2. Sodium and calcium chlorides, singly and in combination in the concentrations used depress the buffer ratios, particularly in the physiological zones of P_H.

3. The acidic P_H values at which the buffering power 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.[1] 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.

212 (2172)

The influence of certain electrolytes upon the electrical charge of bacteria.[2]

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In connection with an extensive series of studies on the effect of electrolytes upon the various properties of the bacterial cell, we have measured the electrical charge of vegetative cells of *B. cereus* (chosen on account of its large size) by the direct microscopic method described by Northrop.[3] In conducting these experiments a voltage of known magnitude (112 v.) is applied to non-polarizing zinc-zinc sulphate electrodes and the direction and velocity of migration of the bacteria in unbuffered suspensions determined by observing through the microscope the time taken by the bacterial cells to cross a definite space on the

1 Michaelis, *Deut. med. Wochensch*, 1911, xxxvii, 969; Eisenberg, *Centr. Bakt.*, 1919, lxxxiii, 70, 472, 561; Northrop and DeKruif, *J. Gen. Physiol.*, 1922, iv, 639.

2 Studies here reported were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

3 *J. Gen. Physiol.*, 1922, iv, 629.

ocular micrometer. In order to avoid theoretical assumptions we have expressed all results in terms of this observed velocity, a high velocity in general presumably signifying of course a greater charge. The average results obtained with varying hydrogen ion concentrations generally based on four or more observations at each P_H value are indicated below.

TABLE I.

Velocity of Migration—Micra per Second.

P_H	1	1.-1.9	2.-2.9	3.-3.9	4.-4.9	5.-5.9	6.-6.9
	—1.2	+0.3	—0.1	—1.8	—7.8	—10.3	—10.3
P_H	7.-7.9	8.-8.9	9.-9.9	10.	11.	12.	13.
	—11.7	—10.8	—12.8	—13.8	—2.2	0	0

It appears that these bacteria maintain a fairly high and reasonably consistent negative charge through the wide range of reaction between P_H 5 and P_H 10, rising somewhat with increasing alkalinity. On the acid side of this range the charge drops steadily to an isoelectric point at about P_H 2.0, the charge beyond this point being slight and variable and sometimes reversed. At reactions more alkaline than P_H 10 the charge diminishes even more rapidly to a second (alkaline) isoelectric point at about P_H 12.

The influence of sodium and calcium salts, alone and in combination, in the concentrations studied, was to depress the velocity of migration very greatly at all P_H values and to narrow the zone of maximum velocity on the alkaline side, bringing the alkaline isoelectric point to P_H 9.0-10.0 (Table II). A solution of 0.145 M $CaCl_2$ is more potent in diminishing velocity than is 0.725 M NaCl and there appears to be an antagonistic effect of the two salts at reactions between P_H 3.0 and P_H 7.0.

TABLE II.

Velocity of Migration—Micra per Second.

P_H	0.725 M NaCl	0.145 M $CaCl_2$	0.580 M NaCl + 0.145 M $CaCl_2$
1.5	—0.8	+1.0	—0.5
3.0	—2.7	—0.8	—2.3
5.0	—3.9	—1.9	—3.5
7.0	—4.9	—3.8	—4.4
8.0	—4.2	—2.6	—2.1
9.0	—0.8	—0.7	0
10.0	0	0	0

In general the curves parallel in a general way those obtained for the viability of bacteria in water, so far as the general influence of electrolytes is concerned except that here antagonism occurs in a more acid zone. They also correspond well to the observation of Falk and Shaughnessy[1] on the buffering power of suspensions of bacterial cells, particularly in the demonstration of a second alkaline isoelectric point. In general it appears that within the zone of hydrogen ion concentration which favors the viability of bacteria in water the bacterial cell exerts a high buffering power and maintains a normal electrical charge. In more acid or more alkaline solutions, or in solution of favorable reaction but in the presence of sodium and calcium salts in toxic concentration, the buffering power fails, the electrophoretic charge is reduced, and the bacteria die.

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The role of phosphate and potassium in carbohydrate metabolism following insulin administration.

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Following the administration of large doses of insulin to several diabetics, to two patients with diabetic coma, to a normal fasting individual, to a fasting dog, and to fasting rabbits, and coincident with the drop in the blood sugar which regularly occurs, a marked drop has been noted in the concentration of inorganic blood serum phosphate and serum potassium. A sharp drop in the urinary output of phosphorus and of potassium accompanies the drop in the blood serum concentration, and is later followed (3-12 hours) by a well marked compensatory increase in the urinary excretion of these substances, so that the total excretion over daily periods is not much altered.

1 PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 426.