STUDIES ON SALT ACTION.

X. The Influence of Electrolytes upon the Viability and Electrophoretic Migration of Bacterium coli.*

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INTRODUCTION.

Among the numerous contributions dealing with the effect of electrolytes upon bacterial physiology (exhaustively reviewed by Falk, 1923) there are two sets of studies which bear directly on the work here reported.

Holm and Sherman (1921) showed that the growth of *Bacterium coli* in 1 per cent peptone solution is accelerated by certain anions and by certain cations in .2 M concentration and retarded by others. Sodium chloride, KCl, and NH_4Cl were particularly favorable, Na fluoride and $CaCl_2$ particularly unfavorable. It was of special interest to note that NaCl and Na_2SO_4 widened the zone of hydrogen ion concentration within which the organism would develop, making its growth fairly rapid at pH 5.2 and at pH 8.2. On the other hand more toxic salts, such as Na citrate, showed an additive rather than an antagonistic effect, narrowing the zone of acidity and alkalinity within which the organisms would develop freely. Sherman and Holm (1922) and Sherman, Holm, and Albus (1922) confirmed and extended these results.

A second series of papers, from our own laboratory, has dealt with the same general problems. Hotchkiss (1923), using the method employed by Sherman and his colleagues, worked out the inhibitive concentration of twenty-three cations in union with chlorine. The

* Based on an essay presented by the junior author in candidacy for the Certificate in Public Health at Yale University.

monovalent ions were in general least toxic, followed by the alkaline earth metals and then by the heavy metals. Fifteen of the twenty-three salts studied, including such toxic substances as $HgCl_2$, $CeCl_3$ and $ZnCl_2$, proved stimulating, rather than inhibitive, in very low concentration. Winslow and Falk (1923, *a*) obtained more detailed data on the influence of NaCl and $CaCl_2$ in varying concentration and at various pH values upon the viability of *Bacterium coli* in water. The quantitative effects in this case would naturally be expected to be quite different from those observed in a peptone culture medium. These observers found that .0145 M NaCl and .00145 M $CaCl_2$ were stimulating, while .725 M NaCl and .435 M $CaCl_2$ were distinctly toxic at all reactions. Calcium chloride in .145 M strength proved toxic only in an unadjusted alkaline solution, where its action was apparently due to interference with the normal power of the bacteria to adjust the reaction of a zone of contiguous liquid to a favorable pH value. In a second paper (Winslow and Falk, 1923, *b*) these investigators show that in a mixture of four parts of NaCl to one part of $CaCl_2$, with a total tonicity of .725 M, the inhibitive effect which either salt would by itself exert, disappears as a result of mutual antagonism. This antagonistic action, however, takes place only on the alkaline side of the neutral point.

In the present study we desired to repeat some of the work of Winslow and Falk and to supplement it by observation of the effect of what physiologists consider a more completely balanced solution, using for this purpose a Ringer-Locke solution (.155 M NaCl, .003 M KCl, and .002 M $CaCl_2$)—of slightly more than isotonic strength.

We also desired to compare the influences produced by the electrolytes studied upon viability with the effects exerted upon migration in the electrical field. Northrop and De Kruif (1921–22), Winslow, Falk, and Caulfield (1923–24) Winslow and Shaughnessy (1923–24), and Winslow and Fleeson (1925–26) have shown that electrolytes markedly influence electrophoretic charge; and Eggerth (1923–24) has suggested that such changes run more or less parallel to simultaneous changes in viability, at least in buffered solutions. The work of Northrop and De Kruif and of Winslow and Fleeson on the other hand strongly suggests that the effects produced on electrophoretic charge are only remotely connected with vital phenomena. There are, however, certain

suggestive parallelisms between the curves for cataphoresis and those for viability which made it seem worth while to test the point somewhat more fully.

Technique.

Our work was all carried out with a single strain of *Bacterium coli* isolated in this laboratory in 1924 from New Haven sewage. It was cultivated on standard nutrient agar in Kolle flasks for 20–24 hours at 37°C. The growth was washed off in distilled water, filtered through absorbent cotton, then centrifuged, and rewashed three times, always under sterile conditions. The final washed suspension was shaken with glass beads and seeded into flasks of the appropriate menstruum. The seeded flasks were kept for 4 hours at room temperature and plates were made immediately after the first seeding and at the end of the 1st, 2nd, and 4th hours. At each of these periods the reaction of each flask was readjusted to its original reaction, to compensate for the buffering influence of the bacterial cells, using the indicators of Clark and Lubs, at pH values 2.0, 6.0, and 8.0 and the indicators of Prideaux (1917) with Northrop and De Kruif's buffer solution at pH 11.0.

The tests of electrophoretic velocity were made according to the methods previously used in this laboratory and described by Winslow, Falk, and Caulfield (1923–24) and by Winslow and Fleeson (1925–26).

The fundamental menstrua used were distilled water, Ringer-Locke solution, sodium chloride in four concentrations (1.45 M, .725 M, .145 M, and .0145 M), and calcium chloride in the same four concentrations—each solution being studied at four different reactions (pH 2.0, pH 6.0, pH 8.0, and pH 11.0).

Results in Regard to Viability.

The results of our study, so far as viability is concerned, are presented in the form of averages in Tables I to X and in Figs. 1 and 2.

It is at once apparent from tables and charts that strongly acid solutions (pH 2.0) were highly toxic under all conditions, the vast majority of the bacteria being no longer viable at the end of the 1st hour. A highly alkaline solution (pH 11.0) was almost as deadly, except in distilled water where one-third of the bacteria survived for 2 hours (Table I).

TABLE I.

Viability of Bacterium coli in Distilled Water.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	100.0	100.0	100.0	100.0
1	1.0	195.0	171.0	79.0
2	1.0	146.0	109.0	35.0
4	1.0	115.0	120.0	4.0
No. of experiments...	5	5	4	3

TABLE II.

Viability of Bacterium coli in Ringer's Solution.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	100.0	100.0	100.0	100.0
1	1.0	101.0	73.0	0.0
2	2.0	107.0	141.0	0.0
4	1.0	198.0	65.0	0.0
No. of experiments...	4	5	5	3

TABLE III.

Viability of Bacterium coli in Sodium Chloride Solution. 1.45 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	100.0	100.0	100.0	100.0
1	13.0	243.0	4.0	0.0
2	3.0	118.0	1.0	0.0
4	2.0	65.0	0.0	0.0
No. of experiments...	3	4	3	2

TABLE IV.

Viability of Bacterium coli in Sodium Chloride Solution. .725 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	—	100.0	100.0	—
1	—	120.0	36.0	—
2	—	131.0	29.0	—
4	—	41.0	4.0	—
No. of experiments...	0	4	3	0

TABLE V.

Viability of Bacterium coli in Sodium Chloride Solution. .145 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	—	100.0	100.0	—
1	—	90.0	88.0	—
2	—	49.0	58.0	—
4	—	88.0	112.0	—
No. of experiments...	0	3	3	0

TABLE VI.

Viability of Bacterium coli in Sodium Chloride Solution. .0145 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	100.0	100.0	100.0	100.0
1	0.1	133.0	90.0	0.0
2	0.1	108.0	55.0	0.0
4	0.1	91.0	60.0	0.0
No. of experiments...	3	4	4	2

TABLE VII.

Viability of Bacterium coli in Calcium Chloride Solution. 1.45 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	—	100.0	100.0	—
1	—	12.0	18.0	—
2	—	24.0	0.0	—
4	—	0.0	0.0	—
No. of experiments...	0	3	2	0

TABLE VIII.

Viability of Bacterium coli in Calcium Chloride Solution. .725 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	—	100.0	100.0	—
1	—	52.0	9.0	—
2	—	526.0	5.0	—
4	—	98.0	2.0	—
No. of experiments...	0	3	2	0

TABLE IX.

Viability of Bacterium coli in Calcium Chloride Solution. .145 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	—	100.0	100.0	—
1	—	155.0	50.0	—
2	—	151.0	8.0	—
4	—	64.0	10.0	—
No. of experiments...	0	3	2	0

TABLE X.

Viability of Bacterium coli in Calcium Chloride Solution. .0145 M.

Hrs.	Per cent alive at pH			
	2	6	8	11
0	100.0	100.0	100.0	100.0
1	0.3	130.0	173.0	0.0
2	1.0	40.0	50.0	0.0
4	0.4	65.0	45.0	0.0
No. of experiments...	2	3	3	2

In comparing the effect of the various salts at the two intermediate pH values results are less consistent, particularly after the 1st and 2nd hours (note the sharp rise at pH 6.0 in Tables III and VIII). At the end of 4 hours, however, after the electrolytes have had time to exert their full effect, the figures are reasonably consistent, as indicated in Figs. 1 and 2.

At pH 6.0, the Ringer-Locke solution is most favorable to viability, followed by the distilled water and then by the salt solutions in approximate order of concentration.

The .725 M concentration alone happens to be out of place in both charts, appearing to be more toxic than it should in the case of NaCl and less toxic in the case of $CaCl_2$. Such aberrations must be expected in work of this kind unless a very large series of experiments have been averaged.

The most striking point brought out by these charts is, however, that at pH 8.0 the curve of viability in distilled water does not drop but instead rises slightly while all the salt solutions (except .145 M NaCl) fall sharply; and even the Ringer-Locke solution which was more favorable than water at pH 6.0 falls far below it at pH 8.0. Apparently, in a solution with adjusted reaction and unbuffered (except for the action of the cells themselves), the harmful effect of Na or Ca is accentuated by slight alkalinity and Ringer solution, which is favorable when acid, becomes unfavorable.

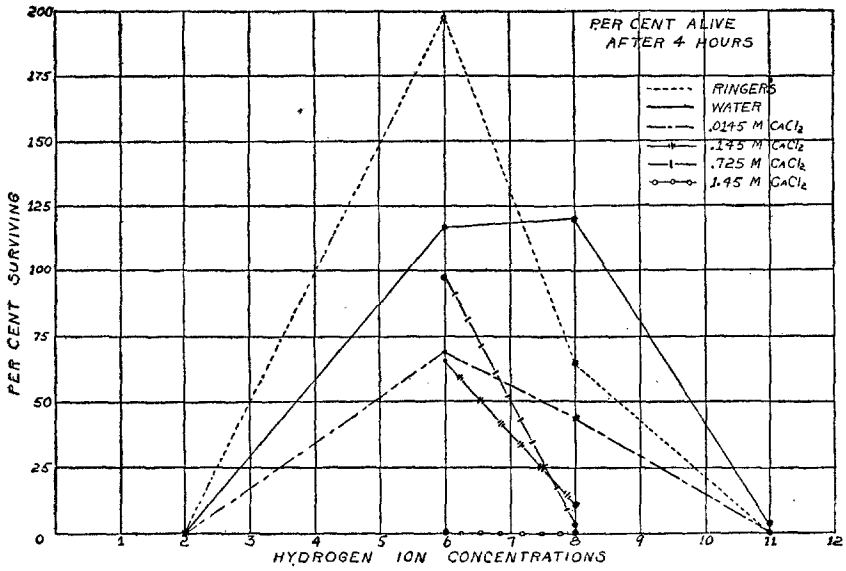
PER CENT ALIVE
AFTER 4 HOURS
RINGER'S
WATER
.0145 M CaCl2
.145 M CaCl2
.725 M CaCl2
1.45 M CaCl2
PER CENT SURVIVING
HYDROGEN ION CONCENTRATIONS

FIG. 1.

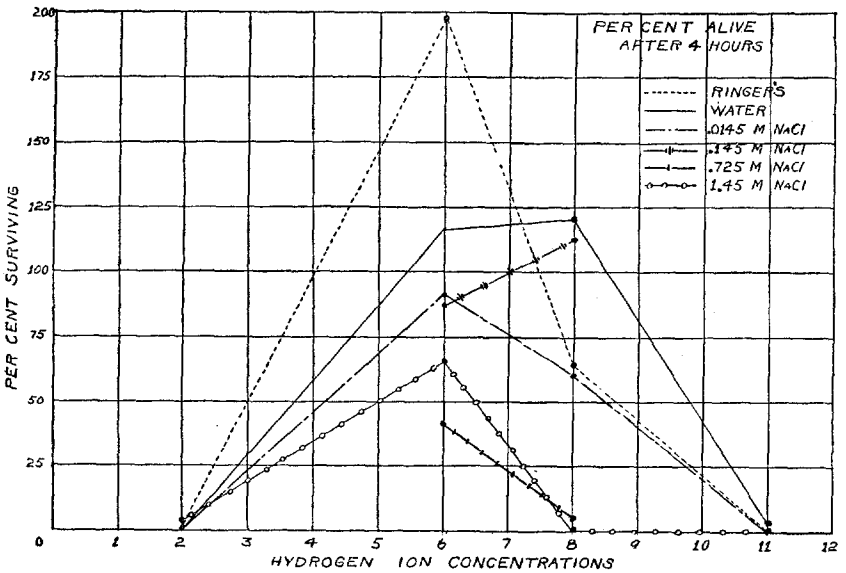
PER CENT ALIVE
AFTER 4 HOURS
RINGER'S
WATER
.0145 M NaCl
.145 M NaCl
.725 M NaCl
1.45 M NaCl
PER CENT SURVIVING
HYDROGEN ION CONCENTRATIONS

FIG. 2.

Results in Regard to Electrophoretic Charge.

Our results on migration velocity in the electrical field are presented in Tables XI to XV and in Fig. 3. The figures represent velocity of migration in micra per second in a microscopic cell connected by zinc zinc-sulfate electrodes with a current giving a potential gradient of 12 volts per cm. (see Winslow, Falk, and Caulfield, 1923–24, and Winslow and Fleeson, 1925–26, for description of the technique employed).

A + sign indicates migration toward the cathode; in the absence of any sign, migration is toward the anode.

The time element in these unbuffered solutions proved of no significance, the electrophoretic charge after 1 hour being essentially the same as that observed after 4 hours.

TABLE XI.

Electrophoretic Velocity of Bacterium coli in Distilled Water—Micra per Second.

pH.....	2	6	8	11
Hrs.				
0	+.5	17.3	19.7	8.9
1	+.5	23.6	24.9	13.2
2	+.5	13.8	14.1	13.3
4	0	20.1	20.6	17.0
No. of experiments...	4	4	3	1

TABLE XII.

Electrophoretic Velocity of Bacterium coli in Ringer's Solution—Micra per Second.

pH.....	2	6	8	11
Hrs.				
0	0	4.0	4.0	6.7
1	0	4.1	4.4	7.0
2	0	3.9	4.7	7.2
4	0	3.9	3.9	7.5
No. of experiments...	4	3	2	1

TABLE XIII.

Electrophoretic Velocity of Bacterium coli in Sodium Chloride Solution. 1.45 M. *Micra per Second.*

pH.....	2	6	8	11
Hrs.				
0	0	1.4	4.7	5.0
1	0	1.2	4.3	5.7
2	0	1.6	4.6	5.1
4	0	3.1	6.9	7.0
No. of experiments...	1	1	1	1

TABLE XIV.

Electrophoretic Velocity of Bacterium coli in Sodium Chloride Solution. .0145 M. *Micra per Second.*

pH.....	2	6	8	11
Hrs.				
0	0	9.8	12.1	11.9
1	0	12.2	12.3	11.8
2	0	11.2	12.7	9.8
4	0	12.8	13.5	12.0
No. of experiments...	3	3	2	1

TABLE XV.

Electrophoretic Velocity of Bacterium coli in Calcium Chloride Solution. .0145 M. *Micra per Second.*

pH.....	2	6	8	11
Hrs.				
0	0	1.9	1.5	13.6
1	0	1.6	3.3	Aggl.
2	0	1.2	2.8	"
4	0	1.4	2.7	"
No. of experiments...	2	2	1	1

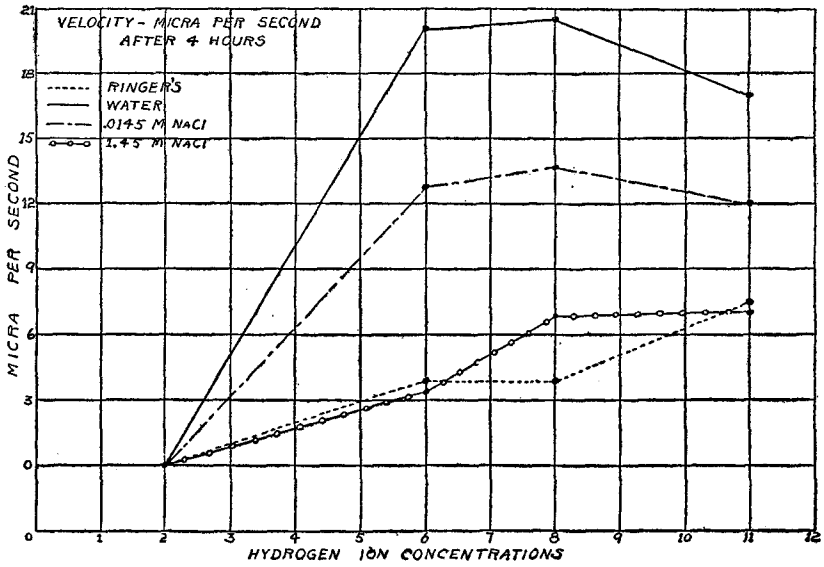
VELOCITY - MICRA PER SECOND
AFTER 4 HOURS
RINGER'S
WATER
.0145 M NaCl
1.45 M NaCl
MICRA PER SECOND
HYDROGEN ION CONCENTRATIONS

FIG. 3.

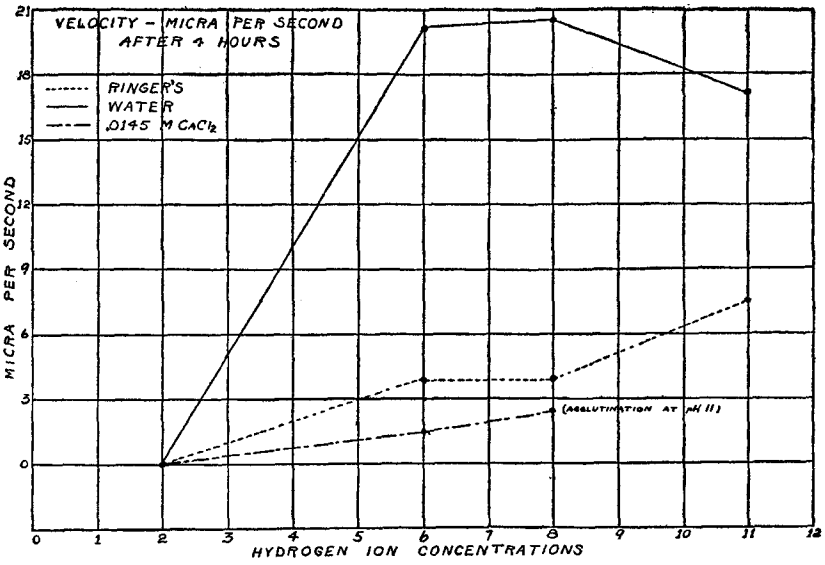
VELOCITY - MICRA PER SECOND
AFTER 4 HOURS
RINGER'S
WATER
.0145 M CaCl2
(AGGLUTINATION AT pH 11)
MICRA PER SECOND
HYDROGEN ION CONCENTRATIONS

FIG. 4.

The highest velocity of migration, as is usual in such experiments, was observed in water at pH 8.0 although water at pH 6.0 gave almost the same value. At pH 2.0 the velocity was greatly reduced, obliterated, or reversed, which might appear to be related to the death of the cells at this reaction. At pH 11.0, however, which is a reaction almost as inimical to viability, the electrophoretic charge falls but slightly. In Ringer solution and the NaCl solutions, indeed, the velocity is greater at pH 11.0 than at pH 8.0. NaCl in .0145 M strength depresses the velocity of the cells only to a moderate degree while Ringer's solution (favorable to viability) depresses it about as does the toxic 1.45 M NaCl and .0145 M $CaCl_2$.

SUMMARY.

1. The strain of *Bacterium coli* used in these experiments multiplies in distilled water at pH 6.0 and pH 8.0 and in Ringer-Locke solution at pH 6.0. Under all the other conditions studied the numbers decrease with the passage of time.

2. The electrophoretic charge of the cells is highest in distilled water at pH 6.0 and pH 8.0. Under all other conditions studied the velocity of migration is decreased, but the decrease is immediate and is not affected by more prolonged exposure.

3. A strongly acid solution (pH 2.0) causes a rapid death of the cells and a sharp decrease in electrophoretic charge, sometimes leading to complete reversal.

4. A strongly alkaline solution (pH 11.0) is almost as toxic as a strongly acid one, although in distilled water the organisms survive fairly well at this reaction. Electrophoretic charge, on the other hand, is only slightly reduced in such an alkaline medium.

5. In distilled water, reactions near the neutral point are about equally favorable to both viability and electrophoretic charge, pH 8.0 showing slightly greater multiplication and a slightly higher charge than pH 11.0. In the presence of salts, however, pH 8.0 is much less favorable to viability and somewhat more favorable to electrophoretic charge than is pH 6.0.

6. Sodium chloride solutions, in the concentrations studied, all proved somewhat toxic and all tended to depress electrophoretic charge. Very marked toxicity was, however, exhibited only in a con-

centration of .725 M strength or over and at pH 8.0, while electrophoretic migration velocity was only slightly decreased at a concentration of .0145 M strength.

7. Calcium chloride was more toxic than NaCl, showing very marked effects in .145 M strength at pH 8.0 and in 1.45 M strength at pH 6.0. It greatly depressed electrophoretic charge even in .0145 M concentration.

8. Ringer-Locke solution proved markedly stimulating to the growth of the bacteria at pH 6.0 while at pH 8.0 it was somewhat toxic, though less so than the solutions of pure salts. It depressed migration velocity at all pH values, being more effective than NaCl in this respect, but less effective than $CaCl_2$.

9. It would appear from these experiments that a balanced salt solution (Ringer-Locke's) may be distinctly favorable to bacterial viability in water at an optimum reaction while distinctly unfavorable in a slightly more alkaline solution.

10. Finally, while there is a certain parallelism between the influence of electrolytes upon viability and upon electrophoretic charge, the parallelism is not a close one and the two effects seem on the whole to follow entirely different laws.

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