ON THE INFLUENCE OF THE RELATIVE CONCENTRATION OF CALCIUM IONS ON THE REVERSAL OF THE POLAR EFFECTS OF THE GALVANIC CURRENT IN PARAMECIUM. BY FRANK W. BANCROFT. (Three Figures in the Text.)

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Introduction. For a number of years Loeb has been investigating the effects of electrolytes on muscle, nerve, and other tissues in order to find an explanation of the stimulating effects of a constant current; since it was evident that the current could act only through the ions by which it is conducted[1].

One of the conclusions of these investigations is stated by Loeb (1906, German edition, pp. 151—152) as follows: "Since, upon making the current, the increase of excitability takes place at the cathode; and since, upon making the current the contraction also starts from the cathode, one might think that the relative concentration of certain cations might be responsible for both processes....Now, as we have seen, the experiments showed, that when a frog's muscle is put in *m*/8 NaCl solution[2] at room temperature the twitchings begin in about an hour. If the solution used is somewhat more concentrated, viz. *m*/6 to *m*/4 then the twitchings begin at once. The results do not depend on the Anion, as $NaNO_3$, or the sodium salts of the fatty acids act similarly or even stronger. This simple experiment shows that a sudden increase in the relative concentration of the Na-ions in the muscle produces contractions. If a sufficient quantity of a Ca- or Mg-salt is added to the NaCl solution then these twitchings do not take place. The experiments, then, lead to the possibility that the make con-

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[1] These results are summarized in *The Dynamics of Living Matter*, chap. v. pp. 71—105.

[2] Concentrations of solutions are stated in fractions of the gram-molecular solution. This is obtained by dissolving the molecular weight plus the water of crystallization in grams in a litre of water.

traction produced by the galvanic stimulation of the muscle or nerve depends upon the fact that the ratio $\frac{C_{\mathrm{Na,K}}}{C_{\mathrm{Ca,Mg}}}$ is increased." Loeb (1901) also discovered in this series of experiments that when the nerve of a frog's muscle nerve preparation is immersed in solutions of Na salts whose anions tend to diminish the ionized calcium (oxalate, citrate, fluoride, etc.) that a very marked increase of excitability takes place, similar to the increased excitability (katelectrotonus) produced at the cathode during the flow of the galvanic current. Conversely, when the nerve is immersed in a solution of a calcium or magnesium salt a condition of diminished excitability results, similar to the decreased excitability (anelectrotonus) produced at the anode during the flow of the galvanic current. In a later paper (1905) he proposes a theory of the electrotonic effects of the galvanic current based upon these facts; supposing that the galvanic current also acts by decreasing the free Ca and Mg ions at the cathode, and increasing them at the anode. He says: "The changes in the concentration of the various ions which will occur in a nerve at the electrode depend primarily upon the velocity of the migration of ions. This velocity varies considerably for the anions found in nerve and muscle. While the Cl ion has a very high velocity (65·4) the higher fatty acid anions, such as oleate, have a very small velocity, probably below 30. The same must be true for palmitate and stearate [and many other organic anions]. These anions of the higher fatty acids must be found in nerve and muscle. It is known that these anions precipitate Ca and it has been shown directly by Friedenthal that sodium oleate acts like sodium oxalate and sodium fluoride in the production of muscular twitchings and otherwise, when injected into an animal. On account of this enormous difference in the velocity of the Cl and oleate ions, the galvanic current must cause more Cl ions to leave the cathode in the unit of time than oleate, palmitate, etc., ions and consequently, in the region of the cathode, the current must bring about a relative increase in the concentration of the Ca precipitants such as oleates, etc., and a relative decrease in the concentration of $CaCl_2$, which is not only very soluble, but also highly dissociated. It is therefore obvious that the effect of the galvanic current at the cathode region must be the same as if we put the nerve into a solution of sodium oxalate, fluoride, citrate, etc. At the anode just the reverse must occur. In the unit of time comparatively more Cl than oleate ions must migrate and collect there and the result will be an increase in the concentration of free Ca and Mg ions."

During an examination of the effect of a number of chemical substances on the galvanotropism of paramecium in order to test Coehn and Barratt's (1905) theory of galvanotropism I noticed that in $\frac{m}{10}$ and $\frac{m}{20}$ sodium citrate the infusoria were entirely unaffected by the current. This showed that it was possible to influence galvanotropism in an entirely unsuspected way, and led to a systematic examination of a considerable number of chemical substances. The investigation led to the conclusion that it was possible by means of suitable concentrations of many salts to produce at will migration forwards towards either the cathode or the anode, migration backwards towards the anode, or the total absence of galvanotropism. A short account of some of the results and of other experiments proving the untenable nature of Coehn and Barratt's theory of galvanotropism was published last spring (1906). Further experiments showed that the most potent influence in producing all the variations of galvanotropism mentioned above as well as some of their modifications was the relative amount of calcium present. Finally, in attempting to explain these phenomena it was seen that all of these apparently contradictory modes of behaviour could be easily explained on the basis of Loeb's theory if it is assumed that galvanic stimulation depends upon the presence of a certain definite relative proportion of calcium ions and does not occur if the relative concentration of the calcium ions is either greater or less.

Methods. The paramecia that served for most of the experiments were a hay infusion culture about 2½ years old that has been kept up by adding pieces of bread from time to time. Professor Geo. E. Colby, to whom I wish to express my best thanks, very kindly analysed the inorganic constituents of the culture fluid with the following results:

Analysis of Culture Fluid.

	Parts per million
K_2SO_4	128·8
Na_2SO_4	188·9
NaCl	417·8
Na_2CO_3	114·5
$MgCO_3$	168·0
$CaCO_3$	40·3
$CaSO_4$	28·0
$Ca_3P_2O_8$	13·7
Iron salts	trace
Silica	90·0
Much organic matter and chemically combined water ...	520·0
Total	1710·0

As the analysis shows, there are considerable quantities of salts present in the fluid, and hence an indispensable precaution in determining the effect of most salts is to carefully wash the paramecia in distilled water. If this is not done the salts contained in a small drop of the culture fluid containing the paramecia will obscure or prevent the characteristic action of the substance to be tested. Some of the more vigorously acting salts like sodium oxalate, Na_2CO_3, and $BaCl_2$ will produce their effect in the presence of small quantities of other salts. Other salts, however, such as NaCl and KCl, may easily have their action prevented by the salts in the culture fluid. Accordingly, unless the opposite is stated, all statements here will refer to paramecia that have been washed once or twice in water distilled from glass; that have been concentrated by centrifuging and have been kept in the distilled water. No difference could be detected in the behaviour of paramecia that had just been washed and those that had been kept in the distilled water for 24 hours or more.

The current used was the 110 volt power circuit of the university. It was led through a water rheostat, milliammeter, pole changer, and Porter's non-polarizable boot-electrodes. The electrodes were filled in many cases with the substances that were being tested instead of with the $ZnSO_4$ to prevent any contamination by Zn and SO_4 ions. The troughs in which the paramecia were tested were made of paraffine and glass, and were usually 60 × 13 × 2 mm. In a short trough the characteristic effects of some of the chemicals cannot be so easily demonstrated. Usually the strength of the current was not found to make much difference. In several cases, however, the nature of the reaction changed entirely with a change in the intensity of the current. Thus on one occasion in $\frac{m}{10}$ NaCl anodal galvanotropism was obtained with a current of 2 milliamperes while with one of 6 and 7 milliamperes an absence of galvanotropism was obtained. On another occasion currents of 9 to 12 milliamperes sometimes gave a cathodal and sometimes an anodal galvanotropism while a current of 2 milliamperes always produced anodal galvanotropism. On account of these results a current of from 1—2 milliamperes was generally used. In the troughs employed these currents had a density of from about 38 to 77 δ.

ANODAL GALVANOTROPISM.

By anodal galvanotropism is meant an orientation of the paramecia with their anterior ends towards the anode. By cathodal galvanotropism is meant an orientation with the anterior end towards the cathode, no matter whether the infusoria are moving forwards to the cathode or backwards to the anode.

Of the substances tested acids and alkalis failed to produce any decided change in the nature of the galvanotropism. The following salts, however, at the concentrations indicated in the table, all produced anodal galvanotropism:

$BaCl_2$
KCl
K_2 Oxalate
LiCl
Na Acetate
$NaHCO_3$
Na_2CO_3
Na_3 Citrate
NaCl
NaF
Na_2 Oxalate
Na_2HPO_4
Na_2SO_4
NH_4NO_3

Though all of these salts were effective in bringing about anodal galvanotropism the character of the galvanotropism differed greatly with different salts, and with different concentrations of the same salt. There was an optimal concentration for each salt, at which the anodal galvanotropism was obtained most distinctly; and this concentration varied even with the same culture at different times so that it was always necessary to experiment with a series of concentrations. The higher concentrations either killed too rapidly or caused a total absence of galvanotropism. In the weaker concentrations there was cathodal galvanotropism. Between these two extremes was a series of concentrations that caused anodal galvanotropism (see table).

Even in these solutions the anodal galvanotropism was not a permanent phenomenon, but developed, gradually reached a maximum and then disappeared, being followed by cathodal galvanotropism or absence of galvanotropism. In the more concentrated solutions the anodal galvanotropism appeared very rapidly and disappeared rapidly, sometimes in 30 seconds. In the weaker solutions it sometimes took 5 minutes for the anodal galvanotropism to appear, and it lasted about 10 minutes longer. In the experiments of Loeb on muscular twitchings (1899, *General Studies*, p. 552) the same time relations were observed for solutions of various concentrations. Loeb concluded that the rapid cessation of the muscular twitchings in the more concentrated solutions was due to an injury of the muscle. But in the case of paramecium it did not appear that the cessation of the anodal galvanotropism was due to an injury of the animal. Two instances in point may be given. In *m*/20 NaCl the anodal galvanotropism did not last more than about 15 minutes. After that the normal cathodal galvanotropism supervened and the paramecia swam normally and appeared to be in good condition for an hour or more longer. In sodium citrate the paramecia

TABLE I. *Effect of chemicals on galvanotropism.*

A = Anodal galvanotropism.
C = Cathodal galvanotropism.
K = Kills before observation can be made.
0 = Total absence of galvanotropism.

Concentrations in fractions of gram molecular weights...	*m*/10	*m*/20	*m*/40	*m*/80	*m*/160	*m*/320	*m*/640	
$BaCl_2$	K	K	K	K	A	A	A	A in *m*/1280.
$CaCl_2$	C	C	C	C				C not normal, orientation poor, reversal slow.
$CaH_4(PO_4)_2$	K	K	K	K	K	K	K	K in *m*/1000, *m*/2000, C in *m*/8000, *m*/32000.
$FeCl_3$	K	K	K	K	K	K	K	K in *m*/1000—*m*/16000, C in *m*/64000.
KCl	A	A	A	C	C			
K_2 Oxalate	0	0	0	A	A	C	C	
KH_2PO_4	0	0	0	0	C			A in *m*/100.
LiCl	A	0	C					At first motionless, later they swim.
$MgCl_2$	K	C	C	C				C like that in $CaCl_2$.
Na Acetate	C	C						A in *m*/5.
$NaHCO_3$	A	A	C					
Na_2CO_3	K	K	A	A	A	C		
Na_3 Citrate	0	A	A	C				
NaCl	A	A	C					
NaF	0	A	C					
Na_2 Oxalate	0	A	C					
Na_2HPO_4	0	A	A	A	C	C		
Na_2SO_4	0	A	A	C				
NH_4NO_3	A	A	A	C				
Boracic Acid	C	C						
CO_2								C in 20 %—100 % Shasta water.
Citric Acid	K	K	K	K	K	K	K	C in *m*/20000, *m*/50000.
Oxalic Acid	K	K	K	K	K	K	K	K in *m*/1000, *m*/4000, C in *m*/8000 to *m*/80000.
$Ca(OH)_2$				K	K	K	K	C in *m*/1000, *m*/2000.
KOH	K	K	K	K	K	C	C	
NaOH	K	K	K	K	K	C	C	

also swam about normally long after the anodal galvanotropism had disappeared, but in this case they exhibited no galvanotropism, being entirely unaffected by the current. Some rough experiments on the effect of temperature were made which showed that with a rise of about 10 degrees the time required for the appearance of the anodal galvanotropism was increased two or three times. All these facts point strongly to the conclusion that the anodal galvanotropism occurs during the course of some chemical reaction, and not after chemical equilibrium has been established.

In addition to the effects of different concentrations, the various salts differ much as regards their effectiveness in producing anodal galvanotropism, and in the character of the galvanotropism produced. $BaCl_2$ was by far the most effective salt. It caused anodal galvanotropism even at the dilution $m/1280$. Moreover the character of the galvanotropism produced by the optimal concentration of $BaCl_2$ (usually $m/320$) was quite different from that seen with any other salt. When first put in the solution the infusoria became motionless and all appeared dead, but as soon as the current was made they all swam towards the anode with perfect orientation. When the current was reversed the paramecia all stopped short and remained whirling about their long axes for a considerable time, perhaps moving very slowly forwards or backwards. Then a few slowly got turned around and started off to the anode again. Na_2HPO_4, Na_2CO_3, and $NaHCO_3$ seemed to belong to the next group so far as effectiveness and the character of the reaction was concerned. In their optimal concentrations these salts produced practically universal anodal galvanotropism, but the orientation was not so exact as in $BaCl_2$, and with a reversal of the current there was a comparatively rapid turning of the animals. Na_2HPO_4 caused an especially rapid turning so that the anodal galvanotropism might be mistaken for the normal cathodal galvanotropism if one did not know the poles.

All the other salts producing anodal galvanotropism belong to the third group so far as the character of the reaction is concerned. Some were found to be effective at lower concentrations than others, but in all of them the orientation was rather poor, many animals were not affected by the current, or swam towards the cathode, so that it was often necessary to wait half a minute after the current was made, and to examine a considerable number of individuals to make sure of the nature of the galvanotropism. When the current was reversed some of the anodal paramecia did not turn at all, and those that did, turned so slowly that one usually had to wait for 30 seconds or more to see that they had turned.

When compared with other cases in which salts have been found to influence the activities of animal tissues it is seen that the salts which cause anodal galvanotropism correspond in general to those that produce muscular twitchings (Loeb, 1899, 1902), contact irritability in muscle, increased irritability in the motor nerve (Loeb, 1901), and increased peristalsis and secretion of the intestines (MacCallum, 1903, 1904). In all of these cases the salts tending to diminish the concentration of free calcium ions are particularly active; and, in the case of the muscular twitchings and intestine, $BaCl_2$ was also found to be very effective.

REVERSAL OF PFLÜGER'S LAW.

In a recent paper (1905) I have shown that when paramecium is subjected to the influence of the constant current the cilia of the cathodal end of the animal are in the stimulated condition while the cilia of the anodal end are in the unstimulated condition and that hence Pflüger's law holds for the galvanotropic reactions of this animal. That the anodal cilia were really in an unstimulated condition and not in a forced condition was made evident by the observation that when the paramecia swim backwards toward the anode (Loeb and Budgett 1897, p. 532) in solutions of NaCl and other substances, the anodal cilia also assume the stimulated condition ; that is, they point towards the anterior end of the animal, and strike more strongly forward.

Now, since the cilia are the locomotor organs of paramecium, the nature of the galvanic stimulation which causes the anodal galvanotropism described above could only be determined by an examination of the cilia. This was determined by examining them in the viscid fluid obtained from Irish moss (Chondrus) recommended by Statkewitsch (1904), to which some india ink had been added, as recommended by Jennings (1904). In this medium the direction of the effective stroke of the cilia could be easily detected by watching the direction of the migration of the ink particles nearest the cilia in question (Fig. 1). These experiments were rendered difficult because of the salts contained in the Irish moss, and so it was attempted to determine the action of the cilia in only three of the more powerfully acting salts. The best results were obtained with $m/30$ $BaCl_2$ and $m/20$ Na_2CO_3 in Chondrus jelly. Both of these concentrations were much higher than was necessary to produce anodal galvanotropism in the absence of the

Chondrus jelly, in fact they would have been fatal in its absence. At these concentrations a decided anodal galvanotropism was obtained, and it was seen many times that in the anodal paramecia the cilia of the anode half of the animal were propelling the neighbouring india ink particles forwards while the cilia of the cathode half were propelling the particles backwards (Figs. 1*a*, 2*a*). It was shown in the previous paragraph that the essence of Pflüger's law in paramecium consisted in the forward stroke of the cathodal cilia, which shows that they are in the stimulated condition. During the anodal galvanotropism on the other hand the anodal cilia are in the stimulated condition, and consequently Pflüger's law has been reversed.

In this medium the anodal galvanotropism did not last long. When the current was left on and the same paramecium watched continuously it was seen to swim slowly towards the anode for about three minutes and then suddenly to move backwards, all the cilia having their effective stroke forwards (Fig. 1 *a* and *b*). A few individuals turned around and swam towards the cathode with the cathodal cilia striking forwards. Most of them, however, continued swimming backwards until they died. If, however, the current was reversed, they were then headed towards the cathode and immediately showed the normal cathodal galvanotropism (Fig. 1 *c*). When the current was reversed during the migration to the anode (Fig. 2) there was not a change in the effective stroke of both anodal and cathodal

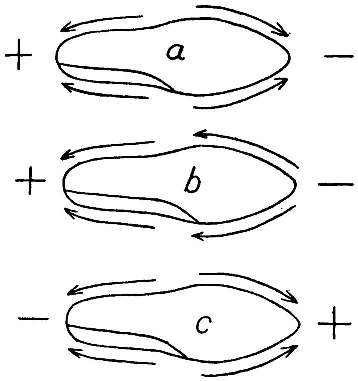

Fig. 1. Diagram showing the effect of the galvanic current in $BaCl_2$ or Na_2CO_3. The arrows indicate the direction in which the cilia move the neighbouring particles of india ink. *a*, condition during the first 3 minutes, anodal galvanotropism. *b*, backward swimming towards the cathode, after about 4 minutes. *c*, cathodal galvanotropism after the current is reversed.

cilia, as is the case in the normal cathodal galvanotropism (Fig. 2 *d, e, f*), but only the posterior cilia changed and beat forwards so that all the cilia were striking forwards and the animal was swimming backwards to the new anode (Fig. 2*b*). When the current was kept flowing in the second direction long enough the condition of cathodal galvanotropism came on and the paramecium swam forward to the cathode with the characteristic stroke of the cilia (Fig. 2*c*).

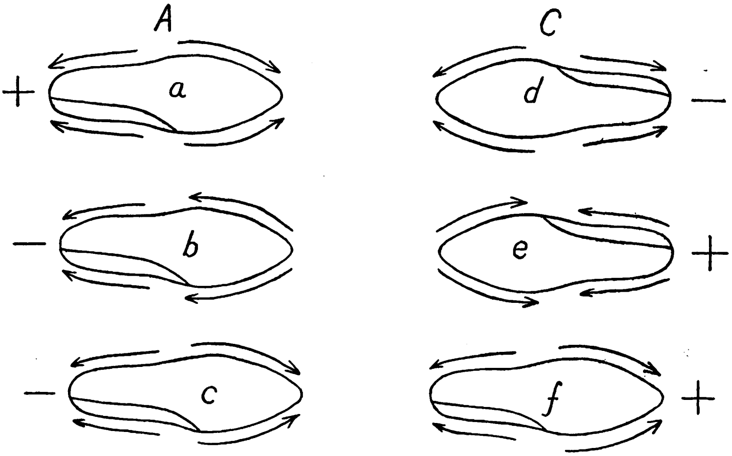

Fig. 2. Diagram showing behaviour of anodal paramecia A, and cathodal paramecia C, under the influence of the galvanic current. The arrows show the direction in which the cilia move the neighbouring india ink particles. When the current is reversed the anodally galvanotropic paramecium swims backwards for a little while, *b*; and then swims forward to the cathode with the characteristic position of the cilia. In the cathodal paramecium all the cilia change direction upon reversal of the current and then the animal turns around.

The behaviour of the cilia was also studied in $m/20$ sodium oxalate dissolved in Chondrus jelly. In this solution fair anodal galvanotropism was easily obtained, but in no case were any of the cilia seen to beat forwards while the paramecia were swimming towards the anode. When the current was reversed they usually turned slowly toward the new anode; but before this turning was accomplished it could be distinctly seen in many cases that the posterior anodal cilia were moving particles forward, and hence were in the stimulated condition (Fig. 3).

These facts show that, although during the anodal galvanotropism the behaviour of the anodal cilia is not exactly the same as the behaviour of the cathodal cilia during cathodal galvanotropism, yet in all cases where some of the cilia are in the unstimulated condition (striking backwards) and others are in the stimulated condition (striking forwards), the stimulated cilia are always at the anodal end of the animal. Thus it is evident that there is in this case a reversal of Pflüger's law, so that the stimulus which normally takes place at the cathode here occurs at the anode. In the later sections it will be shown that this remarkable reversal is connected with the diminution of the calcium in the infusorian.

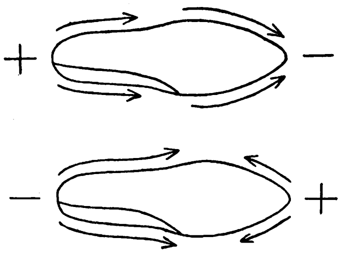

Fig. 3. Diagram showing the effect of the current on paramecia in sodium oxalate. The arrows indicate the direction in which the cilia move the neighbouring india ink particles. When the current is reversed the posterior cilia which are then anodal move the particles anteriorly.

COMPLETE ABSENCE OF GALVANOTROPISM.

It might be thought that the absence of galvanotropism is brought on because the paramecia have been so seriously injured that they cannot respond to the current. An absence of galvanotropism has been seen in several cases that was evidently due to injury. In both $m/160$ KOH and $m/160$ NaOH the animals lived for a little while, but failed to respond to the current. It was very evident, however, that they were being injured for there was no normal locomotion, but merely a series of violent motor reflexes with all sorts of variations. On the other hand, in many solutions that kill rather rapidly the paramecia exhibited an orderly galvanotropism until the very end. The salts that are credited in the table with bringing about an absence of galvanotropism do not kill rapidly, and do not cause violent motor reflexes. The paramecia swim about quietly, in a perfectly normal way, and it is only when the current is made that anything unusual is detected. Thus in the $m/10$ sodium citrate the great majority of the animals lived and behaved normally for 10 minutes, and many of them were swimming normally after 15 minutes. But from the first there was no response to the current.

An inspection of the table will show that the absence of galvanotropism was produced by neither acids nor alkalis, but only by certain salts. These salts were potassium oxalate, KH_2PO_4, sodium citrate, NaF, sodium oxalate, Na_2HPO_4, Na_2SO_4[1]; all of them except the sodium citrate being among the stronger calcium precipitants. All of the salts also produced anodal galvanotropism when their concentration was less. Moreover, in these weaker concentrations anodal galvanotropism was observed only during the first few minutes and was followed later on by an absence of galvanotropism, so that it appeared that the anodal galvanotropism was only a stage in the absence of galvanotropism. But this sequence was not invariable for in the case of some other salts the anodal galvanotropism was followed after a few minutes by cathodal galvanotropism.

ROLE OF CALCIUM.

It has already been seen that many of the salts that produce anodal galvanotropism, and the absence of galvanotropism tend to reduce the amount of ionized calcium in the infusoria, and a direct examination of the effect of calcium shows that its presence is of the greatest importance in determining the character of the galvanotropism.

In pure $CaCl_2$ of the concentration $m/10$ and weaker, the paramecia always exhibited the cathodal galvanotropism, but it was not of the normal character. In $m/10$ $CaCl_2$ they contracted slightly, swam more rapidly than usual, and at first glance did not appear to be affected by the current at all. Many individuals were seen swimming in all directions, and when the current was reversed no infusoria were seen to change the direction of their migration. If, however, the current was continued in one direction for about a minute, and was then reversed and the poles watched, it was seen that a considerable number of individuals had collected at the old cathode and were now leaving it for the new cathode; while at the opposite pole no animals had collected. With $m/20$ and $m/40$ $CaCl_2$ and with still weaker solutions the cathodal galvanotropism was found to become increasingly more pronounced, until its presence became evident at first glance. In $MgCl_2$ also, the current produced a cathodal galvanotropism similar to

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[1] These results were obtained with paramecia that had been washed once in distilled water. When they had been washed twice $m/10$ KCl and $m/10$ NaCl would frequently bring about a complete absence of galvanotropism. It is probable that with still higher concentrations all the salts producing anodal galvanotropism would also produce an absence of galvanotropism.

that described for $CaCl_2$: but the salt was much more toxic and the peculiar nature of the galvanotropism did not appear so clearly. $CaCl_2$ and $MgCl_2$, then, act entirely differently from the other salts tried, all of which except the strongly toxic ones $CaH_4(PO_4)_2$ and $FeCl_3$[1] produced either an absence of galvanotropism, or anodal galvanotropism, or both according to their concentration.

MIXTURES OF SALTS.

When the effect of the mixtures of various salts on the galvanotropism was tested the unique position of $CaCl_2$ was brought out still more strongly. The effect of the following binary combinations of salts on the galvanotropism was tested :

$BaCl_2$ and $CaCl_2$
$BaCl_2$ „ NaCl
KCl „ $CaCl_2$
KCl „ $MgCl_2$
KCl „ Na_3 citrate
KCl „ NaCl

KCl and Na_2 oxalate
K_2 oxalate „ $CaCl_2$
K_2 oxalate „ Na_2 oxalate
Na_3 citrate „ $CaCl_2$
Na_3 citrate „ NaCl
NaCl „ $CaCl_2$
NaCl „ Na_2 oxalate

I always worked with a series of concentrations, and in all the later experiments determined in advance the effect of the pure solutions on the particular sample of paramecia to be tested before trying the mixtures of salts. It was only in the case of combinations containing $CaCl_2$ that particularly instructive results were obtained. In the case of the other combinations solutions producing a similar result continued to produce it when combined, and with solutions producing different results the antagonistic effect of one solution was not found to be very much greater in one salt than the other. But with the mixtures containing $CaCl_2$ a very striking antagonism between this salt and all the others tried was observed. One example will show the nature of the experiments, and the results obtained.

Effects of $CaCl_2$ and KCl. Paramecia from culture 1, washed twice in distilled water. Current 2*ma*.

m/10 KCl. Total absence of galvanotropism.
m/20 KCl. Anodal galvanotropism, rather poor.
m/40 KCl. Good anodal galvanotropism during the first 2 minutes, later no galvanotropism.

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[1] Even these two salts showed some indications of producing an absence of galvanotropism, but I could not be sure that this absence was not due to injury.

$m/80$ KCl. Cathodal galvanotropism predominates, some anodal galvanotropism.

$m/10$ $CaCl_2$. Characteristic cathodal galvanotropism.

$50\,^0/_0$ $m/10$ $CaCl_2 + 50\,^0/_0$ $m/10$ KCl. Cathodal galvanotropism, strong and fairly normal, but they swim too fast and reverse too slowly. The longer they stay in the solution the more they act like animals in pure $CaCl_2$.

$25\,^0/_0$ $m/10$ $CaCl_2 + 75\,^0/_0$ $m/10$ KCl. 30 secs. after mixing the paramecia with the solution: cathodal galvanotropism, but they swim backwards to anode. 1 min. 15 secs. after mixing: cathodal galvanotropism, they swim forwards, fairly normal.

$12\frac{1}{2}\,^0/_0$ $m/10$ $CaCl_2 + 87\frac{1}{2}\,^0/_0$ $m/10$ KCl. 15 secs. to 1 min. after mixing: no galvanotropism, motor reflexes too strong. 1 min. 30 secs. to 3 mins. after mixing: cathodal galvanotropism, slow and halting, complicated by constant motor reflexes.

$6\frac{1}{4}\,^0/_0$ $m/10$ $CaCl_2 + 93\frac{3}{4}\,^0/_0$ $m/10$ KCl. Similar to above. At first they all swim backwards irregularly. Then there is cathodal galvanotropism, which is slow and halting, but which gradually becomes stronger.

$3\frac{1}{8}\,^0/_0$ $m/10$ $CaCl_2 + 96\frac{7}{8}\,^0/_0$ $m/10$ KCl. Similar to above.

$1\frac{9}{16}\,^0/_0$ $m/10$ $CaCl_2 + 98\frac{7}{16}\,^0/_0$ $m/10$ KCl. Similar to above.

$\frac{25}{32}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{7}{32}\,^0/_0$ $m/10$ KCl. Similar to above.

$\frac{25}{64}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{39}{64}\,^0/_0$ $m/10$ KCl. Similar to above.

$\frac{25}{128}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{103}{128}\,^0/_0$ $m/10$ KCl. Similar to above, but the cathodal galvanotropism never gets to be really good.

$\frac{25}{256}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{231}{256}\,^0/_0$ $m/10$ KCl. 15 secs. to 4 mins. after mixing: anodal galvanotropism, good and distinct. 4 mins. after mixing: anodal galvanotropism begins to get indistinct, and after that no galvanotropism.

$\frac{25}{512}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{487}{512}\,^0/_0$ $m/10$ KCl. Anodal galvanotropism good.

$\frac{25}{1024}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{999}{1024}\,^0/_0$ $m/10$ KCl. Anodal galvanotropism not so good as above, more absence of galvanotropism.

$\frac{25}{2048}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{2023}{2048}\,^0/_0$ $m/10$ KCl. Absence of galvanotropism predominates, but there is a slight anodal galvanotropism.

$\frac{25}{4096}\,^0/_0$ $m/10$ $CaCl_2 + 99\frac{4071}{4096}\,^0/_0$ $m/10$ KCl. Absence of galvanotropism predominates greatly, but there is still some slight anodal galvanotropism.

$m/10$ KCl. Complete absence of galvanotropism, no anodal galvanotropism.

This experiment shows us that in a mixture of $m/10$ $CaCl_2$ and $m/10$ KCl the $CaCl_2$ had to be diluted to $m/81920$ before the effect of the pure $m/10$ KCl became the predominant one, and even at the concentration $m/163840$ the $CaCl_2$ still had a noticeable effect on the result produced by the KCl. At the lowest concentration of $CaCl_2$ used the ratios of the molecular concentrations was $\frac{C_{CaCl_2}}{C_{KCl}} = \frac{1}{16383}$. It is thus seen that a very small amount of $CaCl_2$ may have a comparatively great influence in antagonizing the effect of the KCl.

But still more important than these results is the fact that as the proportion of the $CaCl_2$ in the mixture is reduced, we have not one change, but a whole series of changes in the galvanotropism manifesting itself. Another simpler experiment will serve better to illustrate these changes.

Effects of mixtures of *m*/10 KCl + *m*/10 $CaCl_2$.

Pure *m*/10 KCl. No galvanotropism.

100 c.c. *m*/10 KCl + 0·1 c.c. (1⅓ drops) *m*/10 $CaCl_2$. Universal anodal galvanotropism.

100 c.c. *m*/10 KCl + 1 c.c. *m*/10 $CaCl_2$. Cathodal galvanotropism, slow and halting, complicated with motor reactions.

50 c.c. *m*/10 KCl + 50 c.c. *m*/10 $CaCl_2$. Cathodal galvanotropism, fairly normal.

This complicated effect of $CaCl_2$ clears up many problems previously obscure, and will be treated under four heads.

(1) *Calcium and the Absence of Galvanotropism.* The absence of galvanotropism is not caused solely by reducing the amount of calcium in the surrounding fluid, for after washing in distilled water (even after centrifuging 7 times in water twice distilled from glass) the paramecia always migrated to the cathode in the usual way. But the absence of galvanotropism was always obtained when the ratio $\frac{C_{Ca}}{C_S}$ was as small as possible. Here S indicates any salt, or salts producing an absence of galvanotropism or anodal galvanotropism. It seems probable that these salts have the power of substituting their cations for the calcium of the ion-proteid compounds, and thus really are effective because they diminish the available calcium. This explains why the calcium precipitants produce an absence of galvanotropism at a lower concentration than salts like KCl and NaCl. It also explains why the calcium precipitants can prevent the galvanotropism of even culture fluid paramecia, which contain considerable quantities of calcium, while KCl and NaCl will prevent it as a rule only in paramecia that have been washed two or three times. It also explains the variability of the results with the same salt (for no two washings can get rid of exactly the same proportion of calcium), and the fact that the more the paramecia are washed the weaker is the minimum solution that prevents galvanotropism. Thus I think it may be safely concluded that an absence of galvanotropism is produced when the ratio $\frac{C_{Ca}}{C_S}$ is smallest.

(2) *Calcium and Anodal Galvanotropism.* When the ratio $\frac{C_{Ca}}{C_S}$ becomes slightly greater anodal galvanotropism is produced. This has been proved as described by the direct addition of minute quantities of $CaCl_2$ to KCl; and similar results have been obtained by adding $CaCl_2$ to Na_3 citrate and even to K_2 oxalate. This fact easily explains why the same salts produce an absence of galvanotropism

in their more concentrated, and anodal galvanotropism in their less concentrated solutions. It also explains why the anodal galvanotropism so frequently changes to an absence of galvanotropism after a little time, for the calcium is continuously being replaced by the other metals and the ratio is continually being reduced.

(3) *Calcium and Cathodal Galvanotropism.* A still further increase of the ratio $\frac{C_{Ca}}{C_S}$ produces cathodal galvanotropism.

This has been proved by directly adding $CaCl_2$ to solutions of $BaCl_2$, KCl, NaCl, and Na_3 citrate. The least amount of $CaCl_2$ which would produce the cathodal galvanotropism was found to be as follows:

Minimum concentrations of $CaCl_2$ to produce cathodal galvanotropism.

	$\frac{C_{CaCl_2}}{C_S}$
1 drop $m/160$ $CaCl_2 + 80$ c.c. $m/160$ $BaCl_2$	1/1066
$\frac{25}{128}$ % $m/10$ $CaCl_2 + 99\frac{103}{128}$ % $m/10$ KCl	1/511
$\frac{25}{256}$ % $m/10$ $CaCl_2 + 99\frac{231}{256}$ % $m/10$ NaCl	1/1023
$12\frac{1}{2}$ % $m/10$ $CaCl_2 + 87\frac{1}{2}$ % $m/10$ Na_3 citrate	1/8

This role of calcium explains why cathodal galvanotropism is ordinarily obtained with culture fluid paramecia in most solutions; for the calcium present in the drop of culture fluid added is enough to prevent the usual action of most solutions. It explains why cathodal galvanotropism is obtained with all solutions if sufficiently dilute. For, on account of the extreme difficulty in washing the salts out of some proteids, and the failure of the attempts to wash them out of others we have no reason to suppose that even repeated washings will remove all the calcium from the paramecia. And as the solutions are made more and more dilute a point must be reached at which the effect of the salts will be counteracted by the small amount of calcium present in the animal. At this point they will show cathodal galvanotropism just as they do in distilled water. It also explains why with the most toxic substances only cathodal galvanotropism can be obtained, for if made dilute enough so that they do not kill they are too dilute to counteract the effect of the calcium present within the animal.

(4) *Calcium and the Backward Swimming to the Anode.* I will not attempt to name the different variations of cathodal galvanotropism as the ratio $\frac{C_{Ca}}{C_S}$ changes; but this much was very clear. With mixtures of $m/10$ KCl $+ m/10$ $CaCl_2$ and $m/10$ NaCl $+ m/10$ $CaCl_2$ a certain

value of the ratio was found at which the backward swimming to the anode was pronounced while this feature of cathodal galvanotropism became less distinct as the ratio was changed in either direction from this value. This backward swimming has been interpreted in a previous paper (1905) as a summation of galvanic and chemical stimulation and it is very interesting to note that there were also certain values of the ratio at which the chemical stimulation was very intense. Furthermore the various portions of the motor reflex mechanism was affected differently depending upon the amount of $CaCl_2$ present. At one concentration the animals swam violently backwards, at another they turned violently, and corresponding to these differences the cathodal galvanotropism was backwards to the anode, or slow and halting with wide spirals and constant turning of the infusoria.

Coehn and Barratt (1905, p. 8) in connection with their theory of galvanotropism have supposed that this backward swimming to the cathode must depend only upon the osmotic pressure, and not upon the chemical nature of the solution. I have already (1906) mentioned many facts that speak against this theory, and here again we have contrary evidence. For in the various mixtures of $m/10$ NaCl + $m/10$ $CaCl_2$ we have solutions differing but little in osmotic pressure which have very different effects on the backward swimming to the anode.

THEORETICAL.

It has been shown that by varying the value of the ratio $\frac{C_{Ca}}{C_S}$ in the surrounding fluid an absence of galvanotropism, anodal galvanotropism and cathodal galvanotropism are produced. It has also been shown that in the case of the anodal galvanotropism there is an actual reversal of Pflüger's law, the stimulation taking place at the *anode* instead of at the cathode as is usual. In this section I will attempt to show that all of these different effects can be easily explained by assuming that the galvanic stimulation depends upon the presence, within the part of the paramecium stimulated, of one, and only one, value for the ratio $\frac{C_{Ca}}{C_S}$.

This explanation is based upon the theory of Loeb (1905, p. 15) for the electrotonic effects of the galvanic current mentioned in the introduction. He states in brief that "It seems therefore as if the electrotonic effects of the galvanic current might be due to the comparative increase in the Ca precipitants at the cathode and the comparative increase of the Ca solvents at the anode." In the case of the galvanotropism of paramecium we are dealing not with a condition of increased or decreased excitability, as in the nerve, but with the presence or absence of galvanic stimulation. But it is still possible to explain the facts of galvanic stimulation in paramecium by supposing that: Galvanic stimulation in paramecium is produced by a relative decrease (or increase) of the Ca ions (in comparison with the other free cations) to a certain definite point; and that if the decrease (or increase) proceeds beyond that point then we have again an absence of stimulation. In other words: Galvanic stimulation depends upon the production within that part of the paramecium stimulated, of a certain definite value for the ratio $\frac{C_{Ca}}{C_S}$. If this ratio becomes either greater or less there is no stimulation. In view of the fact that in solutions where the value of the ratio $\frac{C_{Ca}}{C_S}$ is least there is no galvanotropism, this assumption is a very natural one.

Let us see how this theory fits the facts:

During *cathodal galvanotropism* the Ca ions will be diminished at the cathode by the increase of the oleate and other slowly moving organic anions until the stimulating value of the ratio $\frac{C_{Ca}}{C_S}$ is reached. At the anode the concentration of the Ca ions will be increased, and thus there will be no stimulation there. If the surrounding fluid contains very many Ca ions the maximum stimulating value for the ratio may not be quite reached, and the galvanotropism should become less distinct. It has already been shown that this is actually what happens in $m/10$ $CaCl_2$ and mixtures containing much $CaCl_2$.

To produce *anodal galvanotropism* the ratio $\frac{C_{Ca}}{C_S}$ must have been very much reduced. We must suppose that it has been reduced *below* the stimulating value. If this is true then there will be an increase of the Ca ions at the *anode* until the stimulating value of the ratio is reached at this pole, and the stimulation will take place at the anode, as actually occurs. At the cathode there will be a still further decrease in the Ca ions, and therefore no stimulation.

To produce an *absence of galvanotropism* we must suppose the ratio $\frac{C_{Ca}}{C_S}$ has been so greatly reduced that even by means of the increase of the Ca ions at the anode the stimulating value for the ratio cannot be reached, and thus there is no stimulation.

SUMMARY.

1. Paramecia may be made to swim forwards towards the anode if they are washed in distilled water, and then subjected to weak solutions of many salts.

2. Such anodally galvanotropic paramecia have the cilia at their anodal ends beating more strongly forwards, and are oriented by this means.

3. This proves that the pole at which galvanic stimulation occurs has been reversed, and is now the *anode.*

4. Complete absence of galvanotropism in paramecium is caused best by salts tending to diminish the concentration of free calcium ions.

5. $CaCl_2$ in sufficient quantities will prevent both anodal galvanotropism and absence of galvanotropism.

6. The character of the galvanotropism depends upon the relative amounts of free calcium ions present, as follows:

   (*a*) Absence of galvanotropism when the relative concentration of free calcium ions is least.

   (*b*) Anodal galvanotropism when the relative concentration of free calcium ions is but very little greater.

   (*c*) Normal cathodal galvanotropism when the relative concentration of free calcium ions is considerably greater.

   (*d*) Indistinct cathodal galvanotropism when the free calcium ions predominate considerably.

7. These four responses to the galvanic current, together with many of their variations, may be easily explained on the basis of the theory of Loeb, that the electrotonic effect of the galvanic current consists in a relative decrease of the concentration of the free calcium ions at the cathode, and in their increase at the anode; by assuming that galvanic stimulation depends upon the presence of some particular relative concentration of the free calcium ions, and cannot take place when this concentration is either greater or less.

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