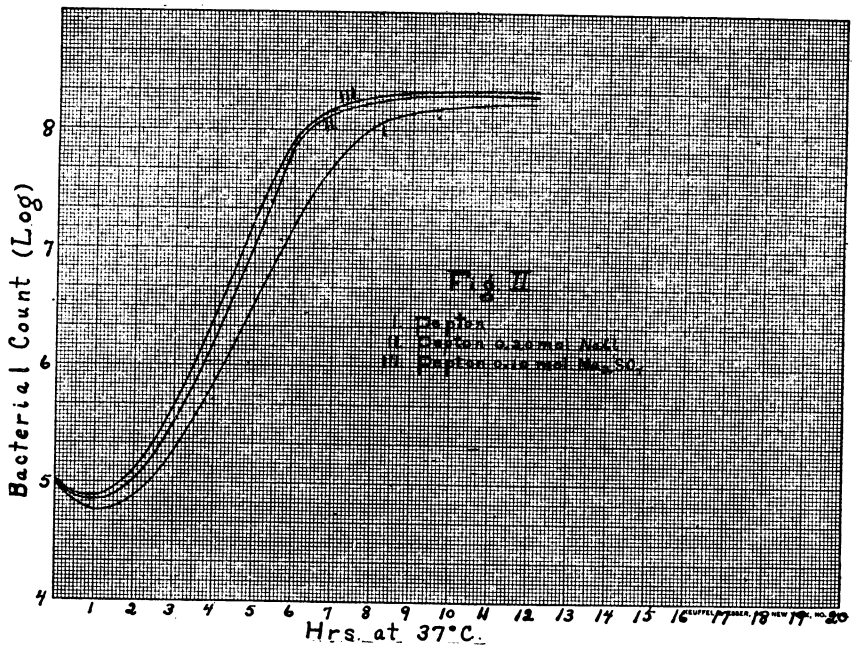
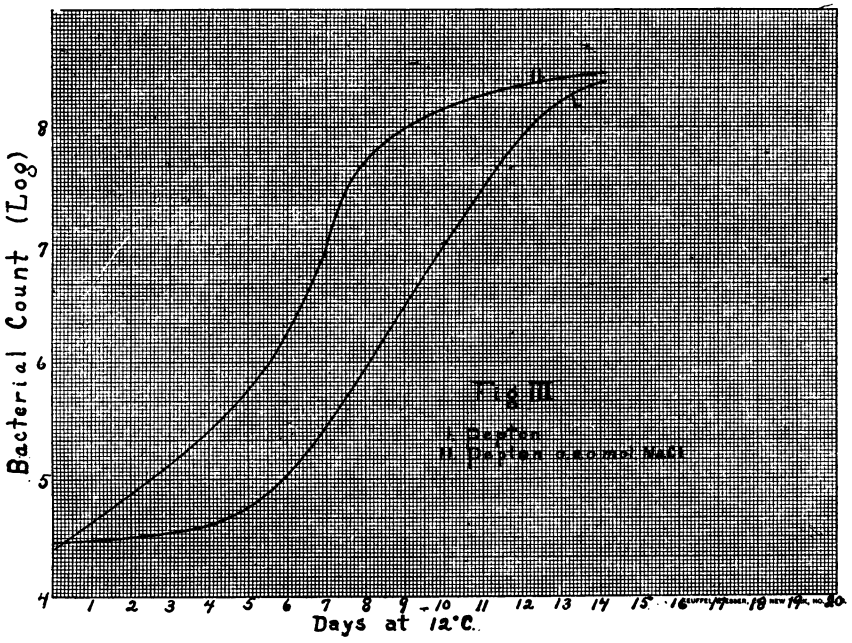

Bacterial Count (Log)
8
7
6
5
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Hrs at 37°C.
III
II
I
Fig I
I Peptone
II Peptone 0.22 mol NaCl
III Peptone 0.17 M. mol Na2SO4


Bacterial Count (Log)
8
7
6
5
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Hrs at 37°C.
III
II
I
Fig II
I Peptone
II Peptone 0.22 mol NaCl
III Peptone 0.17 M. mol Na2SO4

culture at the breaking point of the growth curve is approximately the same in all cases. It also appears that the salts have the effect of shortening the latent period previous to rapid growth. This difference is marked in the case of the sulphate (0.1 M), which appears to have a somewhat greater accelerating effect than the chloride, while in the case of the NaCl (0.2 M) the shortening effect upon the period of lag is not so definite; or may



be entirely lacking, as is indicated by the results of experiment 2.

The effect of NaCl upon the period of lag was therefore extended in experiments 3 to 6 in which plate counts were made at hourly intervals. The results of these additional experiments again show that while NaCl may or may not decrease the latent period, it increases in every case the velocity of growth during the period of active multiplication.

It was thought that it might be possible to magnify the effect of NaCl by incubation at a temperature which allowed only a

slow multiplication of the organisms. This was done in tests in which cultures were incubated at a temperature of 12°C. At this temperature the increments of growth when measured daily are about the same as those taken at hourly intervals at 37°C. The results obtained in these tests (experiments 7 and 8) show the same characteristic increase in the velocity of growth with NaCl, and also a well defined shortening of the lag period. The data from experiment 7 are plotted in figure 3.

SUMMARY

It has been shown that the accelerating effect of certain salts upon the growth of *Bact. coli* is due primarily to an increase in the velocity of growth of the organism during the period of maximum multiplication.

The same salts usually also increase the accelerating effect by decreasing the duration of the preliminary latent period.

REFERENCES

HOLM, G. E., AND SHERMAN, J. M. 1921 Jour. Bact., 6, 511.
SHERMAN, J. M., AND HOLM, G. E. 1922 Jour. Bact., 7, 465.

APPENDIX

The organism used in all of these experiments was a laboratory strain of *Bact. coli.* Inoculations were made from cultures one or more days old in 1 per cent pepton.

The media used for determining the growth rates were put up in 100-cc. amounts; all contained 1 per cent pepton, with the indicated amount of salt, and were adjusted to a reaction of pH 5.4.

In experiments 1 to 6, incubations were at 37°C. and plate counts were made at hourly intervals. Experiments 7 and 8 were conducted at 12°C. and counts made at daily intervals.

Standard extract-pepton agar was used for plating, and the plates were incubated for three days at 33°C. before counting. Triplicate plates of each dilution were made in every case.

Experiment 1

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	Pepton	0.20 M NaCl	0.10 M Na_2SO_4 M Na_2SO_4
0	39,000	41,000	26,000
1	29,000	28,000	24,000
2	17,000	23,300	33,700
3	16,000	48,000	86,000
4	17,000	163,000	473,000
5	33,000	620,000	2,630,000
6	47,000	4,700,000	12,300,000
7	66,000	29,300,000	76,000,000
8	117,000	135,000,000	153,000,000
9	210,000	164,000,000	149,000,000
10	410,000		183,000,000
11	780,000	188,000,000	198,000,000
12	1,560,000	208,000,000	183,000,000
13	4,300,000	231,000,000	220,000,000
14	12,100,000		
16	67,000,000		
18	104,000,000		
20	121,000,000		

Experiment 2

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	Pepton	0.20 M NaCl	0.10 M Na_2SO_4 M Na_2SO_4
0	99,000	95,000	103,000
1	58,000	79,000	78,000
2	77,000	106,000	125,000
3	300,000	181,000	471,000
4	766,000	1,750,000	2,630,000
5	3,730,000	8,500,000	16,300,000
6	17,900,000	74,000,000	74,000,000
7	49,500,000	172,000,000	168,000,000
8	109,000,000	181,000,000	191,000,000
9	149,000,000	169,000,000	201,000,000
10	148,000,000	190,000,000	209,000,000
11	162,000,000	207,000,000	214,000,000
12	175,000,000	214,000,000	215,000,000

Experiment 3

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	54,000	56,000
1	52,000	52,000
2	49,000	76,000
3	86,000	129,000
4	152,000	480,000
5	360,000	780,000
6	790,000	14,100,000
7	3,200,000	53,000,000

Experiment 4

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	56,000	57,000
1	51,000	54,000
2	59,000	59,000
3	94,000	57,000
4	148,000	139,000
5	273,000	1,120,000
6	790,000	20,600,000
7	23,700,000	105,000,000

Experiment 5

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	35,000	44,000
1	41,000	42,000
2	87,000	83,000
3	310,000	203,000
4	1,090,000	423,000
5	8,100,000	23,800,000
6	23,500,000	64,000,000
7	29,200,000	63,000,000

Experiment 6

HOURS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	126,000	119,000
1	128,000	132,000
2	175,000	188,000
3	676,000	940,000
4	2,660,000	4,440,000
5	17,800,000	85,000,000
6	24,500,000	135,000,000
7	45,200,000	205,000,000

Experiment 7

DAYS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	28,200	25,300
1	29,000	37,500
2	38,000	69,000
3	38,600	161,000
4	47,000	240,000
5	46,000	523,000
6	129,000	1,630,000
7	360,000	14,750,000
8	834,000	54,000,000
9	3,100,000	96,000,000
10	9,300,000	150,000,000
11	45,000,000	185,000,000
12	73,000,000	148,000,000
13	156,000,000	280,000,000
14	260,000,000	290,000,000

Experiment 8

DAYS	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	Pepton	0.20 M NaCl
0	26,800	30,000
1	29,500	36,000
2	41,000	57,000
3	36,000	182,000
4	52,000	257,000
5	53,000	340,000
6	186,000	1,480,000
7	860,000	16,600,000
8	2,020,000	54,000,000
9	7,300,000	91,000,000
10	35,000,000	160,000,000
11	56,000,000	160,000,000
12	88,000,000	186,000,000
13	165,000,000	250,000,000
14	250,000,000	270,000,000