THE ALKALINE ISOPOTENTIAL POINT OF THE BACTERIAL CELL.*

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In an earlier contribution from this laboratory (1) it was shown that the cells of *Bacillus cereus* are normally negatively charged, with an isoelectric point at about pH 3.0; that at higher pH values the velocity increases with increasing alkalinity up to about pH 10.0; and that at still higher alkalinity the velocity of migration towards the anode decreases again, apparently falling toward another isopotential point at pH 12.0 or over. In individual instances the cells lost their velocity entirely but at these high alkalinities the results were highly variable.

We have found in more recent work that the reason for this irregularity of results lies in the tendency of the bacteria to buffer the adjacent medium and create in the immediate vicinity of each cell a zone having a pH much below that of the alkaline menstruum in which they are suspended. This general phenomenon of local buffer action has been studied by Winslow and Falk (2) and its relation to the problem now in hand was revealed by the fact that when solutions of high alkalinity were used the cells would often show considerable velocity at first (while they were really exposed to a solution less alkaline than the general medium) and would gradually slow down as their buffering power became exhausted. We found that this difficulty could easily be overcome and very uniform results reached by shaking the suspension for 5 minutes before placing it in the electrophoresis cell. The pH of the suspension as a whole was checked by duplicate determinations on the final effluent liquid and was not lowered more than one-tenth of a pH unit.

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The general apparatus and methods used were the same as those described in our earlier communications (1). At the high alkalinities we desired to study, colorimetric indicators of a satisfactory accuracy are not available, so that all pH determinations were made electrometrically, using essentially the apparatus described by Falk and Shaughnessy (3).

The results of five experiments given in Table I indicate that there really is a clearly marked and definite isopotential point at pH 13.3 and 13.4 and that beyond this point the electrical state of the cells is reversed so that they bear a distinct positive charge which increases with increasing alkalinity.

We have summarized one complete curve for *Bacillus cereus* in Table II. The figures for pH values below 12.0 are based on last year's experiments but differ slightly from those previously published (1) for the following reason. In our earlier tabulations we followed what has, we believe, been the general procedure of averaging observed times taken to cover a given distance at the three levels of the cell and then converting the average times into velocities. It is logically and mathematically somewhat sounder to convert time observations at each level into velocities and then average the three velocities. This method, which gives somewhat different final values, has been used in preparing Table II.

There is obviously a reasonably smooth curve with isopotential points just below pH 3.0 and 13.5, a zone of maximum negative charge at pH 9.0 to 11.0 and a positive charge below pH 2.0 and above pH 13.5.

In order to test the generality of this phenomenon we made five series of tests on *Bacterium coli*, the results of which are presented in Table III.

The results are very similar to those presented in Table I except that the isopotential zone is a little further to the alkaline side (*Bacillus cereus*, 13.3 to 13.4; *Bacterium coli*, 13.6 to 13.8). The enormous positive charge at pH 14.2 is especially to be noted.

It is important to know whether the positive charge observed above pH 13.5 is reversible (as we have previously shown is the case with the positive charge below pH 2.0) or whether the alkaline reversal is accompanied by irreversible chemical changes. We have tested this important point with the following results.

TABLE I.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacillus cereus in Extremely Alkaline Solution.

pH.....	12.0	12.1	12.2	12.3	12.4	12.5	12.6	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5	13.6	13.7	13.8
Exp. 1								−4.9					−4.8		0.0				
2													−3.5		0.0	+2.4			+14.8
3								−4.4					−3.6		0.0				
4													−9.4	0.0	0.0		+6.8		
5	−7.0	−7.3	−5.8		−6.3														

− indicates motion toward anode.
\+ " " " cathode.

TABLE II.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacillus cereus at Different Hydrogen Ion Ranges.

pH.....	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.5	13.6–13.9
No. of observations.....	8	8	8	3	7	13	5	8	5	11	8	6	10	2
Average velocity.....	+0.4	−0.1	−2.6	−9.7	−12.9	−9.9	−15.2	−15.3	−18.6	−18.8	−7.3	−5.9	−1.9	+10.8

TABLE III.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacterium coli in Extremely Alkaline Solution.

pH.....	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5	13.6	13.7	13.8	13.9	14.0	14.1	14.2
Exp. 1					−5.7		−1.9			0.0						
2	−4.7			−5.2		−2.4										
3							−1.5			0.0				0.0		
4						−2.0				0.0			+15.1			+34.4
5									−1.0	0.0		0.0		+12.3		

At pH 13.8 a culture of *Bacillus cereus* showed a velocity of 9.8 micra per second toward the cathode. When readjusted by the addition of normal HCl to pH 6.6, the migration was toward the anode with a velocity of 5.2 micra. In a second experiment the cells moved toward the cathode with a velocity of 13.5 micra at pH 14.1 and an adjustment to pH 9.8 moved toward the anode with a velocity of 4.1 micra. These negative velocities after readjustment from an extreme alkaline reaction are, it will be noted, much less than those recorded for pH values of 6.6 and 9.8 in Table II. This we believe however to be due to the presence of the salt formed in neutralizing the high alkalinity. The final salt concentration was in the neighborhood of a 0.5 molar solution of NaCl which we have previously shown (1) gives a velocity of just about 5 micra with *Bacillus cereus.*

CONCLUSIONS.

Irregularities in migration velocity of bacterial cells in the highly alkaline solutions are due to the buffering effect of the cells upon the immediately adjacent zone of menstruum. Consistent results can be obtained by shaking the suspension thoroughly before placing it in the electrophoretic cell.

When observed in this way both *Bacillus cereus* and *Bacterium coli* show an isopotential point near pH 13.5, that for *Bacillus cereus* being slightly below, and that for *Bacterium coli* slightly above this point. At more alkaline reactions the cells acquire a positive charge which increases with further increase in pH to very high values.

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