THE EFFECTS OF SOME ELECTROLYTES ON THE BUFFERING CAPACITY OF BACTERIUM COLI

H. J. SHAUGHNESSY AND I. S. FALK

Department of Public Health, Yale School of Medicine, New Haven, Connecticut

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I. INTRODUCTION

Studies which have been reported from this laboratory on the relation of hydrogen ion concentration to viability of *Bact. coli* (Winslow and Falk, 1923) have suggested the great importance of certain amphoteric reactions of the bacterial cell. The experiments recorded here were undertaken with the view of studying, from another angle, certain anomalous results of the studies mentioned above. These anomalies are fully explained in the paper cited above but may perhaps be briefly mentioned here. It was found that certain sodium chloride solutions which were initially alkaline and in which the hydrogen ion concentration was not regulated showed toxicities to *Bact. coli* comparable with slightly acid solutions. On the other hand calcium chloride solutions starting at the same unregulated alkalinity showed toxicities which were comparable with those in alkaline rather than those in acidic solution. As a matter of fact the percentage viability in calcium chloride solutions at unknown pH (unknown because the experiments were first conducted without measuring or controlling the hydrogen ion concentration) was not comparable with the viability at any one pH value in similar solutions in which pH was held sensibly constant by repeated adjustments. It appeared that the explanation for these anomalous toxicities was found to lie in the fact that in sodium chloride solutions, as in water, there was a general shifting of the pH of the suspension *toward* neutrality (the most favorable zone for the viability of *Bacterium coli*); and that in these solutions of calcium

chloride, on the contrary, this shifting of pH was very much retarded. It was found, however, that the shift of pH in water and in sodium chloride solution was insufficient to bring the suspension *into* the zone of favorable viability and hence could not account completely for the observed results. The authors then conceived the idea that there might be a minute zone of solution immediately surrounding the bacterial cell which was buffered to a favorable pH by cell metabolites before the bulk of the menstruum had been similarly modified. In a test of this idea made by shaking part of each of a series of suspensions and leaving the remainder of each suspension unshaken, it was found that the pH of a shaken solution was shifted toward pH 7 more rapidly than the pH of its unshaken aliquot, due, according to this theory, to the dissemination of the buffering substances first contained in these minute zones contiguous to the cells throughout the solution in general nearly as rapidly as they were excreted. Experimental findings had already indicated that $CaCl_2$ reduced the rate of this type of buffering more than NaCl. Hence it appeared that the anomalous toxicity of alkaline $CaCl_2$ solutions could be accounted for on these grounds. It therefore seemed to the authors that it was of some importance to find a measure of the buffering capacity of *Bacterium coli* and the effect of hydrogen ion concentration and of calcium and sodium chlorides upon this capacity. The titration curve method was selected and utilized in the studies reported in this paper.

II. METHOD OF MAKING TITRATION CURVES

Titration curves have been used for the measurement of buffer in chemical systems (Clark, 1920, 1922; Cohn, Gross and Johnson 1919-1920, etc.) and in bacteriological media (Brown, 1921), but we have found no reference to their use in regard to the buffer of the bacterial cells themselves. Hence a description of the method of construction may be pertinent, particularly because it became necessary to make some modifications in the technique to meet the requirements of this study. Briefly, a titration curve is a graph made by plotting pH on one ordinate against

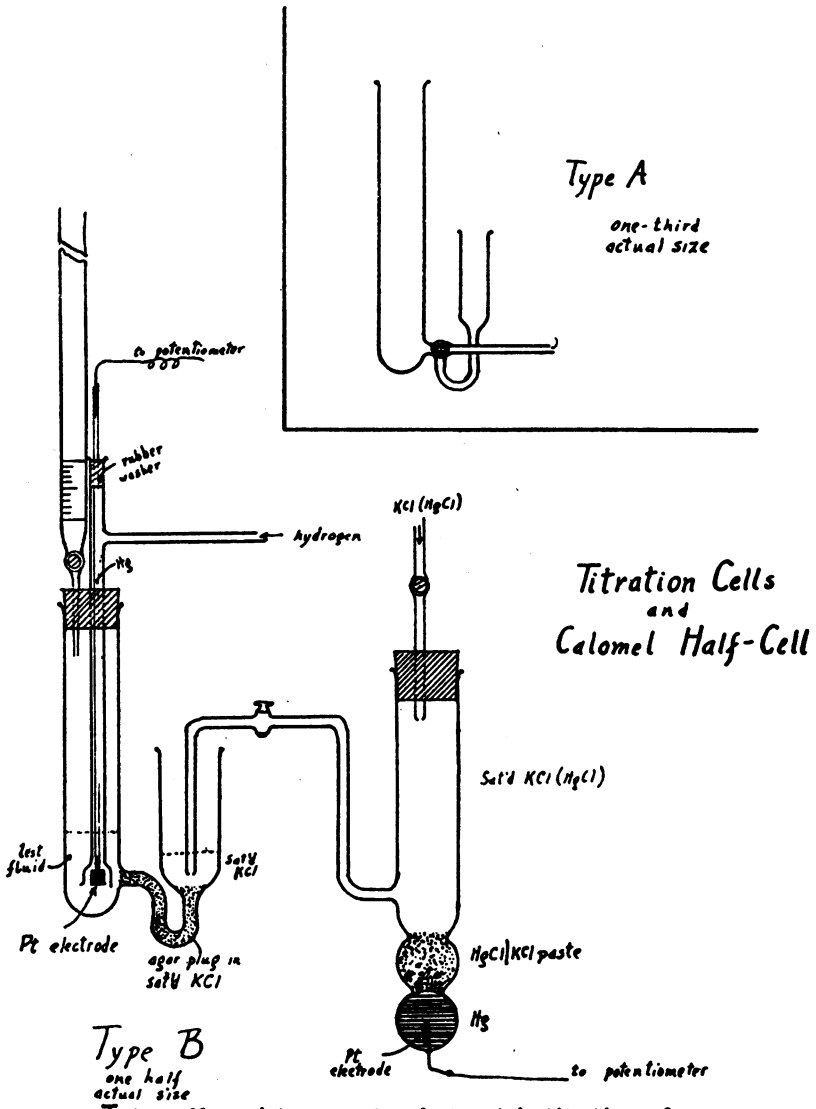

Figure I. The cells used in conducting electrometric titrations of bacterial suspensions.

I.S.F.

FIG. 1

measured amounts of acid or alkali added on the other ordinate. In our experiments the pH was determined by using an electrometric method briefly described by Falk and Shaughnessy (1922-1923). The essential portion of the apparatus differed from the ordinary titration cells as described by other workers in that we were compelled to use a cell which permitted but a minimal diffusion of KCl from the liquid junction solution into the test fluid. In our first experiments we used the chamber shown as type A in figure 1, in which the KCl comes up from the U-tube below to diffuse around the ungreased middle portion of the closed stopcock. The type B was later developed. In this cell an agar plug made by dissolving 1 per cent shred agar in saturated KCl was placed in the U-tube. This arrangement permits the flow of current to the potentiometer and galvanometer, but very little diffusion of the saturated KCl. The test solution was stirred by the escape of hydrogen gas from the modified Hildebrand hydrogen electrode through the solution. Additions of acid or alkali were made to the suspensions in the titration chambers in cubic centimeters or fractions of a cubic centimeter of 1/500 normality until the pH of the solution approached an acidity or alkalinity that required large additions of acid or alkali for further appreciable changes in pH. When that point was reached, we had recourse to the use of N/5 alkali or acid to bring the suspension to higher or lower pH. The quantity of suspension introduced into each chamber was either exactly 20 or 40 cubic centimeters. In the construction of the curve it was necessary to plot the grams of acid or alkali (computed from the cumulative cubic centimeters added) as negative logarithms to keep the size of the graph within bounds. Hence both coordinates are logarithmic. In all cases when calculating acidity the necessary corrections were made for volume changes due to acid and alkali additions. In this respect the data therefore represent titrations at constant volume.

III. MEASURING BUFFER FROM THE CURVES

It has been possible in the past to measure roughly the buffer of an ampholyte by finding the change in the slope of the titration curve (Clark 1919, 1920; Cohn, Wolbach, Henderson and Cathcart, 1918-1919), i.e., the further the slope of the line deviates towards the vertical the greater the buffer when pH units are represented as abscissae. It has been possible for us, however, to develop a method for quantitating buffer by the following means: first, a titration curve of the water or salt solution without bacteria was plotted and the grams of acid, let us say, to move the solution from pH 4 to 3 was read off from the curve; then a titration curve of water or of salt solution of this same strength, as the case might be, containing about 4000 million *Bact. coli* per cubic centimeter was inspected and the grams of acid necessary to move this suspension through the same pH zone, i.e., from 4 to 3, was read off. The ratio of the latter weight to the former gives what we are calling the buffer ratio. A similar ratio has been plotted for each pH zone except where the solution and suspension started at different zones or where part of the shift of one zone was made by alkali and part by acid. If there were no buffer, this ratio would equal unity; if a measurable buffering capacity were present, the ratio would be greater than one, in a degree depending upon the magnitude of this capacity. It is possible also to get a ratio of less than unity, implying perhaps that the cell is liberating its content of acid or alkali or that amphoteric constituents of the cell are dissociating, under the influence of the added acid or alkali, to increase the hydrogen or the hydroxyl ion concentration. However, in this measure of buffer we have a method which, while not postulating any particular theory to account for changes in buffering capacity gives a rather sensitive index of differences in buffer. It is recognized that with the technique employed it is impossible to correct for changes in cell buffer incidental to dilution of the menstruum by the added acid or alkali. Preliminary

TABLE 1a

Electrometric titration of distilled water

Acid portion of the curve

OBSERVED E.M.F.	pH	ACID ADDED (ACCUMULATIVE)	ACID ADDED	LOG OF GRAMS OF ACID ADDED	LOG OF GRAMS OF ACID ADDED
		N/500			
volts		*cc.*	*grams*		
0.560	5.35	0.0	0.0000000	$-\infty$	
0.534	4.91	0.1	0.0000073	$\bar{6}.86$	−5.14
0.513	4.55	0.3	0.0000219	$\bar{5}.34$	−4.66
0.496	4.27	0.65	0.0000475	$\bar{5}.68$	−4.32
0.485	4.07	1.0	0.0000730	$\bar{5}.86$	−4.14
0.470	3.82	2.0	0.0001460	4.16	−3.84
		N/5			
0.428	3.10	0.1	0.0008760	$\bar{4}.94$	−3.06
0.411	2.80	0.3	0.0023360	$\bar{3}.37$	−2.63
0.398	2.59	0.6	0.0045260	$\bar{3}.66$	−2.34
0.375	2.20	1.5	0.0110960	$\bar{2}.04$	−1.96

Alkaline portion of the curve

OBSERVED E.M.F.	pH	ALKALI ADDED (ACCUMULATIVE)	ALKALI ADDED	LOG OF GRAMS OF ALKALI ADDED	LOG OF GRAMS OF ALKALI ADDED
		N/500			
volts		*cc.*	*grams*		
0.560	5.35	0.0	0.000000	$-\infty$	
0.738	8.37	0.25	0.000020	$\bar{5}.30$	−4.70
0.786	9.17	0.55	0.000044	$\bar{5}.64$	−4.36
0.822	9.78	1.10	0.000088	$\bar{5}.94$	−4.06
0.851	10.27	2.00	0.000160	4.20	−3.80
		N/5			
0.898	11.06	0.20	0.001760	$\bar{3}.25$	−2.75
0.927	11.53	0.50	0.004160	$\bar{3}.62$	−2.38
0.951	11.94	1.05	0.008560	$\bar{3}.93$	−2.07

Twenty cubic centimeters of fluid titrated in each chamber.

experiments demonstrated, however, that these were not significant because the measurable buffer is independent of cell concentration within the limits of change which occur with our technique. We may recapitulate the definition of buffer ratio in the following manner:

$$\text{Buffer ratio} = \frac{\text{grams acid or alkali to shift pH } (a \text{ to } b) \text{ in bacterial suspension}}{\text{grams acid or alkali to shift pH } (a \text{ to } b) \text{ in suspension fluid}}$$

IV. GENERAL PROCEDURE

The procedure was to use sixteen to twenty-four hour old cultures of *Bact. coli* of the same strain which had been used to study viability, grown at 37°C. on nutrient agar in Kolle flasks. The growth was washed off with distilled water and

TABLE 1b

Electrometric titration of Bact. coli in distilled water

Acid portion of the curve

OBSERVED E.M.F.	pH	ACID ADDED (ACCUMULATIVE)	ACID ADDED	LOG OF GRAMS OF ACID ADDED	LOG OF GRAMS OF ACID ADDED
N/500					
volts		*cc.*	*grams*		
0.647	6.83	0.0	0.000000	$-\infty$	
0.642	6.75	0.1	0.0000073	$\bar{6}.86$	−5.14
0.588	5.72	0.5	0.0000365	$\bar{5}.56$	−4.44
0.491	4.17	1.5	0.0001095	$\bar{4}.04$	−3.96
0.461	3.67	3.0	0.0002190	$\bar{4}.34$	−3.66
N/5					
0.406	2.72	0.1	0.0009490	$\bar{4}.98$	−3.02
0.360	1.95	1.0	0.0075190	$\bar{3}.88$	−2.12

Alkaline portion of the curve

OBSERVED E.M.F.	pH	ALKALI ADDED (ACCUMULATIVE)	ALKALI ADDED	LOG OF GRAMS OF ALKALI ADDED	LOG OF GRAMS OF ALKALI ADDED
N/500					
volts		*cc.*	*grams*		
0.645	6.80	0.0	0.000000	$-\infty$	
0.652	6.92	0.1	0.000008	$\bar{6}.90$	−5.10
0.705	7.81	0.5	0.000040	$\bar{5}.60$	−4.40
0.808	9.55	1.5	0.000120	$\bar{4}.08$	−3.92
0.821	9.77	3.0	0.000240	$\bar{4}.38$	−3.62
N/5					
0.893	10.97	0.1	0.001040	$\bar{3}.04$	−2.96
0.959	12.08	2.0	0.016240	$\bar{2}.21$	−1.79

the resulting suspension was washed three times by centrifugalization at high speed. The work of Loeb (1922) suggests that it would have been desirable to have washed the organisms and to have started the titration curves at the isoelectric point of *Bact. coli*. This was not done primarily because we were desirous of finding, if possible, how living cells react

TABLE 1c

Titration curve of distilled water. Derivative data

pH	LOG OF GRAMS PRESENT	LOG OF GRAMS PRESENT	PRESENT	ADDED
		Acid portion		
			grams	*grams*
5	−5.25	$\bar{6}$.75	0.0000056	
5–4				0.000083
4	−4.05	$\bar{5}$.95	0.000089	
4–3				0.0012
3	−2.90	$\bar{3}$.10	0.0013	
3–2				0.019
2	−1.70	$\bar{2}$.30	0.020	
		Alkaline portion		
6	−5.72	$\bar{6}$.28	0.0000019	
6–7				0.0000033
7	−5.28	$\bar{6}$.72	0.0000052	
7–8				0.000010
8	−4.83	$\bar{5}$.17	0.000015	
8–9				0.000025
9	−4.40	$\bar{5}$.60	0.000040	
9–10				0.00008
10	−3.93	$\bar{4}$.07	0.00012	
10–11				0.0013
11	−2.85	$\bar{3}$.15	0.0014	
11–12				0.0075
12	−2.05	$\bar{2}$.95	0.0089	

to the changes mentioned below. When salt solutions were used as the menstrua, the bacteria were washed twice in water, once in the salt solution to be studied and were finally suspended in the salt solution. The titrations were started immediately when salts were used. But in the case of those experiments in which water was used for the dispersion medium,

the suspension were often left on ice over night for the purpose of getting the material buffered to as near neutrality as possible. The salt solutions were all adjusted to between pH 6 and 7 before seeding because of the importance of the initial pH on the viability of the cells. Tables 1a, 1b, 1c, cd, 1d, and figure 2 are introduced to illustrate the experimental and de-

TABLE 1d

Buffer ratios of Bact. coli in distilled water

pH	LOG OF GRAMS PRESENT	LOG OF GRAMS PRESENT	PRESENT	ADDED	BUFFER RATIO
			Acid portion		
			grams	*grams*	
6	−4.62	$\bar{5}.38$	0.000024		
6–5				0.000039	
5	−4.20	$\bar{5}.80$	0.000063		
5–4				0.00008	0.96
4	−3.85	$\bar{4}.15$	0.00014		
4–3				0.00049	0.41
3	−3.20	$\bar{4}.80$	0.00063		
3–2				0.0056	0.30
2	−2.21	$\bar{3}.79$	0.0062		
			Alkaline portion		
7	−5.05	$\bar{6}.95$	0.0000089		
7–8				0.000036	3.60
8	−4.35	$\bar{5}.65$	0.000045		
8–9				0.000040	1.60
9	−4.07	$\bar{5}.93$	0.000085		
9–10				0.00023	2.88
10	−3.50	$\bar{4}.50$	0.00032		
10–11				0.0008	0.62
11	−2.95	$\bar{3}.05$	0.0011		
11–12				0.012	1.60
12	−1 90	$\bar{2}.10$	0.013		

rivative data of a representative experiment. Figure 3 illustrates the case when no buffering capacity is present.

V. RESULTS OF EXPERIMENTS

The materials first used as menstrua for these studies were water, the two concentrations of electrolytes which had been found to be just toxic in the experiments of Winslow and Falk, viz., 0.725 M NaCl and 0.145 M $CaCl_2$, and a mixture

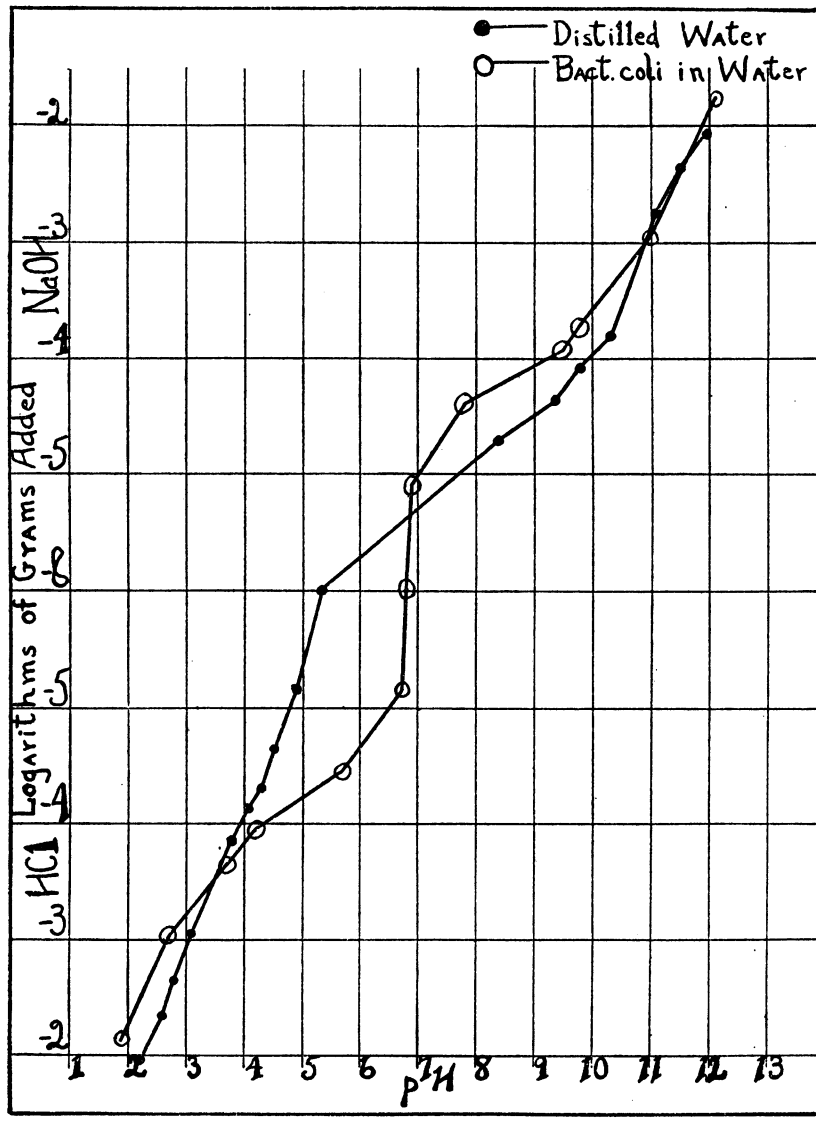

FIG. 2. TITRATION CURVES OF DISTILLED WATER AND OF BACT. COLI IN DISTILLED WATER

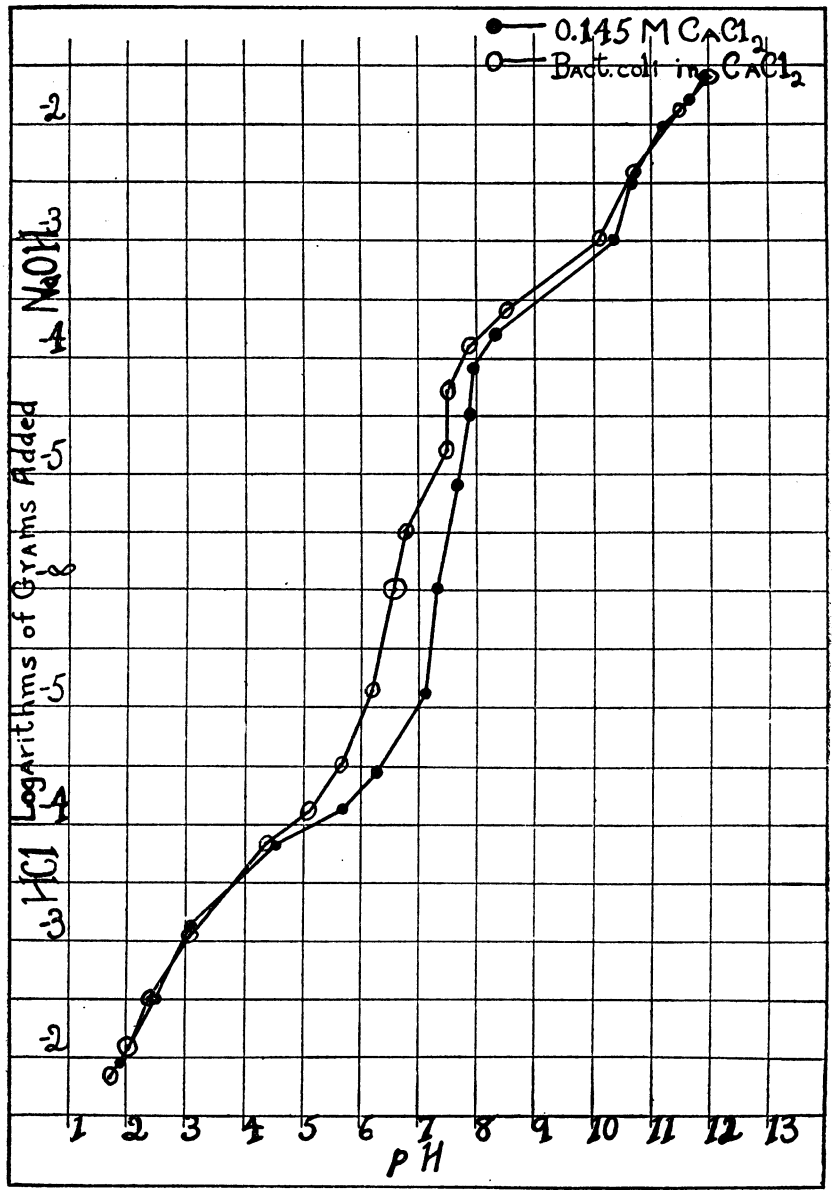

FIG. 3. TITRATION CURVES OF 0.145 M $CaCl_2$ AND OF BACT. COLI IN 0.145 M $CaCl_2$

of the two salts in which antagonism had been demonstrated, i.e., 0.580 M Nacl + 0.145 M $CaCl_2$. The results of these experiments were summarized elsewhere (Falk and Shaughnessy, 1922–1923), somewhat as shown in table 2.

It appears from the data that:

1. In distilled water suspensions of *Bact. coli* possess very marked buffering capacities in the pH zone from 4 to 10. These capacities are strongest near the zone of greatest physiological interest, i.e., from pH 6 to 7, are appreciable from pH 4 to 10, and approach unity at the pH zones 3 to 4 and 10 to 11.

TABLE 2

Average buffer ratios for Bacterium coli in water and in salt solutions

pH ZONE	RATIO FOR BACT.COLI IN:			
	Water	0.725 M NaCl	0.145 M $CaCl_2$	0.580 M Na Cl + 0.145 M $CaCl_2$
3–2	0.85	1.5	1.0	1.2
4–3	0.77	1.0	1.0	1.3
5–4	1.7	1.8	0.75	1.4
6–5			1.0	1.0
7–6	8.2	1.9		1.3
7–8	4.7	1.4		
8–9	1.7	0.75	1.2	1.3
9–10	2.4	0.88	1.5	0.75
10–11	0.59	0.92	0.92	0.91
11–12	0.93	0.68	0.91	1.0
Number of experiments.....	7	3	3	4

2. The concentrations of sodium and calcium chlorides used markedly depress the buffering capacities of *Bact. coli*. It is significant to note that a 0.145 M $CaCl_2$ solution depresses the buffer ratio more than does sodium chloride in five times that concentration.

3. The solution of 0.580 M NaCl + 0.145 M $CaCl_2$ (a physiologically balanced solution with respect to influence upon viability) acts upon buffer capacity like the unmixed $CaCl_2$ solution.

4. The acidic pH values at which the buffering capacity of *Bact. coli* becomes insignificant are approximately those at which this organism is known to be spontaneously agglutinable and to be isoelectric with the menstruum (Michaelis, 1911; Eisenberg, 1919; Northrop and DeKruif, 1922; Winslow, Falk and Caulfield, 1923). It is therefore significant to note that a similar reduction in the buffer ratio is attained at alkaline as well as at acidic reactions. This observation suggests the existence of a second—an alkaline—isoelectric point for bacteria.

It seemed that logically the next step should be to try the effect of varying the concentrations of the two salts used.

TABLE 3

SOLUTION	NUMBER OF EXPERIMENTS	BUFFER RATIOS IN pH ZONE									
		2 to 2.9	3 to 3.9	4 to 4.9	5 to 5.9	6 to 6.9	7 to 7.9	8 to 8.9	9 to 9.9	10 to 10.9	11 to 11.9
Distilled water	7	0.50	1.22	**3.47**	**7.88**	**12.27**	**9.60**	**3.90**	**4.34**	0.78	0.53
0.0725 M NaCl	3	0.89	**2.83**	**3.07**	**4.47**		**3.88**	**2.42**	1.47	1.00	1.13
0.145 M NaCl	3	1.00	1.11	**1.73**	**2.12**	**3.77**	**1.92**	**1.64**	1.00	0.56	0.81
0.3625 M NaCl	1	1.27	1.46	**1.76**			**2.65**	1.02	0.76	1.00	1.22
0.725 M NaCl	3	1.45	1.00	**1.78**		**1.87**	1.40	0.75	0.88	0.92	0.68
1.450 M NaCl	5	1.27	1.28	**2.03**	**1.82**		**2.59**	**1.85**	**1.59**	0.96	0.87

The buffer ratios were calculated as before, using the titration curve of each concentration of salt for the basis of the buffer ratio for that molarity. The results of the sodium series are shown in table 3 and figure 4. It has seemed to us that it would be useless to consider any slight divergence from a buffer ratio of 1.00 as significant because of the errors inherent in the biological material and perhaps to a lesser degree in the method. (A difference of 0.2 of a pH unit as between 6.0 and 6.2 or 6.8. and 7.0 is equivalent to a difference of 37.5 per cent in hydrogen ion concentration because of the logarithmic nature of the pH symbols.)

With due regard to the errors in the material and method, we have arbitrarily set a deviation of 0.50 from the ratio of unity as significant. In tables 3 and 4 all values which

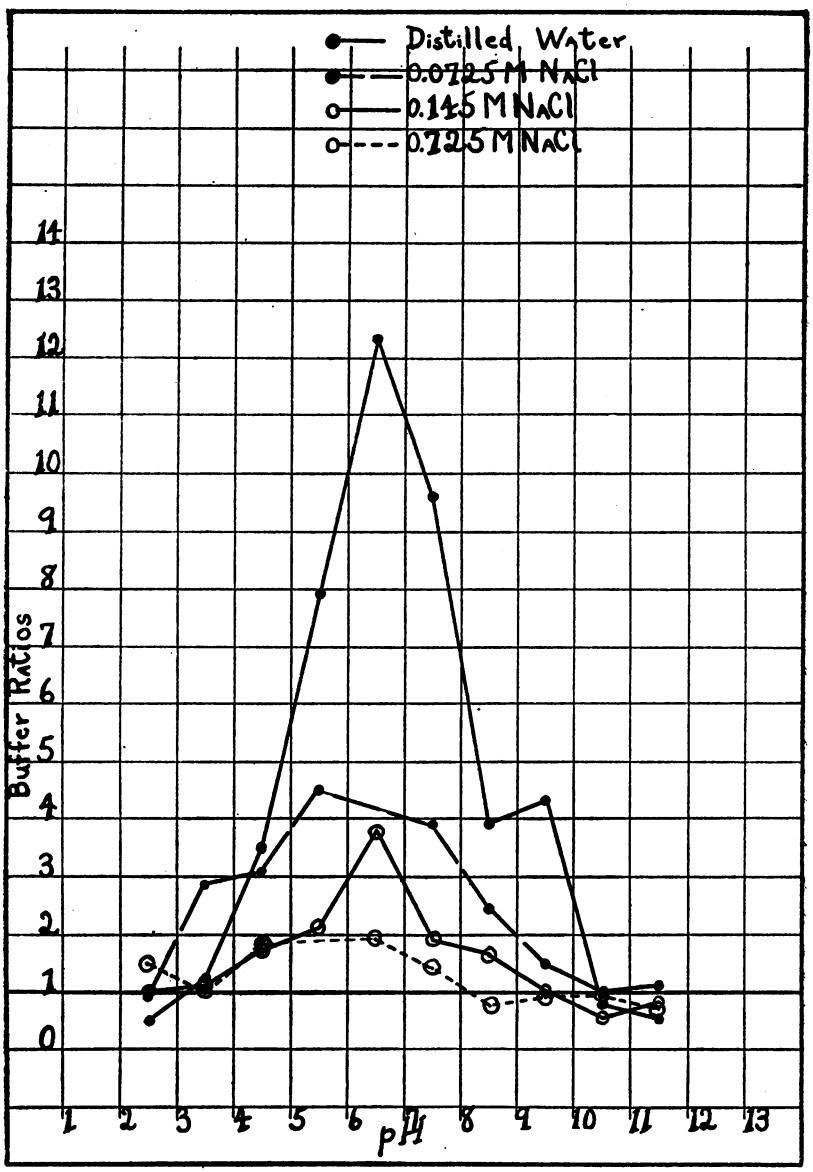

FIG. 4. AVERAGE BUFFER RATIOS OF BACT. COLI IN DISTILLED WATER AND IN SODIUM CHLORIDE SOLUTIONS

exceed 1.50 are set in boldface type. The water curve figures are presented for comparative purposes. From the table for each experiment a series of curves was drawn, plotting the ratios for each pH zone for the more important concentrations and for water. The data presented in these tables are averages calculated from seven experiments with suspensions in water, fifteen in sodium chloride, and twelve in calcium chloride solutions of various concentrations, and from four with suspensions in a solution containing both salts. From these values it seems to us the following conclusions may be drawn:

1. In water there is an appreciable buffer from pH 4 to 10 with a peak between 6 and 7 and with fairly high values between 5 and 8.

2. In the most dilute solution of sodium chloride (0.0725 M), there is a depression of the buffer at all pH values, but there seems to be an appreciable capacity to unite with hydrogen and hydroxyl ions from pH 3 to 10 (although the deviation from 1.50 at pH 9 to 9.9 is very slight). The broadening of the zone of buffer on the acid side perhaps points to something significant because it has been found that sodium chloride broadens the curve of velocity of electrical migration of *Bact. coli* in a concentration similar to this.

3. From the concentrations of 0.145 M NaCl ("isotonic") to 0.725 M NaCl the area of buffer progressively becomes smaller and smaller until at the latter concentration it is present only in the limited zone from pH 4 to 7. It may be noted that the loss in breadth of the area has been practically all on the alkaline side of neutrality (pH 7.0). Attention may be called also to the fact that as the zone becomes narrower, the significant ratios become smaller.

4. A concentration of 1.450 M NaCl (toxic enough to kill practically all bacteria in from three to six hours) produces what at first seems a surprising result, i.e., both a broadening of the range of significant buffer to the original pH 4 to 10 and an increasing of the individual ratios at the zones of buffer until they are almost as great as in the least toxic

concentrations. This finding can probably be explained by assuming that this concentration of salt (which is 10 × isotonic) may produce liberation of proteins (either by lysis or by extrusion of amphoteric products) which unpublished data on autolyzed *Diplococcus pneumoniae* demonstrate to have higher buffering powers than the organized cell.

The results obtained by varying the concentration of $CaCl_2$ from 0.00725 M $CaCl_2$ to 0.290 M $CaCl_2$ are shown in table 4 and in figure 5. Boldface type is used again to show the buffer ratios which seem to be significant.

TABLE 4

SOLUTION	NUMBER OF EXPERIMENTS	BUFFER RATIO IN pH ZONE									
		2 to 2.9	3 to 3.9	4 to 4.9	5 to 5.9	6 to 6.9	7 to 7.9	8 to 8.9	9 to 9.9	10 to 10.9	11 to 11.9
Distilled water	7	0.50	1.22	**3.47**	**7.88**	**12.27**	**9.60**	**3.90**	**4.34**	0.78	0.53
0.00725 M $CaCl_2$	2	1.21	1.21	1.44	**1.76**		0.82	0.53	1.00	0.84	0.93
0.0145 M $CaCl_2$	1	1.00	1.10	1.39		1.14	0.91	1.38	1.20	1.40	1.20
0.0725 M $CaCl_2$	1		1.47	**1.57**	1.29		**1.70**	1.44	0.89	0.81	0.85
0.145 M $CaCl_2$	3	1.04	1.04	0.75	1.02			1.20	1.46	0.92	0.91
0.290 M $CaCl_2$	5	1.23	1.05	1.37	**2.06**		**3.22**	**2.40**	**1.49**	1.21	0.92
0.580 M NaCl + 0.145 M $CaCl_2$	4	1.21	1.27	1.38	1.00	1.32		1.32	0.75	0.91	1.00

These findings may be summarized as follows:

1. The only concentrations of $CaCl_2$ which seem to show buffer are the 0.00725 M $CaCl_2$ at pH 5 to 6, the 0.0725 M $CaCl_2$ at 4 to 5 and 7 to 8, and the 0.290 M $CaCl_2$ at from pH 5 to 9.

2. Of these concentrations the first is perhaps significant of a true buffering capacity on the acid side although it is the average of but two curves and is so narrow a zone as to be somewhat questionable.

3. The buffer values in the 0.0725 M $CaCl_2$ are questionable because they are small in value, rather widely separated and the results from but one experiment.

4. The values for buffering capacity in 0.290 M $CaCl_2$ are probably to be explained on the same basis as the values

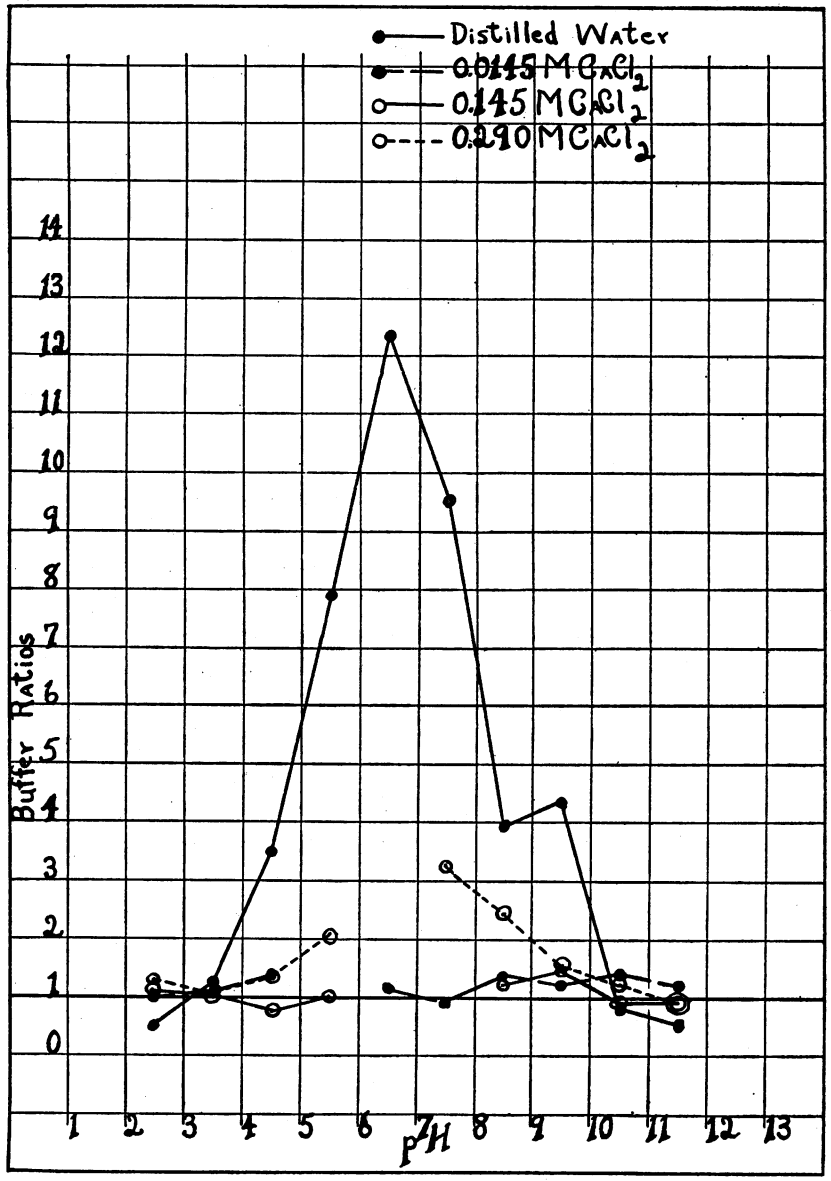

FIG. 5. AVERAGE BUFFER RATIOS OF BACT. OCLI IN DISTILLED WATER AND IN CALCIUM CHLORIDE SOLUTIONS

in the very toxic concentration of NaCl (1.450 M NaCl), i.e., that the salt caused bacterial constituents to be liberated in some way into the solution so that the recorded values are those for the buffer ratio of the proteins or other cell products rather than for the bacterial cells.

5. The values for the 0.580 M NaCl + 0.145 M $CaCl_2$ are the same as those previously reported and the same results are therefore apparent, namely, that there is no appreciable buffer in this "physiologically balanced" solution.

VI. SUMMARY AND CONCLUSIONS

Numerous studies have clearly established that the bacteria are extremely sensitive to the hydrogen ion. The implication from the conclusion of general physiology and the generally accepted views on the fitness of the environment for the maintenance of viable protoplasm is that the bacteria, like higher forms of plant or animal life, are in possession of a mechanism for the regulation of acidity in the fluids in which they are suspended. It is also implied that aqueous menstrua—because water may be considered a very weak acid or base—provide the desirable opportunities as well as necessities for the operation of such mechanisms. From the data which have been presented in this paper it appears that the buffering capacities of a typical bacterial species (*Bacterium coli*) can be measured by the methods described here and that when so measured they are found to be significantly large in water and in certain salt solutions. For the purposes of this analysis it is supposed that the underlying mechanism of buffer action in the fluid contiguous to a bacterial cell is not essentially different from that which is generally proposed to account for similar action in the body fluids of higher organisms.

It is of interest to recall that whenever in our experiments the zone of effective buffer was narrowed by a salt the loss was especially marked in neutral or alkaline solutions. This observation is entirely in harmony with deductions which may be drawn from other observations on physico-chemical characteristics of the bacterial cell. Thus, it has been clearly

established (vide Northrop and DeKruif, 1922; Winslow, Falk and Caulfield, 1923) that the bacterial cell in neutral solutions is electronegative to water. Whether this charge on the cell is to be accounted for on the basis of a selective adsorption of electronegative ions or on the theory of membrane equilibria or both is immaterial for the present purposes. It remains true that the bacterial cell or its principal constituents are especially reactive with electropositive ions and (on the basis of either theory) should be the more reactive the more alkaline the solution up to certain limits. The differences in the observed behavior of sodium and calcium chlorides must be considered as due to the specific properties of the cations of these salts which dissociate to give a common anion. When the menstruum is rendered acid the eletronegativity of the cell is reduced (and is abolished when the acidity attains the specific value which is termed the "isoelectric point"). Its reactivity with cations—it may be expected—will be diminished. Hence the reactions with the sodium or calcium cations which are evidenced by depression of the buffer ratio should be, as they are, more marked in neutral or alkaline than in acid solutions.[1]

We have observed from the data which were cited that the buffer ratio is appreciably depressed by calcium chloride in non-toxic concentrations. This observation suggests that interference with the mechanism of buffer action may become physiologically significant only when the reaction of cation and cell or cell constitutents has proceeded to some specific point and that viability may be maintained although the buffering capacity has been considerably reduced or nearly abolished.

Our specific experimental findings may be recapitulated as follows:

1. In distilled water *Bact. coli* possesses distinct buffering capacities which are greatest in the most favorable zone for viability—pH 6 to 6.9—and which are significant in the range which is of physiological importance, the pH range from 4 to 10.

[1] The experiments of Stearn and Stearn (1923) suggest a similar explanation for the reaction of bacteria with acidic and basic dyes.

Above pH 10 and below pH 4 the buffer ratio approximates unity and further may be so significantly below unity as to suggest that there is actual liberation of, rather than reaction with, the hydrogen or hydroxyl ion which predominates in the surrounding medium. The existence of a second, alkaline isoelectric point is suggested because the buffer becomes insignificant several pH units away from the acid isoelectric point (which is below pH 1.0 for this strain) and likewise becomes insignificant at certain alkaline pH values.[2]

2. In sodium chloride solutions the buffering capacities were depressed with all the strengths used (0.0725 M to 1.450 M); but it seems that the breadth of the significant buffer zone and magnitudes of the individual ratios increase as the dilution (or decrease as the concentration) increases up to the point of just appreciable toxicity of the salt for the bacteria. Beyond that point the buffer is somewhat increased but is still lower than the buffer in distilled water or in very dilute sodium chloride solution. In these concentrated solutions it is probable that we are dealing with unorganized cell constituents rather than with bacterial cells.

3. In calcium chloride solutions (0.0145 M to 0.290 M) the buffering capacities were practically abolished in all the solutions which were non-toxic at favorable pH. It is possible that there is an appreciable buffer in the pH zone 5 to 5.9 in the most dilute solutions (0.00725 M). The toxic concentration showed the same increase in buffer as did the toxic concentration of sodium chloride and very probably for the same reason.

4. The "physiologically balanced" solution of NaCl + $CaCl_2$ (0.580 M + 0.145 M) showed no material increase in buffering capacities over that of the solution containing calcium chloride alone.

[2] The existence of this alkaline isoelectric (isopotential) point for bacterial cells has been demonstrated by the data recently reported by Winslow and Shaughnessy (1924).

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