STUDIES ON THE ACTION OF ELECTROLYTES ON BACTERIA.

PART I.

THE ACTION OF MONOVALENT AND DIVALENT SALTS ON THE CONDUCTIVITY OF BACTERIAL EMULSIONS.

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(With 8 Charts.)

CONTENTS.

I. INTRODUCTION.

To the bacteriologist the problem of how chemical substances enter and leave the cell presents many features of special interest. It is well known that singularly few bacteria produce true exotoxins, the majority forming endotoxins, but we possess little information as to the conditions under which these pass the cell-wall. In some instances it would seem that some form of autolysis enters in the matter to a considerable extent[1].

In the case of the meningococcus, Gordon (1918) has pointed out that different strains of this germ possess widely different toxic powers for mice, but that they all have the same minimal lethal dose for these animals when ground up and extracted with distilled water. Thus in this case it would look as if toxicity depended on the ease with which the endotoxin diffuses out of the cell; while some races of the germ allow it to escape freely, others only permit it to pass in small quantities, but that all strains possess the same amount. Similar conditions probably hold for many other pathogenic bacteria.

The striking part played by capsule formation in the acquisition of virulence by many races of bacteria clearly points to the importance of this struc-

[1] These studies had their origin in an attempt to determine some of the conditions of autolysis in the meningococcus.

https://doi.org/10.1017/S0022172400007531 Published online by Cambridge University Press

ture in preventing the penetration of many substances into the bacterial cell. Danysz (1900) has drawn attention to the fact that anthrax bacilli frequently possess arsenic and serum resistant qualities which are always coupled with their ability to form capsules. It has been shown that organisms like the pneumococcus and some streptococci, which sometimes acquire this power, are invariably most virulent in the capsulated condition. The capsule would seem to play a rôle similar to the action of one colloid on another, in preventing aggregation and flocculation, and the notorious difficulty always experienced in agglutinating these forms amply confirms this contention. This is supported by Porges's (1905) demonstration that they are easily agglutinable, if the capsules are first removed by heating a short time in weak acid.

It is clear that the question as to how endosmosis and exosmosis takes place in living bacteria is one of fundamental importance, and its solution is bound up with many questions of infection, virulence and immunity. It is remarkable that its investigation so far has attracted little attention from bacteriologists. If we turn to the domain of general physiology, we find a large and ever-growing literature dealing with the subject. It has been attacked from many sides, and the application to its study of some of the recent discoveries of colloid and physical chemistry have added very much to our knowledge, and our methods of dealing with the problem have been greatly extended and improved.

The question is necessarily a complex one, in which a large number of related and interdependent actions are simultaneously taking place. It involves the passage of substances from the external medium, through the cell-wall to the cytoplasm, across the intermediary boundary phases. In a balanced solution, that is, one that contains two or more salts in definite proportions, so that their specific individual action does not come into play, but is antagonised, living cells can remain for relatively long periods without suffering any injury. If the salts are sufficiently numerous, such solutions can even act as nutrient fluids, in which many plants and bacteria can actively grow and reproduce. It has been pointed out that under these conditions the cell-wall must be semi-permeable, that the passage through it of salts must be conditioned to a large extent by the chemical changes going on within the cell, and that the physical properties of the cell-wall play a minor part in the matter. Regarded in this light the problem is a dynamical one, in which equilibrium is constantly being adjusted between the external and the internal conditions.

If we place living cells, on the other hand, in weak but pure solutions of these salts we find we are dealing with a different problem. The fundamental conditions are now altered and the normal stability of the cell-wall has been destroyed. In such a solution we are studying the unantagonised action of a salt on the cytoplasm.

The results obtained from the investigation of this side of the question are of great experimental interest. It is particularly interesting to apply

experiments of this kind to bacteria, as there is no other class of organisms in which surface conditions and the properties of the cell-wall play a greater part, as they present relatively such a great extent of surface for a given mass, on account of their small size, surface conditions on all occasions determine their behaviour. The following paper for these reasons is mainly devoted to an investigation of the action of various salts in pure solutions in altering the cell-wall and surface conditions of bacteria.

It has been shown by Oker-Blom (1900), that if we estimate the relative conductivity of various mixtures of sand and a solution of NaCl, we find this is proportional to the quantity of sand in the solution. As an electric current is carried through a solution by its ions, the rate at which these will conduct the current is dependent on two factors, first the potential gradient, and secondly the friction or resistance offered by the solution to the passage of the ions. If the potential gradient is kept fixed, then any additional obstruction to the passage of the ions will necessarily increase the resistance and lower the conductivity. In a mixture of 61 parts quartz sand and 39 parts NaCl solution, he found the conductivity was 24·5 per cent. that of the NaCl solution without sand. The ions of such a mixture were forced by the grains of sand to take a zigzag course in passing from one electrode to another, and the resistance is proportionally increased.

In a similar manner the conductivity method has been used by Róth (1897), Bugarsky and Tangl (1897), Oker-Blom (1900), Stewart (1899), Woelfel (1908) to determine the proportion of blood corpuscles to plasma. If we measure the comparative conductivity of blood serum, whole blood, and corpuscles alone, we find the plasma has the highest figure, the corpuscles the lowest, and the whole blood an intermediate position. Thus these workers conclude, that the cellular elements of the blood, like the sand grains in the previous instance, offer considerable resistance to the passage of the ions of the plasma.

Stewart (1910), as the result of extensive studies on the conductivity of normal as compared with laked blood, comes to the conclusion that the cell "envelopes" of the corpuscles are relatively impermeable to the ions with which they are normally in contact. He found, moreover, that any strong cytolitic agent, such as saponin, which rapidly destroys the envelop membrane, at once increases the conductivity of the corpuscles.

This conclusion would seem to be supported by Höber's (1913) experiments, where he has shown that if a conducting body is placed in the axis of a coil of wire, through which a rapidly alternating current is being passed, it will dampen or diminish this current in proportion to its power of conductance. The internal conductivity of blood cells is therefore greater than their conductivity as determined in the ordinary way. This indicates that the cell-wall or plasma membrane of the corpuscles offers considerable resistance to the passage of ions. He also found that saponin, which undergoes no dissociation, has little effect on the internal conductivity, while its cytolitic

action on the cell-wall greatly increases conductivity as determined by the Kohlrausch method; thus confirming Stewart's previous discovery.

If instead of sand grains or blood cells we add living bacteria to a clear solution of Ringer's fluid, we find in a similar way, that the resistance will increase and the conductivity decrease in proportion to the number of bacteria added. If sufficient bacteria are added to turn the fluid a white milky colour, the resistance is usually double that of the clear solution; if we add enough bacteria to turn the mixture into a thick paste the resistance may be trebled, while if we centrifuge the germs down in a solid mass, the resistance of the bacterial deposit will now be five or six times that of the original plain fluid. By placing bacteria in a similar manner in various salt solutions, if we take the precaution to make these solutions of the same conductivity as the Ringer's solution, we can determine the specific action of these solutions in altering the normal conductivity of the bacterial cell as originally determined in the Ringer's solution.

The work of Osterhout (1913), McClendon (1910), and Gray (1916) has shown that the Kohlrausch conductivity method is readily applicable to the study of the action of salts, in this manner, on living plant tissues and the animal egg-cell. Moreover the work of Perrin (1904), Girard (1910), (1919 a), (1919 b), Mines (1911) and Brooks (1917), on the passage of electrolytes through artificial and natural membranes, form a series of researches of remarkable interest, when compared with the results obtained by the conductivity method.

The conductivity method seems to have been first employed in the investigation of living cells by a number of independent workers about the same time. Among these are Róth (1897), Bugarsky and Tangl (1897), and Stewart (1910). Stewart found as already mentioned that the conductivity of blood plasma was greater than that of the whole blood, and that the resistance rose rapidly with an increase in the number of corpuscles. The action of saponin, in lowering the resistance of the corpuscles, was noted and he drew attention to the fact that it produced this effect as well on dead corpuscles. McClendon (1910) was the first to apply the method, using special electrodes, to the estimation of the changes taking place in the conductivity of the Echinoderm egg on fertilisation. This work was elaborated still further by Gray (1916), who demonstrated the marked action of the trivalent salts in altering the conductivity of these eggs in sea-water. The method was applied about the same time independently by Osterhout (1915), to determine the conductivity of the tissues of the marine alga *Laminaria* to salts in pure and balanced solutions.

It was found difficult working with bacteria to obtain resistances as high, even with the thickest emulsions or solid masses of bacteria, as those obtained by these workers. Osterhout (1918) using a special apparatus, and placing a large number of discs of *Laminaria* tissue one against another, like a roll of coins, was able to obtain resistances well over a thousand ohms; while Gray, using eggs that had been specially washed to remove the jelly-like

outer membrane, obtained resistances of 250–350 ohms, while the resistance of the same quantity of sea-water under the same conditions was only 16 ohms.

II. DESCRIPTION OF EXPERIMENTS.

It was found possible, using bacteria made up into thick emulsions, to obtain very similar consecutive readings of their resistances, if after three or four preliminary washings in Ringer's fluid they were centrifuged down into a solid mass, and then made up in a thick paste with the same quantity of fresh fluid each time. In the following experiment (Table I) is given a

Table I.

Experiment giving a series of consecutive readings of resistances made on the same meningococcus emulsion, in Ringer's solution. The bacteria were centrifuged down into a solid mass each time and then made into a thick paste with the same quantity of Ringer's sol. Temp. 25° C. Cell constant $= 4{\cdot}22 \times 10^{-1}$.

1st time	104 ohms resistance	6th time	108 ohms resistance
2nd	106	7th	108
3rd	105	8th	107
4th	106	9th	107
5th	108	10th	108

series of ten consecutive readings taken in this way, on the same emulsion of the meningococcus. It will be seen that they agree with one another very well, and only differ within a margin of a few ohms. There is a slight tendency for the resistance to rise slightly towards the end of the experiment. This is doubtless due to the washing away of salts brought over with the germs, despite the preliminary washings from the culture medium. It can be neglected as it is always slight, and the resistances for the most part in the following experiments have to deal with a fall not a rise. Only fresh 24 hrs. cultures were used, 24–30 plates of trypagar being sufficient to furnish enough material for one experiment. The bacteria were washed off the plates in a large quantity of neutral Ringer's solution[1] and centrifuged down and rewashed three times in succession in considerable quantities of fresh fluid each time, before being used for any of the experiments as already mentioned.

All measurements were made in a thermostat tank, at a fixed temperature of 25° C., which did not vary more than a twentieth of a degree. A direct reading Kohlrausch bridge was employed. In the earlier experiments the resistances were determined with a large Hamburger cell. This was made to fit into the tubes of the centrifuge direct and the emulsions were centrifuged down in the conductivity cell itself. In this way it was possible to get particularly thick emulsions having about 110 ohms resistance, while the same quantity of Ringer's solution had only 26 ohms resistance. In the later experiments a much smaller cell was employed and the emulsions were made

[1] M/8 KCl ... 25 c.c. 0·0031 M. 0·024 % KCl.
$CaCl_2$ ... 15 0·00187 M. 0·0208 % $CaCl_2$.
NaCl to ... 1000 0·12 M. 0·7 % NaCl.

considerably thinner, which gave slightly more uniform readings, and also saved time in the preparation of the material itself. In this case resistances of 150–200 ohms could be obtained, while the same quantity of Ringer's solution had about 85 ohms resistance. The cell constants for these cells are given in the tables giving the data of the various experiments[1].

If sufficient care was taken to get the original emulsion fairly thick, resistances of 110 ohms could be pretty constantly obtained with the meningococcus and slightly higher resistances with *B. coli*. The same quantity of Ringer's solution in the same cell under the same conditions of temperature having about 26·7–37 ohms resistance. Thus about three-quarters (or a little less) of the resistance of the above solution is due to the presence of the living bacteria. It was found that dead bacteria offer no resistance to the passage of the ions of a solution. Emulsions of dead bacteria have almost the same resistance as that of the fluids in which they are suspended. If the above emulsion of bacteria in Ringer's solution were killed by adding a drop of formalin or warming the emulsion to 55° C. for a few minutes, then on washing the emulsion several times in fresh changes of Ringer's solution to get rid of the formalin or of any salts derived from the dead bacteria, it will be found that the resistance has fallen from 104 or 108 ohms to 26·7 or 30 ohms, the resistance of the Ringer's solution alone. Thus there would seem to be something about the living condition which produces the resistance, and that dead cells offer no more than would so much agar or gelatine.

Living bacterial emulsions undergo little change in conductivity on standing for several days in Ringer's solution, showing a slight tendency to fall on account of adsorption of a little of the salts by the bacteria; this fall is always slight.

If, however, we make up the bacterial emulsion in pure NaCl* instead of Ringer's solution, the NaCl employed having the same conductivity as that of the Ringer's solution, *i.e.* one in which the resistance is 26·7 ohms (which is not very far removed in strength from a 0·85 per cent. NaCl solution or 0·124 M.), we obtain as in the case of the same emulsion in Ringer's solution an initial resistance of 110 ohms. This gradually drops within a short time and at the end of 30 or 40 minutes becomes the same as that of the NaCl solution without bacteria, *i.e.* 26·7 ohms. (See Table II A and Curve I.)

Thus pure NaCl of about the same concentration as that present in the blood gradually destroys the resistance offered by the bacterial cell. If the germs are allowed to lie in the NaCl for several hours, it will be found that at the end of this time they are dead. If they only remain in the NaCl a short

[1] Unfortunately in the case of the large cell this was not determined until most of the experiments had been made and the electrodes had been damaged by a slight accident. The cell constant was determined after the cell had been repaired and only applies approximately to this cell.

* All solutions used in the following experiments were made from water that had been distilled three times from glass, and Kahlbaum salts were employed.

time, and are then transferred to Ringer's solution again, they immediately regain their normal resistance and suffer no injury.

In the case of *B. coli* even at the end of one hour in NaCl they are seen to be actively motile, and death only takes place slowly after some time, as the probable result of the gradual diffusion out of the cell of some of the essential chemical substances necessary to the living state.

Table II A.

Sodium chloride Experiment.

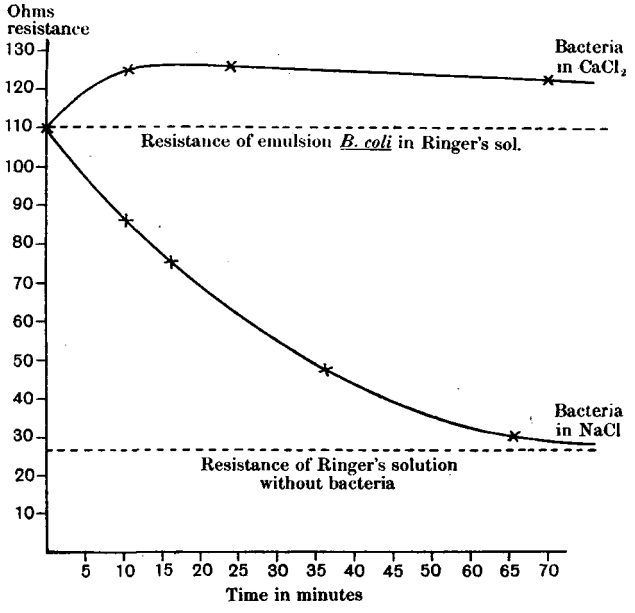
Temp. 25° C. Resistance constant of cell $= 4{\cdot}22 \times 10^{-1}$.

1. Resistance of Ringer's and NaCl (0·124 M.) each ... 26·7 ohms.
2. Resistance of Type III Meningococcus emulsion in Ringer 110 ohms.
3. Resistance due to bacteria, 110 – 26·7 ... ... 83·3 ohms.
4. Resistance of same emulsion in NaCl of the same conductivity as Ringer's sol. after

10 min.	...	90 ohms.
15	...	80
35	...	49
65	...	28

At the end of the experiment the emulsion was subcultured, at the end of 48 hrs. little growth had taken place, showing the emulsion at the time of subculture was practically dead.

Curve I, showing the resistance of emulsion of *B. coli* in pure $CaCl_2$ and NaCl solution of the same conductivity as neutral Ringer's solution. Temp. 25° C. Resistance of all solutions 26·7 ohms. Cell constant $= 4{\cdot}22 \times 10^{-1}$. $CaCl_2 = 0{\cdot}1$ M., NaCl $= 0{\cdot}124$ M.



If, when the resistance of the bacterial emulsion has fallen in the NaCl solution, a little trace of $CaCl_2$ is added, it again returns to its normal conductivity, and suffers no injury. Thus the $CaCl_2$ antagonises the action of the NaCl.

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It was found that a small trace of $SrCl_2$, $BaCl_2$, $CdCl_2$ could also antagonise in a similar manner the action of NaCl. (See Tables and Curves of these experiments.)

Table II B.

Calcium chloride Experiment I. Temp. 25° C.
Cell constant $= 4{\cdot}22 \times 10^{-1}$.

1. Resistance of Ringer's and $CaCl_2$ (0·1 M.)[1] each ... ... 26·7 ohms.
2. Resistance of emulsion of *B. coli* in Ringer's sol. ... 120·0
3. Resistance due to bacteria, 120 – 26·7 ... ... 93·3
4. Resistance of same emulsion washed in 3 changes of $CaCl_2$ of the same conductivity as Ringer's sol. after 10 min. ... 124 ohms.
   25 ... 124
   2 hrs. ... 120

Emulsion grew well on subculture at end of experiment.

Table III.

Calcium chloride Experiment II. Temp. 25° C.
Cell constant $= 9{\cdot}5 \times 10^{-1}$.

1. Resistance of Ringer and $CaCl_2$ solutions each ... ... 85·0 ohms.
2. Resistance of emulsion of *B. coli* in Ringer ... ... 135·0
3. Resistance due to bacteria, 135 – 85 ... ... 50·0
4. Resistance of same emulsion in $CaCl_2$ sol. (0·1 M.) after
   25 min. ... 167 ohms.
   30 ... 161
   35 ... 161
   2 hrs. ... 161

Emulsion grew well at end of experiment on subculture.

Table IV.

Calcium nitrate Experiment. Temp. 25° C.
Cell constant $= 9{\cdot}5 \times 10^{-1}$.

1. Resistance of Ringer and calcium nitrate sols. each ... 85·0 ohms.
2. Resistance of emulsion of *B. coli* in Ringer ... ... 120·0
3. Resistance due to bacteria, 120 – 85 ... ... 35·0
4. Resistance of same emulsion in $CaNO_3$ isotonic with Ringer 124·0
5. Resistance of same emulsion in $CaNO_3$ after 1 hour ... 123·0

Emulsion grew well at end of experiment on subculture.

Further experiments with other salts of the monovalent group, such as KCl, LiCl, RbCl, CsCl, showed that each of these in turn possessed in a varying degree the power of reducing the resistance offered to the passage of the ions of a solution, in a manner similar to NaCl. In all instances this increase of conductivity was reversible, the bacteria returning to their normal condition on being transferred to any balanced solution, such as Ringer's, sea-water, or van't Hoff's solution. The addition to any of the above monovalent solutions of a slight trace of a divalent salt such as $CaCl_2$, $SrCl_2$, or $BaCl_2$, $CdCl_2$, prevents the fall in resistance in the monovalent solution from taking place.

[1] The molecular strengths of the solutions have only been roughly calculated from the conductivity.

https://doi.org/10.1017/S0022172400007531 Published online by Cambridge University Press

Table V.

Lithium chloride Experiment. Emulsion of *B. coli*. Temp. 25° C.
Cell constant = $4{\cdot}22 \times 10^{-1}$.

1.	Resistance of Ringer and LiCl (0·195 M.) each	26·7 ohms.
2.	Resistance of *B. coli* emulsion in Ringer's sol.	147·0
3.	Resistance due to bacteria, 147 – 26·7	120·3
4.	Resistance of same emulsion in LiCl sol. (0·95 M.) after 25 min.	113·0
5	Resistance of same emulsion in LiCl sol. (0·95 M.) after 35 min.	100·0
6.	Resistance of same emulsion in LiCl sol. (0·95 M.) after 50 min.	86·0

Table VI.

Rubidium chloride Experiment. Emulsion of *B. coli*. Temp. 25° C.
Cell constant = $9{\cdot}5 \times 10^{-1}$.

1.	Resistance of Ringer and RbCl	85 ohms.
2.	Resistance of emulsion of *B. coli* in Ringer	120
3.	Resistance due to bacteria, 120 – 85	35
4.	Resistance in RbCl sol. after 40 min.	105
5.	Resistance in RbCl sol. after 50 min.	100

Table VII.

Hydrochloric acid Experiment. Emulsion of *B. coli*. Temp. 25° C.
Cell constant = $9{\cdot}5 \times 10^{-1}$.

1.	Resistance of Ringer and HCl (0·049 M.) each	85 ohms.
2.	Resistance of emulsion of *B. coli* in Ringer	110
3.	Resistance of same emulsion in HCl after 45 min. ...	128
4.	Resistance of same emulsion in HCl after 65 min. ...	126

It was possible to transfer the same emulsion from Ringer to NaCl a number of times in succession, and get a fall each time in NaCl and a return to the normal resistance in Ringer. (See Curve II.)

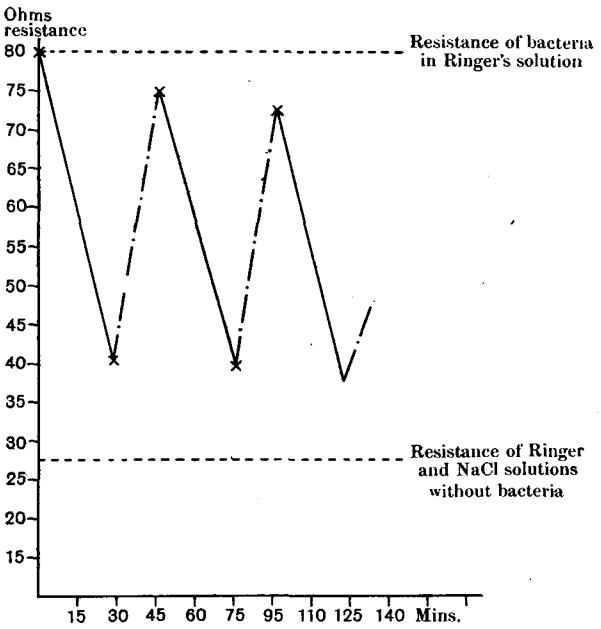
Of the members of the monovalent group, an exception must be made in the case of the H ion. A rapid rise in resistance takes place with all emulsions of bacteria placed in weak solutions of HCl, of the same conductivity as Ringer's solution; as will be seen on consulting the Table and Curve dealing with this experiment.

If, on the other hand, a bacterial emulsion is made up in a solution of some bivalent chloride, such as $CaCl_2$, $SrCl_2$, $BaCl_2$[1], of the same conductivity as Ringer's solution, if the emulsions are sufficiently thick to give fairly high resistances, a slight rise in resistance usually takes place. This is followed after 6 or 9 hours by a slow fall. If, after 12 or 15 hours when this fall is well marked, the emulsion is returned to Ringer's solution, van't Hoff's solution, sea-water, or any balanced solution, it will be found that the emulsion does not regain its normal conductivity, as when a little $CaCl_2$ is added to a monovalent salt. There is this difference between the fall of resistance of an emulsion in a monovalent and a divalent salt, that in the latter the fall is irreversible.

[1] In the $CaCl_2$ and other bivalent salt solutions the emulsions are always more viscid and less fluid than in the monovalent salt solutions.

Curve II. Experiment showing the antagonistic action of $CaCl_2$ in Ringer's solution to the action of pure NaCl solution of the same conductivity as Ringer's solution. Meningococcus emulsion. Temp. 25° C. Unbroken lines represent the meningococcus in NaCl solution, broken lines—same emulsion in Ringer's sol., the emulsion being transferred three times from NaCl to Ringer's sol. In each instance that the emulsion was placed in NaCl the resistance fell to rise again almost to the normal when transferred back to Ringer.

A similar curve was obtained when a pure $CaCl_2$ solution was used of the same conductivity. NaCl = 0·124 M.



If, in place of living, we use an emulsion of dead bacteria for any of the foregoing experiments, none of these changes take place. They are therefore dependent on the cells of the emulsion being alive, as already mentioned; dead bacteria offer no resistance to the passage of the ions of a solution. The gradual fall of resistance which is irreversible in a bivalent chloride is the result of the slow death of the emulsion.

It may be suggested that these effects are to some extent the result of injury to the cell by the electric current or its forcing the ions of the solution through the cells. This cannot be the case, as the action of the monovalent cat-ion is as marked, when the bacteria are placed in these solutions in the absence of any electrical current, as when a current is passing.

In a previous paper (1917) I have demonstrated that the meningococcus is rapidly killed if allowed to remain in 0·85 per cent. NaCl solution for a short time, and that this toxic effect is avoided if a small trace of $CaCl_2$ is added to the solution. Thus in the absence of any current we can get the same results as when a current is employed. There is a large amount of evidence to show

that the characteristic action of a monovalent as compared with a bivalent salt in pure solutions is universal for all living cells.

Loeb (1906) has described similar effects of the action of dilute NaCl on the egg of the marine teleost *Fundulus*. The eggs of this fish develop normally in sea-water. If they are put into pure NaCl having the same concentration as that of the sea-water, none of them develop. If, however, a trace of $CaCl_2$ is added, as many eggs develop as in ordinary sea-water. The same effect is produced if a little Sr, or Ba, is added in place of the $CaCl_2$. He has also shown (1906) that muscle tissue contracts rhythmically when immersed in pure solutions of salts with a monovalent cat-ion, such as Na, Li, Rb and Cs, but that the addition of a small quantity of a bivalent cat-ion inhibits these contractions.

The interest of the foregoing results consists in that they show that the action of electrolytes on bacteria is similar in all respects to their action on plant and animal cells, as determined in different ways by Loeb and Wastenays (1915), Osterhout (1915), Gray (1916), McClendon (1910), Brooks (1917), and other workers.

In *Laminaria*, Osterhout (1915) finds that with $CaCl_2$, and also with $BaCl_2$ and $SrCl_2$, there is invariably a brief temporary rise of resistance when placed in these solutions of the same conductivity as that of the sea-water employed in his experiments. This is followed by a gradual fall. On account of the low resistance used in the present experiments, this preliminary rise of resistance is not so marked. Its presence was frequently demonstrated with $CaCl_2$ and $SrCl_2$, as the data and curves of some of the experiments given in this paper plainly show. In the few experiments done with $BaCl_2$, it was not noticed, but it is clear that this salt can obviously antagonise the action of NaCl, and it undoubtedly belongs to the same group, and probably does not differ from them in this respect.

Table VIII.

Magnesium chloride Experiment.

Temp. 25° C. Cell constant $= 4{\cdot}22 \times 10^{-1}$.

1. Resistance of Ringer and $MgCl_2$ solutions each ... ... 26·7 ohms.
2. Resistance of *B. coli* in Ringer's sol. ... ... 108·0
3. Resistance due to bacteria, 108 − 26·7 ... ... 81·3
4. Resistance of same emulsion in $MgCl_2$ sol. (0·09 M.) after

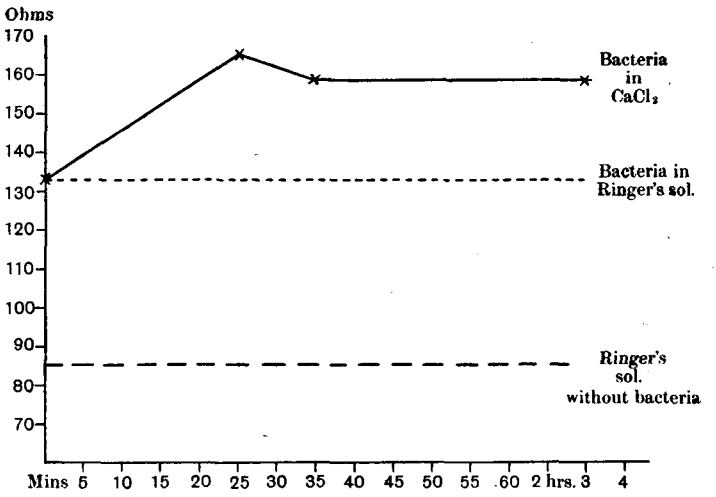
10 min.	...	100 ohms resistance
20	...	88
25	...	80
35	...	72
45	...	72

1 drop 40 per cent. formalin added. Resistance fell to 28 ohms.

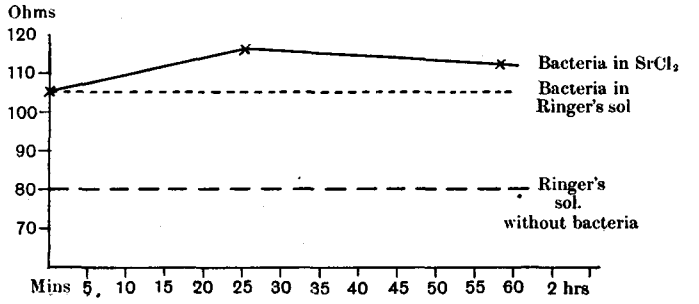
In the light of the present experiments, the time-honoured practice of suspending living bacteria in normal physiological saline (NaCl 0·85 per cent., 29/200 or 0·145 M.) would have little to recommend it, beyond the ease with which most bacteria can be emulsified in such a solution.

As usually employed in practical bacteriology, the action of the NaCl seldom really comes into play, as sufficient $CaCl_2$ is always brought over from the culture medium to completely antagonise the NaCl. In Ringer's solution for instance the amount of $CaCl_2$ present is only 0·00187 M. Thus in an ordinary bacterial suspension, unless this has been washed once with saline, sufficient $CaCl_2$ is present to prevent the specific action of the NaCl. This is doubtless the reason why the injurious action of this salt in ordinary bacteriological manipulation has been so rarely noticed, Flexner (1907) in his classical paper on the meningococcus being one of the few to draw attention to it.

The use of saline solution in practical bacteriological work seems to have originated in a somewhat mistaken opinion of the importance of the rôle of osmotic pressure with regard to bacteria, for which the well-known paper of Fischer (1895), on the plasmolysis, is largely to blame. In distinction to red blood cells, bacteria can easily withstand wide changes in osmotic pressure. In the case of the meningococcus I have shown that this germ can readily survive 24 hours in pure glass distilled water. This is otherwise if the water



Curve III. Calcium chloride Experiment II. $CaCl_2 = 0{\cdot}1$ M.



Curve IV. Strontium chloride Experiment.

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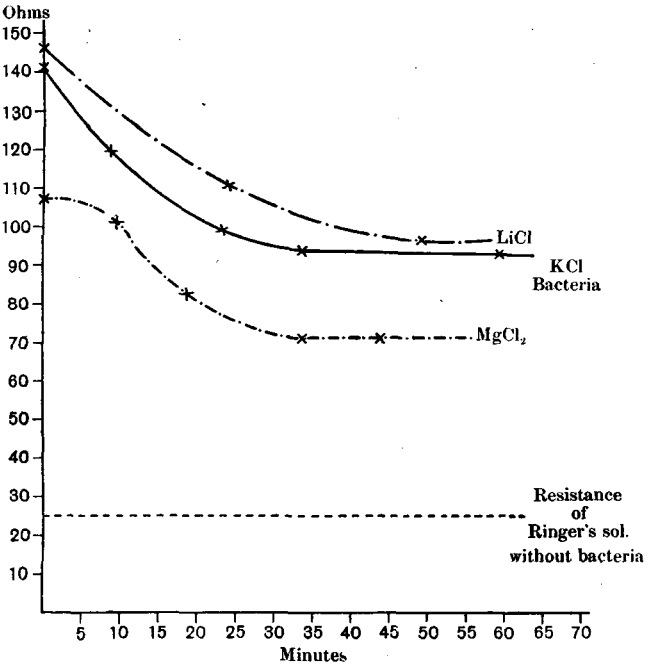
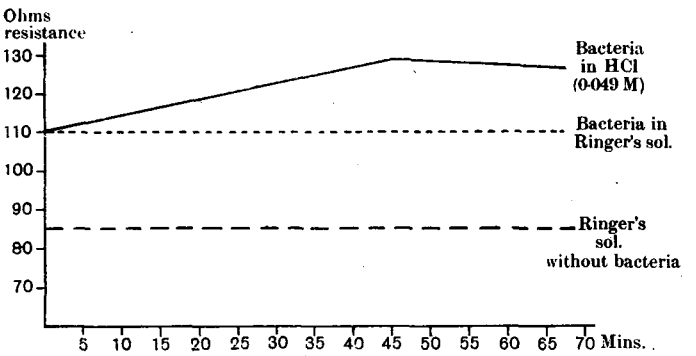
has been obtained from a metal still. Osterhout (1913) has also drawn attention to the fact that glass distilled water has no toxic action on *Spirogyra*, while metal distilled water has a marked action in this respect.

Curve V, showing the resistance of *B. coli* in Potassium chloride (=0·09 M.) solution. LiCl=0·195 M. $MgCl_2$=0·09 M.





Curve VI. Hydrochloric acid Experiment.

In regard to the action of $MgCl_2$, Osterhout (1915) found that a close study of the action of this salt revealed the fact that, although the fall due to its toxic action was very abrupt, resembling at first sight that of NaCl, this salt really acts like $CaCl_2$, $BaCl_2$ and $SrCl_2$, in that it produces a short temporary

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rise in resistance, followed by an irreversible slow fall in resistance. If this should be the case then the fall in resistance obtained with bacteria should be irreversible. The following experiment would seem to show that this is the case, and that $MgCl_2$ in its action is to be classed with the other bivalent chlorides as $BaCl_2$, $SrCl_2$, and $MnCl_2$.

Table IX.

Experiment Demonstrating the Antagonistic Action of $BaCl_2$ to NaCl. Temp. 25° C.

(*a*) To 100 c.c. NaCl of the same conductivity as Ringer's sol. a few crystals of $BaCl_2$ were added until the resistance was 20 ohms.

(*b*) To 100 c.c. Ringer's sol. distilled water was added till the resistance equalled 20 ohms.

1. Resistance of *B. coli* emulsion in (*b*) was 95 ohms.
2. Resistance due to presence of bacteria was 75 ohms.
3. Resistance of same emulsion of *B. coli* (*a*) after 15 min. was 95 ohms.: practically no change.

Table X.

Irreversible action of $MgCl_2$ on bacteria.
Temp. 25° C. Cell constant $= 9{\cdot}5 \times 10^{-1}$.

1.	Resistance of Ringer and $MgCl_2$ solutions each	83 ohms.
2.	Resistance of emulsion of *B. coli* in Ringer	138
3.	Resistance due to bacteria, 138 - 83	55
4.	Resistance in $MgCl_2$ sol. after 25 min.	96
5	Resistance of above emulsion plus 5 drops $CaCl_2$ (0·1 M.)	88
6.	Resistance of above emulsion washed 3 times in fresh Ringer	88

Table XI.

Cadmium chloride Experiment.
Temp. 25° C. Cell constant $= 9{\cdot}5 \times 10^{-1}$.

1.	Resistance of Ringer and $CdCl_2$ solutions each	85 ohms.
2.	Resistance of emulsion of *B. coli* in Ringer	115
3.	Resistance of emulsion of *B. coli* in $CdCl_2$ solution (0·065 M.)	116

Emulsion actively motile at the end of the experiment.

Table XII.

Strontium chloride Experiment.
Temp. 25° C. Cell constant $= 9{\cdot}5 \times 10^{-1}$.

1.	Resistance of Ringer and $SrCl_2$	80 ohms
2.	Resistance of emulsion of *B. coli* in Ringer's sol. ...	105
3.	Resistance of same emulsion in $SrCl_2$ sol. after	
	25 mins. ... 115 ohms.	
	60 ... 112	

In view of the results obtained in the foregoing experiments of the action of the monovalent and divalent salts in altering the normal conductivity of the bacterial cell, it is highly important to determine if these changes are correlated in any way with a modification of pathogenic power. To determine this fresh cultures of *B. anthracis* and the pneumococcus were used. The pneumococcus strain was passed through a number of mice before being used.

Emulsions of these bacteria were treated as in the preceding experiments, with NaCl, $CaCl_2$ and Ringer's solution, and injected into mice and the effects of these solutions on pathogenetic powers observed. In all instances the salt solutions used in these experiments were carefully sterilized before being employed for making up the emulsions of bacteria. The results of these injections are shown in Tables XIV and XV.

Thus a fresh culture of *B. anthracis* was made up in an emulsion in Ringer's solution, its resistance determined, and then transferred to NaCl solution of the same conductivity as the Ringer's solution, being rapidly washed in several changes of NaCl solution to eliminate all trace of the Ringer's solution. It was allowed to remain in the NaCl solution (0·124 M.) for about fifteen minutes, till its resistance had fallen about two-thirds of the way to that of the NaCl solution alone without bacteria, and an estimated dose was then injected into several or more mice. A similar dose of the same emulsion, which had remained in Ringer's solution all the time, was injected into a second lot of mice, of approximately the same weight as those of the first batch. Into a third group of mice a similar dose of the same emulsion was injected in NaCl solution, to which a trace of $CaCl_2$ had been added. All these emulsions were diluted down in their respective fluids, before being injected, so they rendered these fluids slightly turbid. The results obtained from experiments of this kind were invariably uniform.

It was found that when the resistance had fallen in the NaCl solution, the bacteria failed to kill the mice, or only did so after seven or eight days' time, while those mice that had received the same emulsion in a similar dose in Ringer's solution were sometimes dead within 18 hours and were always dead within 24 or 36 hours. The same applies to the mice that had received the germs in NaCl, to which a little $CaCl_2$ had been added. (See Tables XIV and XV.)

It would seem that either the majority of the bacteria in the NaCl solution were dead, when they were injected into the animals, or that the action of the NaCl was such as to render them almost harmless. Similar results were obtained when special care was taken to see that the resistance of the germs in the NaCl had not fallen too low. Subcultures made from these emulsions, at the time they were injected, gave a very good growth after 24 hours' incubation, showing that a fair percentage of the bacilli were still alive. There was therefore some evidence for thinking that the NaCl had a detoxicating action on the germs.

It was found when some of these bacilli were exposed in NaCl solution (0·124 M.) to the action of washed leucocytes, they were taken up by these leucocytes immediately, and within a short time few free bacteria remained outside the phagocytes. The same bacteria in Ringer's solution under similar conditions were taken up at a much slower rate by the leucocytes. This result again may be due to the detoxicating action of the NaCl on the bacteria, rendering them more liable to attack by the leucocytes.

If on the other hand we inject the same bacteria in similar doses in $CaCl_2$ solution (0·1 M.) they kill the mice in even smaller doses than when they are injected in Ringer's solution. In such a solution the preceding experiments show that a slight rise of resistance takes place. It was found however that the $CaCl_2$ solution itself without any bacteria often had an ill effect. In this respect $CaNO_3$ seemed more toxic than $CaCl_2$. If two lots of mice were injected, one with a lethal dose of *B. anthracis* in $CaNO_3$ and the other with the same germ in $CaCl_2$, the batch that had received the bacteria in $CaNO_3$ always were the first to die. In making experiments with $CaCl_2$, control mice were always injected with some of $CaCl_2$ alone without bacteria; it was seldom that these showed any ill effects from the dose of Ca employed in the previous injections. It is clear that in the $CaCl_2$ solutions no detoxication takes place if the bacteria are injected after standing in this solution for 30 minutes, and possibly a slight increase of toxicity takes place.

III. DISCUSSION OF THE BEARING OF SOME OF THE RESULTS OF THE FOREGOING EXPERIMENTS ON THE RÔLE OF CERTAIN SALTS IN THE QUESTION OF WOUND INFECTION.

These results have a direct bearing on some recent research on wound infection and gas gangrene.

Bullock and Cramer (1919) find that the organisms of gas gangrene, when emulsified in saline (NaCl 0·85 per cent. or 0·145 M.) and washed in several changes of this fluid to remove toxins, fail to kill mice and guinea-pigs, while similar doses of broth cultures of these organisms produce a violent gas gangrene which kills the animals in twenty-four hours. In washing the bacteria in several changes of NaCl to eliminate all toxins, it will be seen that they are repeating the conditions of the preceding experiments with this salt, where the NaCl destroys the normal stability of the cell wall and cytoplasm, which has been shown to be correlated by experiments on animals with a loss of toxicity. They find further that this action can be prevented by adding a little Ca salt, either to the culture in saline when it is being injected, or by injecting the animal a short time after with a large dose of Ca. In this case they are simply antagonising the action of the NaCl, and under these conditions, as has been shown in the previous experiments, the conductivity of the bacterial cell returns to its normal condition, and the germs regain their toxic power.

Unfortunately, to determine if the action of the Ca salts is on the bacteria or the tissues of the animal into which the bacteria have been injected, they incubated their cultures for three hours in weak $CaCl_2$; "the suspension was then centrifuged, and the bacteria after washing with saline suspended in NaCl, and the suspension then injected into mice" (p. 521). As would be expected they find that after this treatment the germs are still non-toxic. They conclude from this that the action of the $CaCl_2$ is not on the bacteria, and therefore must be on the tissues of the animal.

To this action they give the name of "kataphylaxis." It would seem, however, that the real point in question has been overlooked and this is the action of the NaCl on the bacteria. In testing the action of the $CaCl_2$ on the germs, they make the mistake of washing this away with NaCl. In washing once in NaCl and resuspending the bacteria in this solution for injection, they have removed all trace of $CaCl_2$, and the unantagonised action of the NaCl again comes into action. The germs are now in the same state as they were previous to treatment with $CaCl_2$, and are unable to kill the animals.

No doubt the injection of the NaCl or the $CaCl_2$ may lead to a certain amount of injury to the tissues at the local point of injection, due to the action of these salts on the tissue cells, but this action is bound to be as great on the bacteria, unless we assume there is a selective action on the tissue cells. Where the $CaCl_2$ is added to the emulsion of bacteria in saline before injection, this cannot take place as a balanced solution results. It is obvious that the action of the salts on the tissues is not the controlling factor, as the result is the same in both cases.

In the light of the previous experiments, Bullock and Cramer's results resolve themselves into a perfectly straightforward problem, involving the action of a bivalent salt in antagonising the destructive action of a monovalent one, on the normal stability of the bacterial cell. It is interesting to note, that they have been able to demonstrate the stabilising action of another bivalent salt, Sr; as this salt in the form of $SrCl_2$ has about the same effect on colloidal aggregation as $CaCl_2$, this is what might be expected. $MgCl_2$ on the other hand has little action in this respect, and they find it has no rupturing action. In regard to the action of $CaCl_2$ on $MgCl_2$, this has also been pointed out by several other observers. Loeb (1906) for instance has called attention to the action of $MgCl_2$ in modifying the action of $CaCl_2$ on the muscular contraction of the disc of the medusa *Polyorchis*, and has pointed out that this is probably not one of real antagonism. The action of Na citrate in destroying the rupturing action of $CaCl_2$ on the tissues is probably a purely chemical reaction in which Ca citrate is formed instead of Na citrate, NaCl also being formed.

An experiment was devised (Table XIII) to determine if Na citrate on being added to $CaCl_2$ would prevent this salt from antagonising the action of the NaCl. A solution of three parts NaCl and one part Na citrate was mixed, and to this a trace of 0·125 M. $CaCl_2$ was added. The solution was then adjusted by the addition of a little distilled water to have the same conductivity as Ringer's solution. A thick emulsion of *B. coli* was then washed in this solution several times and finally allowed to stand in it for an hour. It will be seen on consulting the following table, giving the details of this experiment, that at the end of this period the resistance of this emulsion had dropped very appreciably. It is obvious that the $CaCl_2$ has failed to prevent the NaCl from lowering the resistance of the emulsion. Bullock and Cramer when they use these salts together are undoubtedly repeating the conditions of this experiment.

Table XIII.

Experiment to show the action of Na citrate on $CaCl_2$.

Solutions made up as follows:

3 parts NaCl, 0·124 M. of the same conductivity as Ringer.

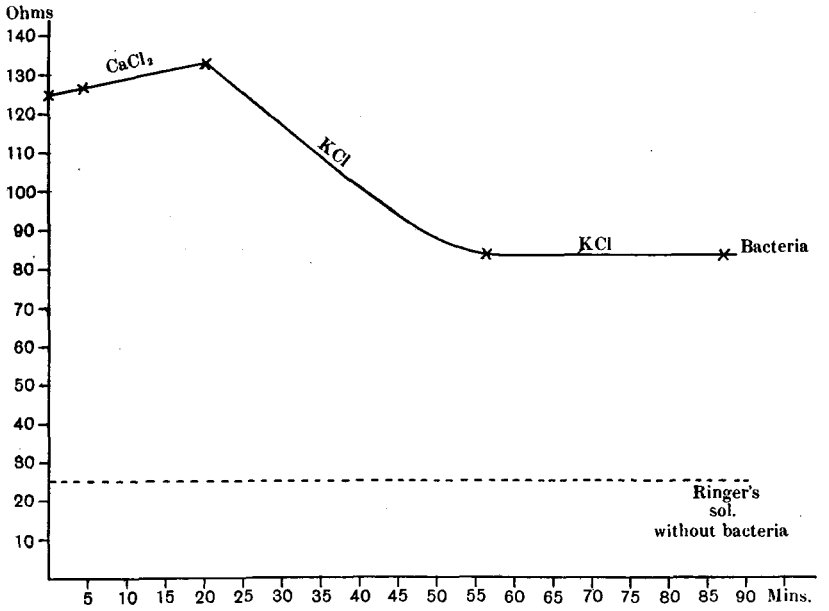
1 part Na citrate of the same conductivity as Ringer.

To 100 c.c. of above solution 1 c.c. of 0·125 M. $CaCl_2$ was added and shaken. Distilled water was then added till the conductivity was the same as that of Ringer's solution.

1.	Resistance of Ringer's sol.	85 ohms.
2.	Resistance of NaCl plus Na citrate plus $CaCl_2$	85
3.	Resistance of *B. coli* emulsion in Ringer	131
4.	Resistance due to bacteria, 131 – 85	46
5.	Resistance in NaCl plus Na citrate plus $CaCl_2$ after 1 hr.	90·6
6.	Drop in resistance in above solution after 1 hr.	40·6

The $CaCl_2$ in the above solution has not antagonised the action of the NaCl on account of the presence of the Na citrate.

Curve VII. Calcium chloride and Potassium chloride Experiment.



In a previous paper Cramer (1918) makes some observations on the action of NaCl and $CaCl_2$ in affecting the growth of cancer cells. He found that if equal volumes (about 12 c.c.) of sterile M/7·5 solutions of NaCl and $CaCl_2$ in tap water were placed separately in two test-tubes, and 0·5 c.c. of a fresh emulsion of cancer cells were added to each and shaken up for an hour or so, the emulsion in $CaCl_2$ on injection into mice failed to grow as well as the control emulsions. The NaCl on the other hand grew at about the same rate as the controls, and the treatment with this salt had no effect in retarding the growth of the cancer cells.

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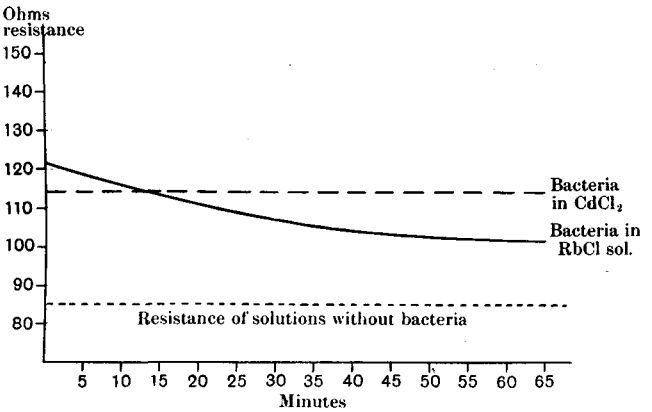
Curve VIII. Rubidium chloride and Cadmium chloride Experiment. Temp. 25° C. Cell constant = $9{\cdot}5 \times 10^{-1}$. Emulsion of *B. coli*.



Cramer does not state if the cancer cell emulsions were first washed several times in distilled water or several changes of NaCl before being finally allowed to remain in the NaCl, to remove all possibility of any Ca being brought over with the emulsion. It is well known that some cancerous growths may contain very considerable quantities of Ca. This amount of Ca combined with that possibly present in the tap water employed might very seriously interfere with the action of the NaCl, especially as the volume of NaCl used was rather small.

All these conditions may have been duly guarded against in this work, but no information is given to this effect in the description of the experiments.

In this paper as well as in the previous one it does not seem to have been realised what a small quantity of $CaCl_2$ is required to antagonise the action of NaCl. The ratio of these salts for instance in van't Hoff's solution being 2·3 to 100, and in the Ringer's solution used in the previous experiments the strength of the $CaCl_2$ is only 0·00187 M. as already mentioned.

The ions of one salt are said to antagonise another, when both are simultaneously present in a solution, and each prevents the other from entering the cell or exerting its specific action on the stability of the cell-wall and cytoplasm. Cramer therefore, in placing an emulsion of cancer tissue in a solution of M/7·5 $CaCl_2$ for a certain time and at the end of this period decanting the cells and resuspending them in M/7·5 NaCl solution for some time, is not strictly speaking dealing with a condition of true antagonism[1]. It is more or less a chance that under these circumstances antagonism takes place.

It is a question, moreover, if cancer tissue can be broken up and emulsified sufficiently to allow the NaCl to act on the cells. I have pointed out in my paper on the action of NaCl on the meningococcus that it fails to kill this

[1] These solutions are not isosmotic as stated in this paper.

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Table XIV.

Experiments with *B. anthracis* on mice, a fresh 24 hr. culture being employed. The bacilli, after their conductivity had been determined in thick emulsions, were diluted down in each case to a strength approximately equal to about one million germs to the cubic centimeter; in this dilution they were injected in varying doses. 0 = animal alive and well, + = animal dead, h = hrs., d = days. All salt solutions used for these experiments had been carefully sterilized.

B. anthracis[1].

No. mouse	Weight mouse	Dose and character of solution injected	Result	Remarks
1	18 grams.	0·25 c.c. in 0·124 M. NaCl sol.	+ 24 h	Small animal
2	22·5 ,,	,, ,, ,,	0 4 d	
3	30 ,,	,, ,, ,,	0 ,,	
4	27 ,,	,, ,, ,,	0 ,,	Slightly ill after 48 hrs.
5	25 ,,	0·5 ,, ,,	0 ,,	
6	28 ,,	,, ,, ,,	+ 3 d	No signs of illness till 3rd day
7	31 ,,	,, ,, ,,	0 4 d	
1	26 ,,	0·25 c.c. in Ringer's sol.	+ 24 h	Some of these had been dead some time when examined at end of 24 hrs.
2	20 ,,	,, ,, ,,	+ ,,	
3	29 ,,	,, ,, ,,	+ ,,	
4	32 ,,	0·5 ,, ,,	+ ,,	
5	27 ,,	,, ,, ,,	+ ,,	
1	20 ,,	0·25 c.c. in $CaCl_2$ solution	+ 24 h	
2	26·5 ,,	,, ,, ,,	+ ,,	
3	21·5 ,,	,, ,, ,,	+ ,,	
4	26 ,,	0·5 ,, ,,	+ ,,	
1	28 ,,	0·5 c.c. $CaCl_2$, without bacteria	0 24 h	No signs of illness
2	30 ,,	,, ,, ,, ,,	0 ,,	
1	19 ,,	0·5 c.c. NaCl + $CaCl_2$ solution*	+ 24 h	
2	29 ,,	,, ,, ,,	+ 48 h	
3	27 ,,	,, ,, ,,	+ 24 h	

* Composed of 200 c.c. 0·85 % sterile NaCl + 0·04 c.c. M/1 $CaCl_2$ and adjusted to have the same conductivity as Ringer's solution.

Table XV.

Experiments with pneumococcus on mice. The pneumococcus strain had been passed through six mice previous to its use in this experiment. All emulsions were diluted down to approximately the same strength before being injected into the animals. 0 = animals alive and well, + = animal dead, h = hours, d = days.

Pneumococcus.

No. mouse	Weight mouse	Dose and character of solution injected	Result	Remarks
1	25 grams.	0·25 c.c. in Ringer's solution	+ 24 h	
2	30 ,,	,, ,, ,,	+ ,,	
3	21 ,,	0·5 ,, ,,	+ ,,	
4	23 ,,	,, ,, ,,	+ 48 h	*
1	31 ,,	0·25 c.c. in 0·124 M. NaCl sol.	0 24 h	Dead 3rd day
2	19 ,,	,, ,, ,,	0 ,,	
3	25 ,,	,, ,, ,,	0 ,,	
4	22 ,,	,, ,, ,,	0 ,,	
5	28 ,,	0·5 ,, ,,	0 ,,	Dead 8th day

[1] The groups of animals to which the three consecutive tables relate were in each case injected with the same bacterial emulsion.

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Table XVI.

B. anthracis.

No. mouse	Weight mouse	Dose and character of solution injected	Result
1	26·5 grams.	0·25 c.c. Ringer's solution	+ 24 h
2	31 „	„ „ „	+ „
3	29	„ „ „	+ 48 h
1	22 „	0·25 c.c. in 0·124 M. NaCl sol.	0 3 d
2	27 „	„ „ „	0 „
3	29 „	„ „ „	0 „
1	30 „	0·25 c.c. in 0·124 M. NaCl + trace Ca nitrate	+ 24 h
2	25 „	0·25 c.c. in 0·124 M. NaCl + trace Ca nitrate	+ „

germ if any clumping of the cocci takes place. On subsequent incubation, the bacteria in these clumps grow and give rise to isolated colonies; the NaCl seem unable to affect the cocci in the interior of the clumps. In order that the NaCl may kill all the bacteria, it is necessary to employ emulsions entirely free from clumps or masses of bacteria. I fail to see how cancer tissue can be emulsified so thoroughly that all the cells are separated, and the NaCl given free scope to act.

I believe this reason, as well as the fact that no special precautions were taken to see that no Ca was introduced with the cancer tissue cells in these experiments, accounts for the failure to get the action of the NaCl. In these experiments the real action of the NaCl on the cancer cells has not been properly demonstrated. There is reason to think that if they were repeated with due attention to these points, the action of this salt on the cancer cell would be the same as on all other animal and plant cells. That sufficiently long exposure to pure solutions of NaCl in a concentration of 0·145 M., say two or three hours, would result in the death of these cells.

Thus the failure to distinguish the characteristic action of NaCl on living cells, and its highly reversible nature, renders the results of Cramer, Bullock and Cramer open to a different interpretation from that given by them.

No evidence has been advanced by Bullock and Cramer to show that the action of the NaCl on the bacteria is not the decisive factor in the matter. If a similar effect could be produced by the injection of the bacteria in distilled water, there might be some ground for believing that the action of the Ca was on the tissues. If on the other hand it can only be obtained by the injection of bacterial suspensions that have been washed several times in NaCl, in the light of the present conductivity experiments the explanation is obvious.

We come finally to the question as to what do these changes in the conductivity of bacterial emulsions signify? Osterhout, in his experiments on plant tissues, believes that they give us definite values for the permeability of the tissues to the ions. This view has been openly challenged by Stiles and

Jørgensen (1918), who point out that this is to a certain extent a pure assumption.

In placing living cells in pure salt solutions, no matter how weak, it is doubtful if we are investigating permeability at all, but simply the destructive action of the salt on the cell, as the result of which a certain amount of exosmosis or endosmosis takes place. Neither Osterhout's experiments, nor those of Stiles and Jørgensen, or Stiles and Kidd (1919), give us any information as to how absorption takes place from a balanced solution. It would seem that the problem is one more for the protein chemist than for the experimental physiologist.

Sørensen (1917), in his extensive studies on the physical properties of proteins, has shown that the capacity of egg-albumen to combine with acids and bases, at a certain hydrogen ion concentration, is a function of the amount of ammonium sulphate present, and is greater as the latter increases.

It is possible on a basis of a measurement of the number of hydrogen ions present in a solution of egg-albumen, having a known composition and containing ammonium sulphate, to estimate the total content of surplus acid, and to determine by formula its approximate distribution between the two phases of the egg-albumen solution.

It will perhaps be from investigations of a similar character, on the action of the ions of Na, K, and Ca, on the physical properties of the proteins and lipoids, that will show us how these ions stabilise the cell membrane and cytoplasm, for it is clear that in the presence of these ions the semi-permeability of the cell-wall remains constant.

Schryver (1913), in a series of researches on the formation of gels from cholate solutions, has shown that Na cholate solutions set to a gel when heated in the presence of Ca. This gel formation is readily inhibited by the presence of relatively small quantities of NaCl. In further experiments, he showed that cholate gels are eroded when immersed in solutions of NaCl and other chlorides, and that this erosive action can be antagonised by very small amounts of $CaCl_2$. Quantitatively, the chloride solutions in their erosive action differ considerably from one another, the order of their action being as follows commencing with the greatest, LiCl, NaCl, $MgCl_2$ and KCl[1]. It will be seen that we are dealing in these experiments with similar conditions to those demonstrated by the previous conductivity experiments on living bacteria.

In order to see if the solutions found by Schryver to produce erosion of a cholate gel would also produce a change in the conductivity of bacteria emulsions, certain experiments were made; they all gave negative results, however, where chloroform and chloral hydrate[2] were used, two substances that gave the highest cholate gel-destroying capacity in Schryver's work.

[1] The effects of the action of these salts on the permeability of vegetable tissues follows the same order, as determined by Stiles and Jørgensen (1915). In the case of bacteria no attempt was made to determine the order of their action, as the resistances employed were too low to allow of any relative comparison.

[2] These experiments need repetition.

IV. SUMMARY.

The action of univalent and bivalent salts on bacteria in affecting the conductivity of thick emulsions of the meningococcus and *B. coli* demonstrates the important fact, that they alter the conductivity of these germs in the living condition, in a very definite manner. In this alteration, the predominant part is played by the cat-ion.

All monovalent cat-ions, with the exception of the H-ion, such as those of Na, K, Li, Rb, produce a rapid increase in conductivity or a fall in resistance. In its early stages this increase in conductivity is readily reversible in these solutions. If allowed to follow its due course, however, it leads finally to death in about two hours, when the bacterial cells no longer offer any resistance to the passage of the ions. The conductivity of the emulsion then becomes that of the fluid in which the emulsion has been suspended.

Bivalent cat-ions and the H-ion, on the other hand, at first produce a slight fall in conductivity or an increase in resistance, followed, secondly, by an irreversible increase in conductivity which is slow and gradual resulting finally in death after 48 or more hours. This is shown by the cat-ions of Ca, Sr, Ba, and Cd.

In a balanced solution such as sea-water, blood plasma, Ringer's solution, van't Hoff's solution, where a certain quantity of $CaCl_2$ antagonises a larger amount of NaCl and KCl, the conductivity of bacterial emulsions undergoes no change, but remains constant.

Dead in distinction to living bacteria offer little resistance to the passage of ions of a solution. It would seem the relatively high resistance of the bacterial cell is due to some condition present in the living and absent in the dead state.

It has been shown by experiments on animals that the condition of increased conductivity of the cell is coupled with a loss of virulence in the case of some pathogenic bacteria. It is not clear from these experiments if this is due to the actual death of the germs in a monovalent salt solution, or to a detoxicating action of these solutions on the germs. It would seem there is some evidence for believing that the latter takes place, as the lost lethal power returns in the presence of a small trace of Ca. In bivalent salt solutions no loss of toxicity takes place. It has been pointed out that the action of monovalent and bivalent salts on bacteria offers a new explanation of certain experiments with gas gangrene organisms, where it is at present considered that the action of the salts are on the tissues of the animal and not on the bacteria.

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