STUDIES ON THE ACTION OF ELECTROLYTES ON BACTERIA.

PART II.

THE INFLUENCE OF THE TRIVALENT POSITIVE SALTS ON THE RATE OF MIGRATION OF BACTERIA IN AN ELECTRIC FIELD, AND THEIR EFFECT ON GROWTH AND VIRULENCE OF PATHOGENIC ORGANISMS.

By C. SHEARER, F.R.S.

(*From the Pathological Laboratory, University of Cambridge.*)

(With Plate I.)

That practically all bacteria in an aqueous solution carry a negative charge and move to the anode in an electric field, has been repeatedly confirmed by different investigators, since the original observations of Nisser and Friedmann (1904), and Teague and Buxton (1906), and Cernovodeanu (1906). In the following paper it will be shown that this charge can be materially altered by various trivalent salts, especially lanthanum and cerium, when these salts are employed in unusually low concentrations.

When the ultramicroscopic method is used, the most satisfactory results are obtained when the type of electrode cell devised by Hardy (1912) is employed. To confine and localise heating effects ample space is left at each end of the field. The motion of the particles is observed in the narrow channel connecting the two chambers. The cell used held about 0·5 c.c. of fluid with the cover-glass in place. The usual procedure was to fill the space at either end with a little Ringer's solution, and add a drop of a thin emulsion of bacteria to the connecting canal between the chambers, and to place the cover-glass in position. The direction and rate of movement of the bacteria was then readily followed under the microscope with dark ground illumination. The distance between the electrodes was 5 cm., a continuous current of 110 volts being used, its direction being reversed every 15 or 20 seconds by means of a key. Below are given the data of a set of observations of this kind.

Table I.

Movement to anode +	Movement to cathode −
B. coli in	
(1) Aq. dest. +.	(7) Ringer plus 3 drops 20 per cent. sod. cit. −.
(2) NaCl +.	(8) Same transferred to fresh Ringer −.
(3) Ringer's sol. +.	(9) Same washed 3 times in fresh Ringer +.
(4) Ringer plus one drop 0·0005 M. La $(No_3)_3$ + very feeble.	(10) Ringer plus 4 drops 0·0005 M. $CeCl_3$ −.
(5) 0·01 HCl +.	
(6) 0·1 M. HCl −.	

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It will be seen from this table that *B. coli*, in distilled water, NaCl, Ringer's solution, carry a negative charge and move to the anode. The addition of a slight trace of La or Ce to any of these solutions immediately slows up this rate of migration. These salts when added in stronger solutions reverse the direction of movement, the bacteria now passing to the cathode. That is, the sign of their electric charge has been changed. In the vicinity of the isoelectric point flocculation takes place and the bacilli show no further motion. In the above experiment it will be seen that the addition of the sodium citrate changes the direction of movement. This result recalls that recorded by Gengou (1909) on the reversal of the direction of movement of $BaSO_4$ particles under the influence of sodium citrate, after the addition of this salt the $BaSO_4$ particles migrate to the cathode. This movement was rendered much more rapid by the addition of some colloid, as gum tragacanth.

Subsequent repetition of this experiment however, with Na citrate, gave contradictory results, sometimes the movement being to the cathode and in others to the anode. Later experiments with the U-tube will show that little if any movement to the cathode takes place when this salt is employed.

In the case of HCl it was possible to demonstrate this change of direction of migration also with the U-tube. In a 0·01 M. HCl solution *B. coli* moved to the anode, while in a 0·2 M. HCl solution it moved to the cathode. This result agrees very well with that obtained by Teague and Buxton (1906), working with sensitised bacteria, the responsiveness of which to the action of electrolytes is about double that of the untreated germs. They observed a change of direction of migration with sensitised *B. pyocyaneus*, using a 0·01 M. HCl solution.

The ultramicroscopic method has several serious drawbacks when applied to the study of the rate of migration of living bacteria in an electric field. In the first place the gas products formed at the poles rapidly interfere with the experiment, and secondly heating effects quickly render the movements of the bacteria very irregular, sometimes entirely reversing the movement.

These difficulties are largely avoided when the U-tube is used. The particular type of this instrument employed in the following experiments was that devised by Hardy (1905). It is provided with a funnel permanently attached, and both limbs of the tube are graduated in millimetres. It was found that under a given potential gradient, the movements of bacteria in it could be more accurately observed than by the former method.

If 10 c.c. of a thick growth of *B. coli* in spleen broth be run into such a U-tube under neutral Ringer's solution of the same conductivity as the broth, then on passing an electric current through the tube, migration takes place towards the anode, the observed velocity being about $4{\cdot}17\times10^{-4}$ cm. per second.

If now to this 10 c.c. broth culture, 1 c.c. of a 0·0005 M. lanthanum nitrate solution is added, it will be seen from Table II that the rate of migration is now $2{\cdot}92\times10^{-4}$ cm. per second, that is the La has slowed down

the rate of movement by almost half. It was found that the addition of the La to the broth did not appreciably alter its viscosity. This was $11{\cdot}69 \times 10^{-3}$ without and with the La.

Table II. *Emulsion of* B. coli *in spleen broth. Distance between electrodes* = 32·5 *cm.*

Time	Temp. ° C.	Voltage sign right electrode	Height bacterial emulsion: Left limb tube cm.	Height bacterial emulsion: Right limb tube cm.	Observed velocity cm./sec.	Absolute velocity in cm./sec./volt per cm.
			Control.			
5 mins. int.	25	×110	*2·5	2·5		
	,,	110	2·4	2·7		
	,,	110	2·4	3·0		
	,,	110	2·3	3·1		
	,,	110	2·1	3·1	$4{\cdot}17 \times 10^{-4}$	$1{\cdot}23 \times 10^{-4}$
		1 c.c. of 0·0005 M. La $(No_3)_3$ added to 10 c.c. *B. coli* in broth.				
5 mins. int.	25	+110	1·6	1·8		
	,,	110	1·5	2·0		
	,,	110	1·5	2·2		
	,,	110	1·5	2·3		
	,,	110	1·5	2·4	$2{\cdot}92 \times 10^{-4}$	$8{\cdot}6 \times 10^{-5}$
			Current reversed.			
5 mins. int.	25	−110	1·5	2·3		
	,,	110	1·6	2·3		
	,,	110	1·9	2·1		
	,,	110	1·9	2·1		
	,,	110	2·0	2·1	$2{\cdot}92 \times 10^{-4}$	$8{\cdot}6 \times 10^{-5}$

* Limbs of tube graduated from the bottom.

If 2 c.c. of the same La solution was added to the same quantity of broth culture, no movement could be observed. The isoelectric point had been reached and flocculation took place.

If in the terms of the Helmholtz-Lamb theory of the double electric layer, we regard the surface tension of the bacterial cell in a solution, as the balance between the cohesive force of the cytoplasm and the disruptive force due to a layer of negatively charged anions attached to the cell surface, the corresponding positive ions being held in the surrounding solution, then the determination of the electric moment due to this charged state can be simply estimated by measuring the velocity with which the bacteria wander towards the anode in an electric field.

If we make use of Perrin's (1914) formula, $\sigma H = n \frac{v}{d}$ where

σ = density of the electric layer on the bacteria,
H = strength of the electric field,
v = the mobility of the bacteria,
n = the coefficient of viscosity,
d = the distance between the double electric layer,

we have $\sigma d = n \frac{v}{H}$, which gives the value of the electric moment of the bacteria.

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The above table (p. 79) furnishes the data from which the value of σd can be calculated. It shows under the heading of observed or absolute velocity, how readily the mobility of bacteria is altered by the addition of an almost infinitesimal quantity of one of the positive trivalent salts.

If we substitute these values in the above formula of Perrin's, we get the following evaluation of $\sigma d = 1{\cdot}45 \times 10^{-6}$, in the case of the broth without La, while in the same broth[1] plus the La, $\sigma d = 1{\cdot}02 \times 10^{-6}$ per cm. per sec. per volt per cm.

Somewhat similar figures were obtained working with $CeCl_3$, using the same concentration as that used with La.

If we turn now to the action of a negative trivalent salt, such as Na citrate, we find that this has little or no influence in altering the rate of

Table III. *Table showing the effect of sodium citrate on the rate of migration of* B. coli *in an electric field. Emulsion of* B. coli *in spleen broth. Distance between electrodes* 32·5 *cm.*

Time	Temp. ° C.	Voltage sign of right electrode	Height of bacterial emulsion: Left limb of tube	Height of bacterial emulsion: Right limb of tube	Observed velocity in cm./sec.	Absolute velocity in cm./sec. per volt. per cm.
		Control, 10 c.c. *B. coli* emulsion in spleen broth.				
5 mins. int.	25	+112	2·0	2·1		
	,,	112	1·9	2·3		
	,,	114	1·9	2·6		
	,,	118	1·8	2·9	$5{\cdot}55 \times 10^{-4}$	$1{\cdot}65 \times 10^{-4}$
		10 c.c. *B. coli* emulsion × 5 drops 20 per cent. sodium citrate solution.				
5 mins. int.	25	−104	2·6	2·4		
	,,	104	2·8	2·3		
	,,	103	3·1	2·2		
	,,	110	3·4	2·1	$6{\cdot}12 \times 10^{-4}$	$1{\cdot}89 \times 10^{-4}$
		Current reversed.				
5 mins. int.	25	+110	3·5	2·1		
	,,	106	3·4	2·3		
	,,	106	3·3	2·4		
	,,	106	3·3	2·7	$4{\cdot}45 \times 10^{-4}$	$1{\cdot}35 \times 10^{-4}$
		10 c.c. *B. coli* emulsion + 5 c.c. per cent. sodium citrate solution.				
5 mins. int.	25	+110	2·6	2·4		
	,,	111	2·6	2·4		
	,,	110	2·6	2·4		
	,,	110	2·5	2·4	$5{\cdot}56 \times 10^{-5}$	$1{\cdot}64 \times 10^{-5}$
		Current reversed.				
5 mins. int.	25	−112	2·4	2·5		
	,,	110	2·5	2·4		
	,,	110	2·5	2·5		
	,,	110	2·5	2·5	$5{\cdot}56 \times 10^{-5}$	$1{\cdot}64 \times 10^{-5}$

[1] The broth used throughout in all these experiments was Cole's spleen broth prepared according to the directions given by him in his paper (*Journ. Pathol. and Bact.* XXI, p 267, 1917). The reaction of the broth was in most instances adjusted to a Ph = 7·5. The Ringer's solution used in the U-tube in connection with this broth was adjusted to have the same conductivity as that of the broth. The growth of bacteria in this broth was in most instances so thick that a definite line could be obtained in the U-tube when the broth was run in under some of the same broth without bacteria.

migration of living bacteria in an electric field. Migration is still in the direction of the anode, even when the concentration of the Na citrate is considerably increased.

In Table III the data of a set of experiments with sodium citrate are given. It would seem that little or no adsorption of the citrate takes place, and this would be in keeping with the fact that an electro-negative surface repels the electro-negative citrate ions. This also agrees with Perrin's observation that in electro-endosmosis, negative ions have no effect in altering a negatively charged wall.

In a recent paper Girard and Audubert (1918), making use of Perrin's formula, give the results of some remarkable experiments with bacteria, in which lanthanum has been employed in very dilute solutions to reduce the normal negative charge on the cell wall. The reduction of this charge they claim is accompanied by a profound alteration in biological properties of the bacteria. It is unfortunate that these authors give hardly any description of their methods and only in one instance do they mention the strength of the lanthanum employed. They found that the addition of small quantities of lanthanum nitrate to various bacterial cultures profoundly influenced their growth and virulence. Their experiments were carried out on the pneumococcus, shiga bacillus, typhoid and paratyphoid A and B, vibrion septique, *B. anthracis*, and the Preiz-Nocard germ or the agent of ulcerative lymphangitis of the horse.

If the value of σd is lowered slightly below the normal, they state that the amount of growth of these bacterial cultures is increased six to sevenfold. In the case of *B. anthracis*, the lowering of this value from $3{\cdot}68 - 2{\cdot}47 \times 10^{-6}$ C.G.S., is equivalent to an increase of five times the usual amount of growth, while the change of this value in the Preiz-Nocard germ from $3{\cdot}60 - 2{\cdot}38 \times 10^{-6}$ sextuples growth. A further lowering of this value in these germs corresponds to their total agglutination. A still further increase in the concentration of the La··· ions in the culture media induces a spore-like condition of the bacteria, and their length of life is greatly prolonged in the culture fluid. If the concentration of La is adjusted so that the value of σd becomes zero, then the surface tension of the bacteria reaches a maximum, and the cells are fixed and killed in a peculiar condition in which they retain their full toxicity. This is well demonstrated in their experiment with the Preiz-Nocard germ. The subcutaneous inoculation of living cultures of this organism in the horse produces local ulceration at the seat of inoculation followed by induration and slow resolution. If killed by being placed for one hour in ether and alcohol, 10 cg. of this germ produce little induration when inoculated under the skin, being completely resolved within six days. The same dose of the germ, however, killed by being placed for 48 hours in dilute lanthanum nitrate (1/5000), produced a reaction in every way similar to that of the injection of the same quantity of living culture, resolution only commencing after the twelfth day and progressing very slowly.

It seems to be clear from the work of these authors, that these various biological effects of the action of lanthanum on bacteria, in producing hypervegetation and lengthened viability, are due to the action of the ions of this salt in lessening the negative sign of the charge carried by these cells. It is only necessary to add a slight excess of trivalent negative ions to any of these cultures, that have been treated with lanthanum, to see the immediate disappearance of these effects.

In view of these results some experiments were made to test the effect of lanthanum nitrate on the growth of the meningococcus and *B. coli*.

In the following experiments the foregoing method of measuring the electric charge on the bacteria was used, and very pure lanthanum nitrate salts were used while the Ph of the culture fluids was carefully controlled by means of "buffer" solutions.

To 10 c.c. spleen broth, 1 c.c. of 0·0005 M. $La(No_3)_3$ was added, the reaction adjusted to Ph – 7·5 and sterilised in a steamer for an hour, a control tube without La was similarly prepared. To each tube 10 drops of a thin emulsion of a freshly isolated meningococcus strain was added. The tubes were incubated for 48 hours at 37° C. Fig. 1 is a photograph of the resulting growth in these tubes at the end of 48 hours.

It will be seen that in the tube that has received the La; a thick growth has taken place, which has settled down in a dense mass in the bottom of the tube (tube A). The yellow pigment of the broth has disappeared to a considerable extent. In tube B only a thin film of growth has settled down and the broth retains its normal slightly opaque condition. On shaking up these tubes and making a count of the number of bacteria in each, it was found that the proportion of cocci in A, as compared with B, was in the ratio of 8 to 1.

A similar experiment was repeated using *B. coli* instead of the meningococcus. In this instance at the end of 48 hours' incubation the cultures were shaken up and 5 c.mm. of each culture taken and diluted 18 times in fresh broth in the manner devised by Wright, the last six of these dilutions for each culture being planted out on agar plates, as shown in Fig. 2. Plate A shows the dilutions of the culture that had received the La, while Plate B is the control. The large number of colonies in Plate A compared with B clearly demonstrates the effect of the La in increasing the amount of growth.

The lowering of the value of σd in this instance was from $1{\cdot}49 \times 10^{-6}$ to $1{\cdot}03 \times 10^{-6}$ cm. per sec. per volt per cm. This result agrees very satisfactorily with the values obtained by Girard and Audubert (1918).

There is, however, a source of grave error in an experiment of this kind where bacteria are planted out on a solid medium. The addition of La, as the preceding Table II shows, renders them more susceptible to agglutination. Thus the addition of 1 c.c. of a 0·0005 M. La solution to 10 c.c. spleen broth reduces the value of σd from $1{\cdot}45 \times 10^{-6}$ to $1{\cdot}02 \times 10^{-6}$, complete agglutination taking place when this value approaches zero. The increased susceptibility to agglutination in the foregoing culture might result in a certain

amount of clumping of the germs, which in turn would result in a larger number of colonies surviving and growing. This objection does not apply with as much force to broth cultures, and a certain amount of evidence furnished by these seems to lend some support to Girard and Audubert's claims.

In some instances it would seem that the effect of the La on the bacteria was most marked during the first 24 hours' growth, and that at the end of this time the effect is not so marked. It is then necessary to add fresh La in order to get a still further increase in growth. It must be admitted that in a large number of experiments, however, I was quite unable to determine that the La added had any effect whatever on the growth of the culture. I was not successful in devising any test for settling this question.

Very similar results were obtained when Yb and Ce chloride were used in place of La. It is well known that Ce chloride has an injurious effect on many living cells and Gray (1916) has shown that it has a decidedly toxic effect on the segmenting egg-cell when used even in a strength of 0·0005 M. On bacteria I could not see that it had any injurious effect and apparently it brought about as much increase in growth as the less injurious La nitrate solution.

In carrying out the experiments with broth cultures it was usual to use six tubes for the controls, and six tubes for each salt tested. There was always considerable variation in the amount of growth in individual tubes so an average could be more easily estimated in this way; the average of the six control tubes being compared with the average of the six experimental tubes.

In Fig. 3 is shown a photograph of the resulting growth in a series of tubes carried out in this manner. The salts tested were $La(No_3)_3$, $YbCl_3$ and $CeCl_3$ and $Na_3(C_6H_5O_7)$ plus $La(No_3)_3$. All the tubes received 10 c.c. sterile spleen broth whose reaction had been adjusted to Ph – 7·5. Six tubes received 0·0005 M. $La(No_3)_3$, and a similar set of six tubes received a like quantity of $YtCl_3$ and $CeCl_3$ respectively. A set of tubes also received the same quantity of $La(No_3)_3$ and 1 c.c. of a 20 per cent. sodium citrate solution. The tubes were all re-sterilised in the steamer for an hour, cooled to 37° C. and 1 c.c. of a thin emulsion of *B. coli* added to each. The photograph shows the resulting growth as a deposit in the bottom of these tubes after 48 hours' incubation at 37° C. Two tubes from each set are shown, these being the ones that approached nearest to the average amount of growth shown by the six. It will be seen that tubes 3 and 4 which received sodium citrate in addition to the La, give about the same amount of growth as the control tubes 5 and 6. Tubes 1 and 2 which had the La, and tubes 7 and 8 the Ce, and 9, 10, 11 the Yb, all show a much heavier and thicker deposit of bacteria than either the controls or the La plus citrate. There seems no doubt that the action of the La in tubes 3 and 4 is counteracted by the citrate.

It was found necessary to employ very much stronger concentrations of citrate to counteract the action of the La, than either the La or the other

simple trivalent salts, the complex citrate ion being far weaker in its neutralising effect. Gray (1916) in working with this salt on the egg-cell found its action almost 20,000 times less active than the La··· or Ce···. To produce any effect it was found necessary to employ at least a 20 per cent. solution of the salt. Morever it was found that 0·5 c.c. of this solution had little effect when added to 10 c.c. of broth to which 0·5 c.c. La had been added.

In Fig. 5 are shown four tubes each of which had received this amount of La; to the first 10 drops of a 20 per cent. sodium citrate solution had been added, to the second 20 drops, and to third and fourth, 1 c.c. and 2 c.c. respectively. It will be seen that in 1 and 2 growth is larger than 3 and 4, being almost the same as the La tubes without citrate. Thus it was found necessary to add at least 1 c.c. or 2 c.c. of a 20 per cent. citrate solution to counteract the action of 0·5 c.c. of a 0·0005 M. La solution.

In another set of experiments the effect of La on the growth of *B. coli* was determined when the bacteria were grown in Erlenmeyer flasks. To 400 c.c. spleen broth sufficient La nitrate was added so that the drift of the bacteria in the broth when placed in an electric field was slightly less than their rate of drift in the same broth in the absence of the La. The value of σd was approximately reduced by one-fourth of its normal value; according to Girard and Audubert a greatly increased amount of growth ought to take place under such a condition.

The Ph of the solution was adjusted to 7·4. The broth was thoroughly sterilised and 25 c.c. of fresh 24 hour culture of *B. coli* added. The flask was then thoroughly shaken and incubated at 37° C. To act as a control a similar quantity of the same broth without La, whose Ph had adjusted to 7·4 had a similar quantity of the same culture of *B. coli* added to it, both flasks were then incubated under similar conditions for the same length of time. The contents of each flask was then centrifuged and the bacterial deposit dried to constant weight in vacuum. The following table gives the results of a number of experiments of this kind in which the dried weight of the bacteria grown in the presence of La is compared with that in a corresponding control culture.

		Weight of dried deposit in gms.	
Exp. I.	Control flask	0·0910	3 days' incubation
	La flask	0·1888	La culture 2·13 times *greater* growth than control
Exp. II.	Control flask	0·0618	4 days' incubation
	La flask	0·1326	La culture 2·14 times *greater* growth than control
Exp. III.	Control flask	0·1358	4 days' incubation
	La flask	0·1192	La culture 1·48 times *less* growth than control
Exp. IV.	Control flask	0·2060	3 days' incubation
	La flask	0·1828	La culture 1·03 times *less* growth than control
Exp. V.	Control flask	0·2697	14 days' incubation
	La flask	0·2181	La culture 1·24 times *less* growth than control

A number of similar experiments were tried in which a range of different concentrations of La ions were used, and the broth cultures incubated for shorter and longer periods of time. On the whole no action could be distin-

guished of any effect through the La increasing the growth in those cultures to which it had been added, as a rule the growth of the control cultures was just as great or in many instances greater in weight than that of the La cultures.

To test the action of La in altering the virulence of bacteria the *Pneumococcus* and *B. anthracis* were used. Care was taken to employ spore-free strains of anthrax, nevertheless it is doubtful if spores were completely excluded. The *Pneumococcus* had been passed through five mice previous to its use for this experiment. In each instance a fresh 24 hours' culture was employed. A very thin emulsion of the germ was prepared in neutral Ringer's solution and this was divided into two equal portions. To one portion a little La nitrate was added—sufficient to make the concentration of the La in the solution equal to 0·0005 M. One mouse received 0·5 c.c. of this emulsion under the skin of the back, while the control mouse received a similar dose of the same emulsion in Ringer's solution to which no La had been added.

A certain amount of inconclusive evidence was obtained as the result of these inoculation experiments which seemed to show that La had some effect on the virulence of these germs. In view of the relatively small number of experiments performed, the small size of the animals (mice) used, and the individual susceptibility of different animals, it would be premature to attach any definite significance to these results. They were as follows:

No. mouse	Weight mouse in gms.	Dose and character of sol. injected	Result	Remarks
1. Control	25	0·5 c.c. *pneumococcus* in Ringer's sol.	+24 hrs.	—
2.	30	,, ,, 0·0005 M. La	○ ,,	+4th day
3. Control	31	,, ,, Ringer's sol.	+24 hrs.	—
4.	29	,, ,, 0·0005 M. La	○ ,,	—
5. Control	32	,, ,, Ringer's sol.	+24 hrs.	—
6.	28	,, ,, 0·0005 M. La	○ ,,	+48 hrs.
		B. anthracis experiments, a much thinner emulsion of bacteria being used		
1. Control	30	0·5 c.c. *B. anthracis* in Ringer's sol.	+24 hrs.	+ at 18 hrs.
2.	30·5	,, ,, 0·0005 M. La	○ ,,	Well at 72 hrs, +6th day
3. Control	32	,, ,, Ringer's sol.	+24 hrs.	—
4.	29	,, ,, 0·0005 M. La	○ ,,	Escaped from cage at 48 hrs.
5. Control	30	,, ,, Ringer's sol.	+24 hrs.	—
6.	31	,, ,, 0·000 M. La	○ ,,	Escaped from cage at 48 hrs.
7. Control	24	,, ,, Ringer's sol.	+24 hrs.	—
8.	26	,, ,, 0·000 M. La	○ ,,	+48 hrs.
9. Control	32	,, ,, Ringer's sol.	+24 hrs.	—
10.	31	,, ,, 0·000 M. La	○ ,,	+at 72 hrs.
11. Control	29	,, ,, Ringer's sol.	+24 hrs.	—
12.	30	,, ,, 0·0005 M. La	+ ,,	—
13. Control	32	,, ,, Ringer's sol.	○24 hrs.	+at 48 hrs.
14.	31	,, ,, 0·0005 M. La	○ ,,	+at 48 hrs.

○ =animal alive and well, + =animal dead

Evans (1913) has drawn attention to the influence of the carbonates of some of the rare earths (Ce, La, Yt) on the growth and rate of division of plant cells. He found that marked effects were produced on the dividing cells of hyacinth rootlets by the addition of small traces of Ce, La or Yt to the

culture medium. The carbonates of La and Ce increased cell growth and division very considerably, while that of Yt seemed to have an unfavourable action. La had a special effect on the flower stalk, causing an increase in length.

SUMMARY.

1. In the foregoing experiments evidence is adduced to show that bacteria in spleen broth or neutral Ringer's solution carry a negative charge and move to the anode in an electric field. The addition of a slight trace of a positive trivalent ion such as La or Ce or Yb to the solutions containing the bacteria immediately lessens or neutralises this negative charge, and if the concentration of these ions is further increased completely abolishes it, and the bacteria undergo flocculation or agglutination. Living bacteria seem much more sensitive to the action of these ions than non-living colloidal particles. The addition of negative trivalent ions to bacterial cultures is without any effect on their movement in an electric field; the negative charge on the bacteria is not increased.

2. Some inconclusive evidence has been brought forward in support of Girard and Audubert's work regarding the influence of the double electric layer of the bacterial cell wall in controlling their biological properties.

REFERENCES.

CERNOVODEANU and VICTOR HENRI (1906). *Compt. rend. Soc. de Biol.* p. 200.
EVANS, W. H. (1913). *Biochem. Journ.* VII. 349.
GENGOU, O. (1909). *Arch. Int. d. Physiol.* VII. 1.
GIRARD, P. and AUDUBERT (1918). *Compt. rend.* CLXVII. 351.
GRAY, J (1916) *Phil. Trans. Roy. Soc. London*, Ser. B, CCVII. 481.
HARDY, W. B. (1905). *Journ. Physiol.* XXXIII. 289.
—— and HARVEY, H. W. (1912). *Proc. Roy. Soc.* Ser. B, LXXIV. 217.
NISSER and FRIEDMANN (1904). *Münchner med. Wochenschr.* LI. 465.
PERRIN, J. (1904). *Journ. d. Chem. Physiq.* II. 601.
TEAGUE, O. and BUXTON, B. H. (1906). *Zeitschr. f. physik. Chem.* LVII. 76.

EXPLANATION OF PLATE I.

Fig. 1. Refer to text, p. 82.

Fig. 2. Refer to text, p. 82.

Fig. 3. Experiment showing the action of La, Yb and Ce in producing an increase in growth of *B. coli* in spleen broth. Tubes 1 and 2 (counting from left to right) La; 2 and 4, La plus Na citrate; 5 and 6 controls plain broth; 7 and 8 Ce; 9, 10 and 11 Yb chloride tubes.

Fig. 4. Experiment similar to that of Fig. 3. Tubes 1 and 2 controls. Tubes 3 and 4 La plus citrate. 5 and 6 Yb. 7 and 8 La. 9, 10 and 11 Ce. Growth in tubes 1–4 about the same, while growth in tubes 5–11 is much greater in every case, these tubes having received 0·5 c.c. of 0·0005 M. solution of the trivalent positive salts.

Fig. 5. Experiment showing the action of various concentrations of sodium citrate in counteracting the action of La. Tube 1, 10 drops citrate. Tube 2, 20 drops citrate. Tube 3, 1 c.c. and tube 4, 2 c.c. citrate in 10 c.c. spleen broth.

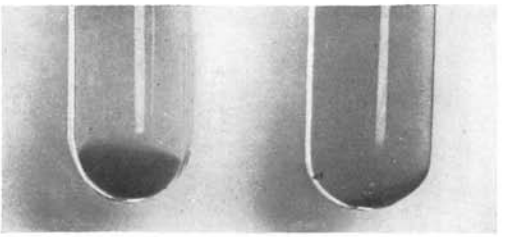

Fig. 1

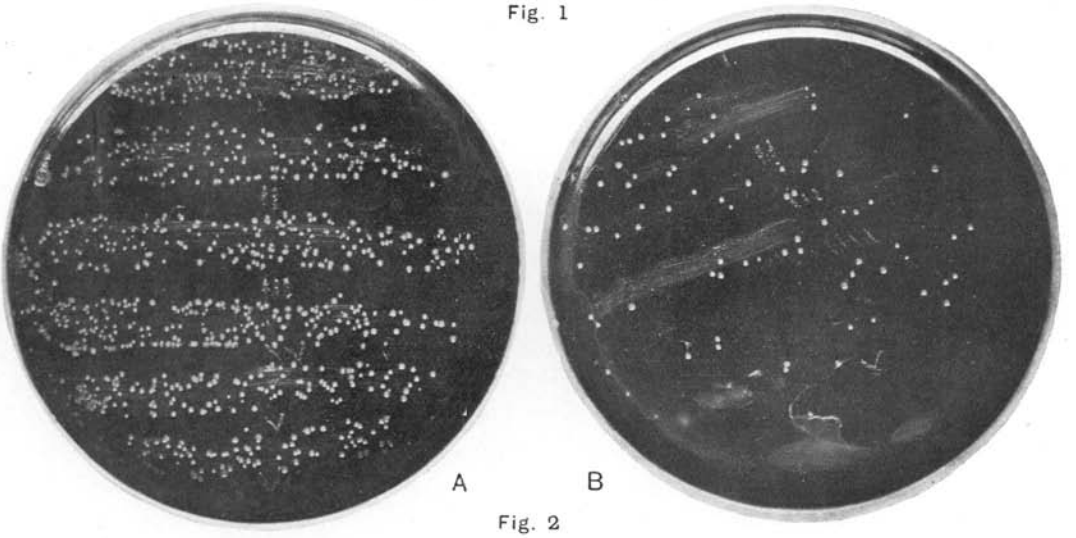

Fig. 2

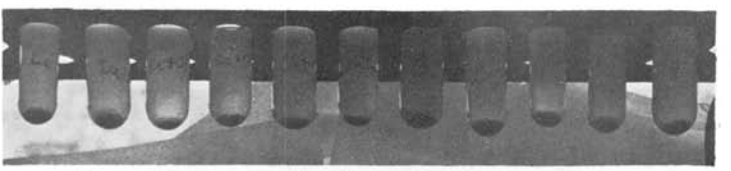

Fig. 3

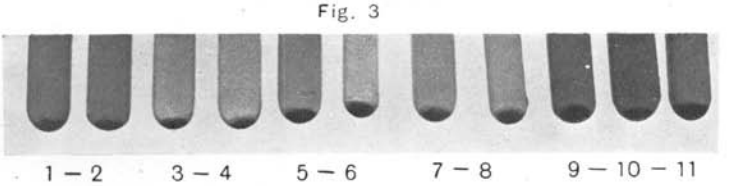

Fig. 4

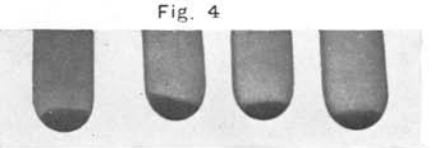

Fig. 5

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