CATION ADSORPTION BY BACTERIA[1]

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At the present time there is a great deal of interest in the mechanism of microbial nutrition. Any knowledge of the manner in which the bacterial cell functions as a physico-chemical unit should assist in elucidating problems of nutrition.

It is a well-known fact that proteins (Docking and Heymann, 1939) as well as inorganic colloids (Jenny, 1932) possess an adsorption capacity for cations. Since the bacterial cell contains proteins, approaches inorganic colloids in size, and is negatively charged (Tittsler, 1938; Kendall, 1925), it seems logical that it might function as an agent in the adsorption of positively charged ions.

Robbins (1926) showed that plants such as *Elodea* and potato tuber would adsorb cations and anions. Stearn and Stearn (1928) indicated from their studies of simple proteins that bacteria might adsorb cations and anions. Jenny and Overstreet (1939) stated that barley roots exhibit a positive cation adsorption capacity of 8 m.e. per 100 grams of plant material. As far as the writer is aware, no one has undertaken to demonstrate such a phenomenon quantitatively for bacteria.

An understanding of the mechanism by which a bacterial cell takes ions from its environment and concentrates them in a limited area around and, possibly, inside the cell, with an electrical force sufficient to limit their freedom of movement, might help to elucidate some of the laws governing the physiological activity of these organisms. For example, knowledge relative to the degree of adsorption of a mineral nutrient by a bacterial cell might help to explain the role of such an element in growth. Only by carefully controlled physico-chemical studies can the

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function and interrelation of the various cations involved in bacterial nutrition, growth, and disinfection be determined.

Since the bacterial cell is known to carry a negative charge and is also known to form acids (carbon dioxide and water) the following equations will serve to indicate possible cation adsorption by bacteria.

$$\text{(Bacterial cell}^{n-}\text{)} + n\text{H}^+ \rightleftarrows \text{(Bacterial cell}^{n-}\text{) (nH}^+\text{)}$$

$$\text{(Bacterial cell}^{n-}\text{) (nH}^+\text{)} + \begin{matrix}(n\text{NaOH})\\(n\text{KOH})\\(\tfrac{1}{2}\ n\text{Ca(OH)}_2)\\(\tfrac{1}{2}\ n\text{Mg(OH)}_2)\end{matrix} \rightleftarrows$$

$$\text{(Bacterial cell}^{n-}\text{)} \begin{matrix}(n\text{Na}+)\\(n\text{K}^+)\\(\tfrac{1}{2}\ n\text{Ca}^{++})\\(\tfrac{1}{2}\ n\text{Mg}^{++})\end{matrix} + n\text{HOH}$$

From the above equations it might be expected that a bacterium growing in a balanced medium would adsorb Na^+, K^+, Ca^{++}, Mg^{++}, etc., from the medium. If, then, an excess of H ions were added to these cells as HCl, it might be expected that the Na^+, K^+, Ca^{++}, Mg^{++}, etc., would be replaced by the H^+. The basic ions released from the adsorption complex would unite with the Cl^- ions to form the respective water-soluble chlorides which could be removed by washing as indicated in the following equation.

$$\text{(Bacterial cell}^{n-}\text{)} \begin{matrix}(n\text{Na}^+)\\(n\text{K}^+)\\(\tfrac{1}{2}\ n\text{Mg}^{++})\\(\tfrac{1}{2}\ n\text{Ca}^{++})\\(\text{etc.})\end{matrix} + n\text{HCl} \rightleftarrows$$

$$\text{(Bacterial cell}^{n-}\text{) (nH}^+\text{)} + \begin{matrix}(n\text{NaCl})\\(n\text{KCl})\\(\tfrac{1}{2}\ n\text{MgCl}_2)\\(\tfrac{1}{2}\ n\text{CaCl}_2)\\(\text{etc.})\end{matrix}$$

The H-bacteria thus formed should be titratable with $Ca(OH)_2$ and the amount of $Ca(OH)_2$ used should be a measure of the H^+ adsorbed, or the cation adsorption capacity of the bacterial cells.

$$(\text{Bacterial cell}^{n-})\ (n\text{H}^+) + \tfrac{1}{2}\,n\text{Ca(OH)}_2 \rightleftarrows (\text{Bacterial cell}^{n-})\ (\tfrac{1}{2}\,n\text{Ca}^{++}) + n\text{HOH}$$

By drying an aliquot of the suspension of bacteria at 105°C. and weighing, values obtained as above could be expressed as m.e. (milligram equivalents) of cations per 100 grams of bacteria.

Theoretically it should be possible to substitute other chlorides such as methylene blue chloride, mercuric chloride, etc., for the hydrogen chloride, and since the methylene blue ion can be measured colorimetrically and the mercury ion functions as a lethal agent, they afford two additional tools for testing out the adsorption hypothesis. Such theoretical reactions are indicated in the following equations.

$$(\text{Bacterial cell}^{n-})\ \begin{matrix}(n\text{Na}^+)\\(n\text{K}^+)\\(\tfrac{1}{2}\,n\text{Mg}^{++})\\(\tfrac{1}{2}\,n\text{Ca}^{++})\\(\text{etc.})\end{matrix} + n\text{MB}^+\text{Cl}^{-}{}^{2} \rightleftarrows (\text{Bacterial cell}^{n-})\ (n\text{MB}^+) + \begin{matrix}(n\text{NaCl})\\(n\text{KCl})\\(\tfrac{1}{2}\,n\text{MgCl}_2)\\(\tfrac{1}{2}\,n\text{CaCl}_2)\\(\text{etc.})\end{matrix}$$

or

$$(\text{Bacterial cell}^{n-})\ \begin{matrix}(n\text{Na}^+)\\(n\text{K}^+)\\(\tfrac{1}{2}\,n\text{Mg}^{++})\\(\tfrac{1}{2}\,n\text{Ca})\\(\text{etc.})\end{matrix} + \tfrac{1}{2}\,n\text{HgCl}_2 \rightleftarrows \begin{matrix}\\\\\\\\\end{matrix}$$

$$\begin{matrix}\\\\\\\\\end{matrix}\ (\text{Bacterial cell}^{n-})\ (\tfrac{1}{2}\,n\text{Hg}^{++}) + \begin{matrix}(n\text{NaCl})\\(n\text{KCl})\\(\tfrac{1}{2}\,n\text{MgCl}_2)\\(\tfrac{1}{2}\,n\text{CaCl}_2)\\(\text{etc.})\end{matrix}$$

should not grow

$$(\text{Bacterial cell}^{n-})\ (\tfrac{1}{2}\,n\text{Hg}^{++}) + \tfrac{1}{2}\,n\text{H}_2\text{S} \rightleftarrows (\text{Bacterial cell}^{n-})\ (n\text{H}^+) + \tfrac{1}{2}\,n\text{HgS}$$

should grow

[2] MBCl refers to methylene blue chloride (Stearn and Stearn, 1930).

On the basis of the above theoretical consideration an effort was made to measure the cation adsorption capacity of *Escherichia coli* and other bacteria by four methods.

EXPERIMENTAL

The organisms were grown in large Blake bottles containing a suitable agar medium. After one to two days the bacteria were washed off the agar with 0.001 molar neutral $CaCl_2$ solution for H^+ adsorption and with neutral distilled water for methylene blue, mercuric chloride and magnesium adsorption.

H-adsorption. To simplify and reduce the cation to as nearly a single ionic system as possible, HCl was added to the aqueous suspension of the bacteria. The amount of HCl to be added was determined by titrating the neutral washed bacteria to a methyl red end point, or about pH 5.6-6.0. This amount of acid was arbitrarily designated as 1H and in suspensions containing about 0.2 to 0.3 per cent bacteria (dry weight) the HCl concentration approximated 0.0004 to 0.0008M, and HCl in slight excess of 1H was then added to the aqueous suspension of bacteria. The pH of such a suspension was approximately 5.0. The bacteria were then thrown out of suspension with the centrifuge and the unadsorbed HCl titrated. The H-bacteria were washed with distilled water and titrated with $Ca(OH)_2$. It was found that nearly all of the HCl added was adsorbed, and that the remainder was recoverable in the supernatant liquid.

TABLE 1

Hydrogen adsorption capacity of E. coli and viability of the H-bacteria

TRIALS	ADSORPTION CAPACITY (M.E./100 GM. BACTERIA)	PER CENT VIABLE H-BACTERIA
1	54	100
2	35	88
3	57	100
4	38	89
5	53	100

The adsorption capacity as measured by this method ranged from 35 to 57 m.e. per 100 grams of bacteria as shown in table 1.

Plate counts carried out in quadruplicates showed no appreciable reduction in the number of viable bacteria by this treatment.

That this adsorption is purely a physico-chemical phenomenon, in no way depending upon the existence of a living protoplast, is indicated by the fact that bacteria killed by heating at 100°C. exhibited, within the limits of error, the same adsorption capacity. (See table 2). The adsorption capacity of *Azotobacter*, *Rhizobium*, and *Bacillus subtilis* compared favorably with that of *E. coli*.

Methylene blue adsorption. The slightly modified method of Kolthoff and Overholser (1939) was used to measure methylene blue adsorption by *E. coli*. Methylene blue, medicinal, of 88 per cent purity was used.[3] A standard solution of 0.01 M concentration was prepared and kept in a paraffined Pyrex flask.

TABLE 2

Adsorption capacity of living and dead bacteria

TRIALS	ADSORPTION CAPACITY (M.E./100 GM. BACTERIA)	
	Dead	Living
1	30	30
2	32	35
3	38	37
4	56	43

In adsorption studies this was added to washed bacterial suspensions so as to give concentrations of 0.0004 and 0.0016 M solution of methylene blue. This mixture was shaken thoroughly and then centrifuged. The amount of methylene blue not adsorbed by the bacteria was measured by comparing the supernatant liquid with a control solution. Quantitatively, the adsorption of methylene blue was, within the limits of error, the same for living and dead bacteria. The adsorption capacity of bacteria as measured with the 0.0004 M methylene blue varied from 6 to 14 m.e. per 100 gm. When the concentration of the methylene blue was quadrupled the absorption was increased as shown in table 3.

[3] Certified by Commission on Standardization of Biological Stains.

Influence of different cations on adsorption of methylene blue. If the physico-chemical behavior of bacteria is similar to that of other colloidal complexes, as regards adsorption and base exchange, then it should be possible to note a difference in the relative ease with which different cations adsorbed by the cell can be replaced, depending upon the position of the cations in the adsorption series. To test this point, 1 ml. of 0.1 N NaCl, KCl, $CaCl_2$, $BaCl_2$, and $HgCl_2$ was added, respectively, to 25 ml. of suspensions of bacteria. After allowing sufficient time for these ions to occupy their positions in the adsorption sphere of the bacteria, 1 ml. of a 0.001 M methylene blue was added to each tube. This was shaken thoroughly and centrifuged. The amount of unadsorbed methylene blue was measured by com-

TABLE 3

Adsorption of methylene blue

TRIALS	ADSORPTION CAPACITY WITH FOLLOWING CONCENTRATIONS (M.E./100 GM. BACTERIA)	
	0.0004 M	0.0016 M
1	13	30
2	14	
3	6	24

paring with a control. About 92 per cent of the methylene blue was adsorbed by the Na and K systems, 75 per cent by Ca and Ba systems, and 50 per cent by Hg systems. Comparable results were obtained regardless of whether the experiments were carried out on living or dead bacteria.

Replacement of adsorbed magnesium. Magnesium sulfate was added to a suspension of *E. coli* to equal approximately 0.001 M solution. After allowing sufficient time for the magnesium to become adsorbed, the cells were washed until free of dissolved magnesium. To 25 ml. portions of the bacterial suspensions were added, respectively, 1 ml. of 0.1 N NaCl, KCl, $CaCl_2$, $BaCl_2$, $MnSO_4$, HCl. These suspensions were shaken thoroughly and then centrifuged. The magnesium was measured by the method of Baver and Bruner (1939). The control showed a

very slight amount of replaced magnesium. Ions like Na and K, which are adsorbed slightly or not at all, replaced such a small amount of magnesium that it was not measurable by this method. Other ions which are more strongly adsorbed replaced the adsorbed magnesium. The values obtained by replacing the adsorbed magnesium compare favorably with those obtained by measuring the adsorbed hydrogen. These values are shown in table 4.

Adsorption of $HgCl_2$ by E. coli and its replacement. If the concept of adsorption of cations in bacterial metabolism is correct, as here advanced, the possibility of explaining the toxic effect of certain electrolytes upon a similar basis would not seem illogical. In fact, an arrangement of metallic ions in the order of their toxicity for bacteria (Salle, 1939) corresponds to the

TABLE 4

Replacement of adsorbed magnesium

TRIALS	AMOUNT OF ADSORBED MG. REPLACED BY VARIOUS CATIONS (M.E./100 GM. BACTERIA)						
	Control	Na	K	Ca	Ba	H	Mn
1	Very slight	Slight	Slight		16.5	26	
2	Very slight	Slight	Slight	38	38	50	38
3	Very slight	Slight	Slight	54	54		54

order of their adsorption ability by colloids (Kruyt, 1930, Jenny and Reitemeir, 1935).

Upon this basis it might be expected that a given bacterium could be subjected to such a concentration of a toxic cation as would prove lethal and that later, it should be possible before death has actually occurred, to displace the toxic ion with one less toxic and by this means preserve the life of the cell. However, if toxicity is correlated with adsorption ability, such displacement would be impossible without increasing the concentration of the exchange ion to such a degree as might render it also toxic. The same end can be attained by suspending the poisoned cell in a solution of an anion for which the toxic cation has a greater affinity than it does for the bacterial cell.

Stearn (1928) and Engelhardt (1922), "showed that staphylo-

cocci, which had been treated for 72 hours in a 1 per cent mercury bichloride solution would, after removal of the mercuric ion by precipitation as sulfide, grow."

A similar experiment was carried out on *E. coli*. To a washed suspension of the organisms containing about 0.2 per cent dry weight of cells, $HgCl_2$ solution was added to give approximately 0.0004 M concentration. After one hour, some of the suspension was diluted and then plated out. Less than 1 per cent of the cells grew. If the Hg ion had not been adsorbed, then in the dilution process the 0.0004 M solution would become a 0.000,000,000,0004 M solution. Salle (1939) stated that Winslow and Hotchkiss (1922) have shown that 0.000,005 M $HgCl_2$ is not only not toxic but is definitely stimulatory to the growth of

TABLE 5

Adsorption of mercuric ion and its replacement

TRIALS	PER CENT VIABLE AFTER 60 MINUTES	
	Hg	Hg removed with H_2S
1	1.0	99
2	0	90
3	1.0	97

E. coli. When H_2S was bubbled through a portion of the same Hg-cell suspension, thereby removing the Hg-ion from the adsorption sphere of the organism, almost complete viability of the organism was observed. These data are given in table 5.

DISCUSSION

The data herewith presented are in agreement with the theoretical consideration upon which the experimental work was predicated. All common media contain various cations in solution. If a bacterial cell is placed in, or develops in such a medium, apparently there is an exchange of ions between the solution and cell until an equilibrium is established compatible with the energy of adsorption of the ions and their distribution in the solution. If an ion is used by the organism and becomes

an intimate part of the cell, it is possible that this process would remove an ion from the adsorption sphere. Consequently, a further shift in the equilibrium would be expected and another ion would be removed from the solution to replace the first.

In bacterial metabolism large amounts of CO_2 are liberated as waste products. The CO_2 in the presence of H_2O forms H^+ and HCO_3^-. The H^+ is adsorbed by the cell. A continuation of this reaction might result in a complete H-bacterial system. In the presence of other ions, however, an exchange of the H^+ for basic ions to equalize their distribution would be expected. Such an adsorption of cations by a bacterial cell from solution intimates that the cell may be able to concentrate and bind these ions as a store for future use.

The presence of an adsorption complex on the surface or in the interior of a bacterial cell indicates that some agent has set up an electrical force capable of holding these ions. A somewhat similar force undoubtedly orients the water molecules immediately surrounding the cell, thus creating a water hull in which the cell is encased. Each ion is likewise hydrated. Even though the ion may be adsorbed by the cell it is still in active motion, oscillating within its sphere of attraction.

The active places for attraction of the cations are not known. An approximate calculation of the number of H ions adsorbed by *E. coli* indicates that there are about 100,000,000 active sites per bacterial cell. Oxygen ions are sites of strong attraction for cations. It is well known that in proteins polar groups orient in the aqueous phase whenever possible. The presence of such groups in the cell or on the surface of the cell would produce an ionizable H which could be replaced by any cation as indicated in the following equation:

$$R - CH^{-}NH_2 - COOH = R - CH^{-}NH_2 - COO^{-} + H^+$$

Such active spots might account for the presence of a cation adsorption complex in a living cell. From the results it appears that when matter becomes living it does not lose its physicochemical properties such as the power to adsorb various materials.

CONCLUSIONS

On the basis of the results obtained in this investigation, the following assumptions seemed well-founded.

1. *Escherichia coli* adsorbed cations and the magnitude of this capacity was measured. The ability of *E. coli* to adsorb cations was demonstrated by four methods.
2. Other bacteria adsorb cations.
3. A proposed mechanism of adsorption is advanced.
4. The adsorbed cations are exchangeable.

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REFERENCES

BAVER, L. D., AND BRUNER, F. H. 1939 Rapid soil test for estimating the fertility needs of Missouri soils. Missouri Bull., **404**, 1–16.

DOCKING, A. R., AND HEYMANN, E. 1939 Studies on the lyotrophic series. II. The adsorption of salts on gelatin. J. Phys. Chem., **43**, 513–529.

ENGELHARDT, H. 1922 The action of mercuric chloride on bacteria. Disinfection, **7**, 63 and 81.

KENDALL, A. I. 1925 Bacteria as colloids. Colloid Symposium Monograph, **2**, 195–203.

KOLTHOFF, I. M., AND OVERHOLSER, L. G. 1939 Studies on aging and coprecipitation. XXIX. The aging of ortho ferric hydroxide in the absence and presence of divalent ions in ammoniacal medium. J. Phys. Chem., **43**, 909–922.

JENNY, H. 1932 Studies on the mechanism of ionic exchange in colloidal aluminum silicates. J. Phys. Chem., **36**, 2217–2258.

JENNY, H., AND REITEMEIR, R. F. 1935 Ionic exchange in relation to the stability of colloidal systems. J. Phys. Chem., **39**, 593–604.

KRUYT, H. R. 1930 Colloids. John Wiley and Sons, New York, N. Y., page 35.

ROBBINS, W. J. 1926 The isoelectric point for plant tissue and its importance in absorption and toxicity. Univ. Missouri Studies, **1**, 1–60.

SALLE, A. J. 1939 Fundamental Principles of Bacteriology. McGraw-Hill Book Company, New York, N. Y.

STEARN, A. E., AND STEARN, E. W. 1928 Studies in the physicochemical behavior of bacteria. Univ. Missouri Studies, **3**, 1–84.

STEARN, A. E., AND STEARN, E. W. 1930 The mechanism of staining explained on a chemical basis. II. General presentation. Stain Tech., **5**, 17–24.

TITTSLER, R. R., AND BERRY, G. P. 1938 The electrophoretic migration velocity of *Esch. coli* after cultivation on media of various composition. II. Observation following changes in inorganic constituents. J. Bact., **35**, 213–222.

WINSLOW, C. E. A., AND HOTCHKISS, M. 1922 Studies on salt action V. The influence of various salts upon bacterial growth. Proc. Soc. Exptl. Biol. Med., **19**, 314–315.