THE EFFECT OF ELECTROLYTES PRESENT IN THE GROWTH MEDIA UPON THE ELECTROPHORETIC MOBILITY OF ESCHERICHIA COLI[1]

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I. INTRODUCTION

A number of articles have been published (Winslow, *et al.* 1923, 1926) in which the effects of electrolytes upon the electrophoretic mobility of bacteria have been reported. In these studies the organisms were grown, separated from the growth medium, suspended in the electrolyte solution under investigation, and the velocity of migration determined. The presence of low concentrations of electrolytes increased the mobility while higher concentrations decreased the mobility. Analogous results for non-living adsorbents are well known.

Would similar results be obtained if bacteria were grown in the presence of electrolytes or would the bacterial metabolism exercise some control over the mobility? This study attempts to determine this point and is of importance in the discussion of possible relationships between electrophoretic mobility and any bacterial property or activity.

II. EXPERIMENTAL PROCEDURE

A. The suspension. *Escherichia coli* was grown in a 2 per cent aqueous solution of Bacto-Peptone to which had been added the

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amount of salt necessary to give the desired concentration. The cultures were kept overnight at 37°C. and centrifuged for one hour. The supernatant liquid was poured off, the cells thoroughly mixed with distilled water, and again centrifuged. After the desired number of washings the bacteria were suspended in a small volume of distilled water, filtered through a small washed plug of cotton, and placed in a refrigerator. Before use each suspension was diluted with distilled water to the concentration most desirable for electrophoretic readings.

B. The determination of mobility. The mobility was determined with a Northrop-Kunitz micro-cataphoresis cell under a potential gradient of approximately 8.8 volts per centimeter. The microscope was fitted with a dark-field condenser and gave a magnification of 460 diameters.

The readings were made in the upper half of the cell at that depth (0.211) which gives actual electrophoretic velocities. For each sample the cell was filled twice, the microscope focused five times and 20 stopwatch readings taken at each focus. The average of these 100 readings was calculated to micra per second per volt per centimeter.

Control determinations were made with untreated bacteria at the beginning and at the end of each set of samples. Because of the excellent agreement between values obtained for the two controls, however, the second came to be considered unnecessary.

C. Washing of bacteria. A study was made of the effect upon the electrophoretic mobility of washing the organisms with water. This was done with bacteria grown in media containing no added electrolyte (blanks) and in media containing $CaCl_2$ and Na_2SO_4. Except in this study all cultures were washed once.

D. Growing time. To ascertain the precision with which the growing time would have to be controlled, an investigation of the mobility of organisms varying in age from 3 to 35 hours was made. All other cultures were grown approximately for 22 hours.

E. pH determinations. These were made colorimetrically using chlorophenol red.

III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Washing studies. Organisms centrifuged from the growth medium and suspended in water were considered to have had "zero" washing. Other samples were washed 1, 2, 3, and 4 times. The results are given in table 1 and the averages in figure 1.

The values for the blanks are practically constant for 1, 2, 3, and 4 washings. For subsequent investigation, therefore, one washing was adopted as the standard procedure.

TABLE 1

Effect of washing upon electrophoretic mobility

	NUMBER OF WASHINGS				
	0	1	2	3	4
Blanks	1.66 1.82	2.23 2.16	2.23 2.22	2.18 2.25	2.34 2.22
Average	1.74	2.20	2.22	2.21	2.28
$CaCl_2$ 0.01 molar	0.94 0.96	1.86 1.60	2.05 1.81	2.27 2.05	2.24 2.20
Average	0.95	1.73	1.93	2.16	2.22
Na_2SO_4 0.5 molar	2.42 2.38	2.75 2.61	2.44 2.48	2.32 2.36	2.29 2.32
Average	2.40	2.68	2.46	2.34	2.31

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

It appears from figure 1 that in the growth media the mobilities of the bacteria were less than those observed for "zero" washing. It also appears that one washing removes the electrolytes in the case of the blank but not where $CaCl_2$ or Na_2SO_4 were used. If the calcium and the sulphate ions were preferentially adsorbed, their gradual removal by washing would explain the difference in behavior noted for $CaCl_2$ and Na_2SO_4.

The fact that the mobilities eventually obtained, through continued washing of the treated and untreated bacteria, were practically identical indicates that the salts had produced no irreversible change in the nature of the bacterial surface.

B. Growing time. Possible changes in electrophoretic mobility due to differences in the growing time were sought with media

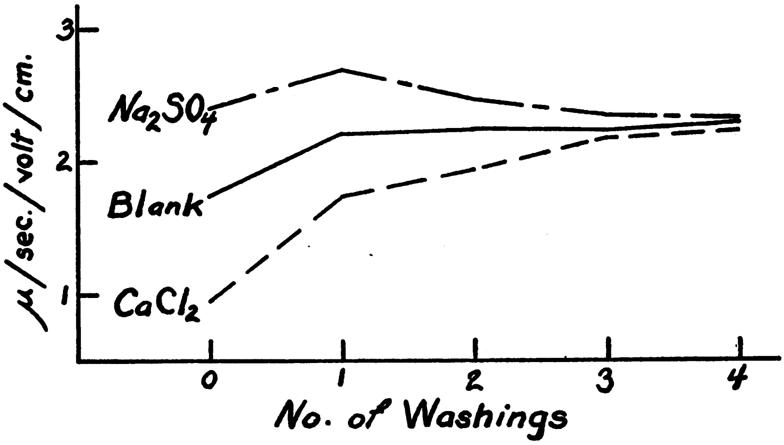

FIG. 1

TABLE 2

Relation of growing time to electrophoretic mobility

	3 HOURS	6 HOURS	10 HOURS	15 HOURS	21 HOURS	28 HOURS	35 HOURS
Blanks	2.29	2.22	2.22	2.26	2.27	2.26	
	2.20	2.22	2.25	2.26	2.24		2.26
Average	2.25	2.22	2.23	2.26	2.26	2.26	2.26
Na_2HPO_4 0.1 molar	1.73	1.61		1.73	1.87	1.90	
	1.70	1.69	1.85	1.84	1.89	1.96	
Average	1.72	1.65	1.85	1.78	1.88	1.93	

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

containing no added electrolyte and with media 0.1 molar with respect to Na_2HPO_4. The results are given in table 2.

The results for blanks show no change with growing time. Although the Na_2HPO_4-containing media produced a slight upward trend in migration velocity, the change is hardly significant when compared with the data reported by Shibley (1924) for a Type 1 *pneumococcus* and for *Salmonella paratyphi* B. He reported an increase in mobility to a maximum at about 10 hours followed by a decrease to a constant value after about 15 hours. Shibley's findings are apparently not of general applicability. Kahn and Schwarzkopf (1931) working with the tubercle bacillus found that younger cultures have a higher mobility than older cultures. The ages of their cultures varied, however, from four days to five weeks so that the results are not strictly comparable.

The increased mobility obtained might have been due to a decrease in the active concentration of Na_2HPO_4 brought about by bacterial metabolism rather than to any direct effect of the age of the culture.

C. pH values. It has not been possible to correlate the pH values secured with any changes in electrophoretic mobility. The pH values varied, as would be expected, with the multiplication of the bacteria; old cultures showed greater changes from the original than young cultures, and luxuriant growth produced greater changes than meager growth. After the growth of the bacteria the values were all on the alkaline side of neutrality with the maximum at pH 8.4. The data of Winslow and Upton (1926) show practically constant migration velocities for coli from pH 6.0 to pH 8.5 and the values obtained all fall within this pH range. Accordingly it is concluded that changes in migration velocity were not produced by changes in pH.

D. Salt studies. 1. Blanks. The results obtained for the blanks (read before the treated samples) show as an average of 2,740 stopwatch readings a migration velocity of 2.29 μ/seconds for unit potential gradient. The second blank (read after the treated samples) gave the same value for the average of 1,240 individual readings. Of the 62 blanks determined only one showed a greater deviation than ± 5 per cent from the average value of 2.29 μ/seconds per · volt/centimeter. This one value was 6.6 per cent too low. The conclusions are therefore drawn that the reading technic is adequate to give reproducible results, that differences in the data are not due to the reading

TABLE 3

Effect of salts on electrophoretic mobility

SALT	CONCENTRATION								CHANGE FROM BLANK AVERAGE 2.29
	0.00001M	0.0001M	0.001M	0.01M	0.05M	0.1M	0.5M	1.0M	
Na_2HPO_4		2.11	2.37	2.22	1.94	1.76	1.33		
		2.14	2.23	2.15	1.85	1.52	1.33		
		2.35	2.36	2.36	1.90	1.76			
		2.34	2.33	2.32	1.88	1.80			
Average...		2.24	2.32	2.26	1.89	1.71	1.33		Decrease
$CaCl_2$	2.23	2.17	2.14	1.83	1.76	1.76			
	2.25	2.13	2.13	1.82	1.52	1.52			
		2.15	2.09	1.79	1.34	1.32			
					1.33	1.65			
					1.75	1.71			
					1.74	1.76			
Average...	2.24	2.15	2.12	1.81	1.57	1.62			Decrease
NaCl		2.24	2.03	2.22	2.18	2.24	2.49	2.56	
		2.25	2.27	2.27	2.46	2.53	2.54	3.19	
			2.37		2.64	2.70	2.58	2.75	
					2.62	2.59	2.59		
Average...		2.25	2.22	2.25	2.48	2.51	2.55	2.83	Increase
Na_2SO_4		2.31	2.21	2.25	2.47	2.67	2.67	3.00	
		2.31	2.23	2.37	2.42	2.55	2.69	2.79	
						2.80	2.69		
						2.52	2.58		
Average...		2.31	2.22	2.31	2.45	2.63	2.66	2.90	Increase
NaI			2.29	2.24	2.17	2.13	2.15*		
			2.27	2.23	2.21	2.14	2.23*		
				2.24	2.26	2.28			
Average...			2.28	2.24	2.21	2.18	2.19*		Slight decrease

* 0.25M.

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

technic, and that the organism can be grown over a long period of time without change in zeta potential.

2. Effect of salts. The results are given in table 3 and figure 2. The lowest values obtained (0.5 M Na_2HPO_4) represent a decrease in migration velocity of 41.9 per cent; the highest value (1.0 M Na_2SO_4), an increase of 26.5 per cent.

The results for the individual samples receiving the same treatment show in most cases a fairly good agreement one with

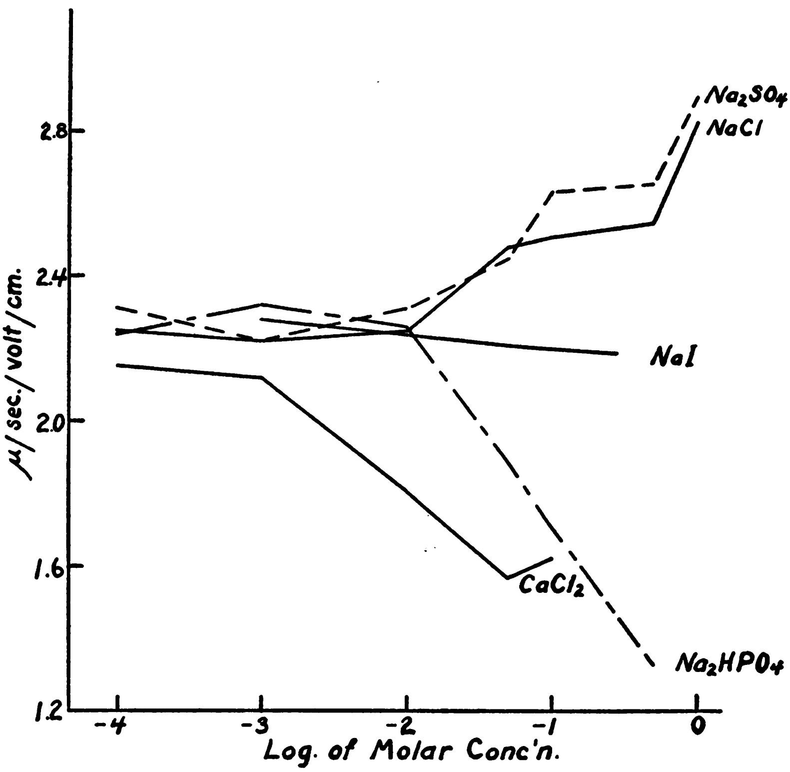

FIG. 2

another, but in a few instances, such as the higher concentrations of $CaCl_2$, considerable differences are evident. Attempts were made to correlate the different values with the age of the culture, the age of the suspension, the age of the distilled water used, and the pH of the growth medium, but no relationship could be established.

No significant change in mobility has been accomplished by the addition of NaI to the growth medium. It is difficult to believe that this salt had no effect upon the mobility of the colon bacilli, but what did happen is not clear.

E. General discussion. Electrophoretic potential varies directly with the thickness of the electrical double layer and with the density of the charge on the particle. Abramson and Müller (1933) indicate that in low concentrations of electrolytes the charge per unit area is the more important factor in determining electrokinetic behavior, while in higher concentrations the thickness of the double layer is the more important factor. The explanation of the results obtained in the washing experiments seems to depend on both of these factors. In the various growth media the thickness of the double layer was very probably considerably decreased and the immediate result of replacing the growth medium by water (zero washing) should be an increase in the thickness of the double layer with a resultant increase in mobility. In subsequent washings the density of the charge on the particles seems to be the factor largely responsible for further changes in mobility.

From the increased mobility encountered with NaCl and Na_2SO_4 it appears that the high concentrations of these two salts in the growth media produce an increased charge density. This high charge density together with the increased thickness of the double layer, brought about by washing, would produce the high mobilities recorded.

The decreased mobility produced with such dissimilar salts as Na_2HPO_4 and $CaCl_2$ would seemingly be due to a decreased charge density. With the $CaCl_2$ a decrease in the negativity of the charge on the bacterial surface undoubtedly occurred, but whether the Na_2HPO_4 acted in a similar manner is not evident even though similar results were noted.

To explain adequately the differences found among the various salts used would require a knowledge of the relative adsorbability of the ions involved and also the active concentrations of these ions in the suspensions studied. In view of these considerations it is not surprising that the data presented do not follow the curves mentioned in the introduction.

The results obtained in this investigation raise a question as to the validity of many of the conclusions arrived at in bacterial electrophoresis. It has been a common practice to suspend washed bacteria in water and to determine the migration velocity of the organisms. From such data conclusions are drawn and classifications are made in attempts to correlate virulence, age of culture, strain of organism, etc., with electrophoretic mobility. The data accumulated here indicate the improbability of any direct relationship existing between the mobility of bacteria suspended in water and the mobility of these same bacteria in their normal environment.

IV. SUMMARY

A study was made to ascertain the effects upon the electrophoretic mobility of *Escherichia coli* of the presence of electrolytes in the growth media. The following conclusions seem justified.

1. The washing with water of colon bacilli grown in peptone broth and in peptone broth containing $CaCl_2$ increases the electrophoretic migration velocity to a constant value.

2. The washing with water of colon bacilli grown in peptone broth containing Na_2SO_4 first increases and then decreases the migration velocity to the same constant value mentioned in (1).

3. Contrary to results reported for other bacteria, the electrophoretic mobility of colon bacilli is not greatly influenced by the age of the culture up to 28 hours.

4. The technic used gives, for colon bacilli cultured over a considerable period of time in peptone solution, average values reproducible within ± 5 per cent.

5. The pH changes which took place did not change the electrophoretic mobility.

6. Under the conditions of this experiment, the electrophoretic mobility can be increased or decreased by the presence of certain salts in the growth medium.

7. The well known mobility-concentration curves determined with salts present in the suspension media were not obtained in this study.

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