The Relation of Spermatozoa to certain Electrolytes.—II.

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The fact that living spermatozoa move towards the positive pole of an electric field has been known for some years. In a note published in 1915, the writer (8) pointed out that this movement is dependent upon a certain concentration of hydroxyl ions, without which the spermatozoa neither exhibit their normal activity nor do they move in an electric field. In the same paper the behaviour of spermatozoa to such trivalent ions as cerium was briefly described. In the present communication these results are enlarged, and the problem briefly discussed from its theoretical aspect.

A considerable mass of evidence now exists to show that the surface charge of a particle or membrane is profoundly affected by the nature of the solution with which it is in contact. Albumen particles, when suspended in an acid medium, are positively charged; when in an alkaline medium they are negatively charged. Perrin (11) has shown that, if a diaphragm separates two phases between which a potential gradient exists, then the gradient can increase or decrease by treating the diaphragm with various agents. In acid solutions a negative diaphragm becomes less negative, and finally positive; in an alkaline solution the negative charge is increased.

Table I.

Diaphragm.	Solution.	Relative Charge.
Carborundum (negative) ...	0·02M HCl	+ 10
	0·008M HCl	0
	0·002M HCl	− 15
	Water	− 50
	0·0002M KOH	− 60
	0·002M KOH	−105
Naphthalene (negative) ...	0·02M HCl	+38
	0·01 HCl	+39
	0·001 HCl	+28
	0·0002 HCl	+ 3
	0·0002 KOH	−29
	0·001 KOH	−60
	0·02 KOH	−60

It should be noted that the greatest change takes place round the neutral point. Perrin also investigated the effects of lanthanum nitrate upon a carborundum membrane.

Table II.

Conc. of La $(NO_3)_3$.	Relative Charge.
0	−60
0·00004 Mol.	−58
0·0002 ,,	−18
0·001 ,,	− 1

These results have been confirmed and enlarged by Mines (9) and 10). This author applied the name of "polarising ions" to those ions which are capable of affecting the surface charge of a membrane or particle to any marked degree. Such ions are the hydrogen ion, hydroxyl ion, and such trivalent ions as cerium, lanthanum, and the citrate ion.

Finally, the work of Chick and Martin (2) on the precipitation of albumen or globulin suspensions by such salts as lanthanum is of great interest. These authors show clearly that the precipitating power of such salts as lanthanum nitrate or sodium citrate depends very closely upon the hydroxyl ion concentration of the colloid suspension. If albumen is dissolved in dilute acid, lanthanum salts do not precipitate the colloid; these salts are, however, powerful precipitants from an alkaline medium. They have also shown that lanthanum is capable of conveying a positive charge to such particles if the salt is sufficiently concentrated. Precipitation only occurs when the "polarising" elements present allow the particles to possess a minimum charge. In other words, precipitation occurs at the isoelectric point.

The application of these principles to physiology was first made by Mines, who investigated the relationship of the vertebrate heart to polarising and other ions. He also showed that red-blood corpuscles suspended in Ringer solution closely resemble the negative particles of an emulsoid colloid.

In 1916 the writer (7) described the effects of lanthanum or cerium upon the electrical conductivity of echinoderm eggs, and reference was made to the possibility that the nature of the surface charge on the egg played an important *rôle* in the phenomena of artificial parthenogenesis. The present experiments with the spermatozoa of *Echinus miliaris* form an attempt to attack the problem of fertilisation from a similar standpoint.

At the outset it is convenient to refer to one or two well-known facts. In the first place, spermatozoa suspended in sea water are surrounded by an alkaline medium whose P_H is about 8·0. In the second place, the interior of the living cell is always more acid than the surrounding medium. Living

spermatozoa or eggs stain bright red with neutral red, whereas sea water gives an orange reaction with this indicator. Finally, living spermatozoa migrate to the positive pole of an electric field. We therefore start from the assumption that spermatozoa resemble a suspension of colloid particles in an alkaline medium. It is maintained that the experiments to be described afford strong support for this hypothesis.

The Effects of Trivalent Positive Ions.

A concentration of 0·0005 M $CeCl_3$ or $La(NO_3)_3$ in sea water quickly causes most marked agglutination of a sperm suspension. The spermatozoa are heaped together in irregular masses clearly visible to the naked eye. These masses quickly sink to the bottom of the tube containing the suspension. If the agglutinated spermatozoa are gently centrifuged and washed with normal sea water little or no change takes place in their appearance even after fairly strong agitation.

If, however, to 10 c.c. of sea water containing agglutinated spermatozoa 1 c.c. of 0·8 M* sodium citrate is added, all trace of agglutination rapidly disappears, and the fluid again resembles the original dispersed sperm suspension. The phenomena are very striking with both cerium and sodium citrate solutions. Similar experiments were performed with lanthanum and thorium nitrates.

Table III.

Mol. conc. of cerium.	Degree of flocculation.
0	0
0·00025	+ +
0·0005	+ + +
0·00075	+ + +
0·0010	+ + +
0·00125	+ + +

Note.—In the above and subsequent tables the following symbols will be used:—

0 represents a suspension of maximum dispersal.

+ + + represents a suspension of maximum agglutination which quickly settles to the bottom of the fluid.

+ + represents a suspension in which the agglutination is well marked, but settles somewhat slowly.

+ represents a suspension in which agglutination is still visible to the naked eye, but which settles very slowly.

⊕ very faint indication of agglutination.

It will be noted that these experiments are an exact parallel to those which can be performed with a suspension of albumen or globulin in slightly

* A solution of this strength depresses the freezing point of water to approximately that of sea water.

alkaline solutions; the phenomena observed are the same in the two cases and are capable of the same explanation.

In the presence of cerium or lanthanum the negative charge on the surface of the cell or the particle is lost and agglutination results. In the presence of citrate, however, the negative charge is restored by the negative trivalent ion, and dispersal consequently takes place.

That the agglutination caused by cerium or lanthanum is completely reversible by sodium citrate is illustrated by the following experiment: 100 c.c. of sperm suspension was agglutinated by means of cerium and the spermatozoa washed in several changes of sea water; 10 c.c. of this agglutinated suspension were placed in several test-tubes to which varying amounts of 0·8 M sodium citrate solution were added. The tubes were *gently* and uniformly shaken:—

Table IV.

Amount of citrate added to 10 c.c. suspension.	P_H.	Agglutination after 15 min.
c.c.		
0	7·9	+ + +
0·5	7·95	+
1·0	8·0	⊕
1·5	8·1	⊕
2·0	8·2	0
2·5	8·3	0
3·0	8·4	0
4·0	8·5	0

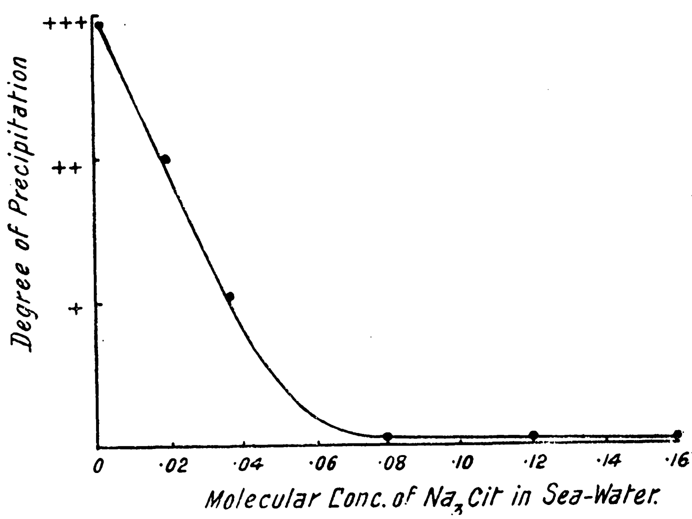

Diagram showing Removal of Cerium Precipitation by Sodium Citrate.

It should be noted that the presence of sodium citrate raises the P_H of the sea water, but this change is insufficient in itself to account for the removal of the effects of the cerium.

That the precipitation of spermatozoa by means of trivalent positive ions depends very closely on the P_H of the solution is shown in the following Table:—

Table V.

P_H of sea water.	Precipitation by 0·0012M Ce···.	P_H of sea water.	Precipitation by 0·0012M Ce···.
7·6	+ + +	3·7	0
7·3	+ + +	3·3	0
6·5	+ + +	2·9	0
6·1	0⊕*	8·2	+ + +
5·6	0	8·57	+ + +
5·3	0	9·3	+ + +
4·5	0		

* Faintest trace of precipitation.

In different samples of sperm the critical value of P_H varied slightly. In one experiment P_H 7·3 gave no precipitation, in another 6·5 gave none. In each case 7·6 gave strong precipitation.

A number of experiments were performed which brought out the interesting fact that exactly the same hydrogen ion concentration is required to render the spermatozoa motionless as is required to prevent their flocculation by cerium.

Table VI.

20 c.c. sea water + c.c. N/10 HCl.	After 15 min.	
	Movement.	Flocculation by cerium.
c.c.		
0	+ + +	+ + +
0·07	+ + +	+ + +
0·14	+ + +	+ + +
0·21	⊕*	0
0·28	0	0
0·35	0	0
0·42	0	0
0·49	0	0
0·56	0	0

* very slight movement.
+ + + = active movement or complete flocculation.
0 = no movement or no flocculation.

Whereas in normal sea water the cerium produces its effect in less than 30 seconds, in slightly acid sea water the reaction is delayed, and it may be several minutes before incomplete precipitation is apparent.

Three drops of undiluted sperm were placed in 20 c.c. of the following solutions, and after 15 minutes were examined for movement, and tested by cerium (conc. 0·001 M).

In this particular experiment the flocculation in the solution containing 0·14 c.c. N/10 HCl was most marked, and more intense than normal. The experiments illustrate very clearly the intense effects of very small changes of hydrogen ion concentration. A very slight change beyond the critical value stops both movement of spermatozoa and their power of being agglutinated by cerium. Both these phenomena are reversible by raising the P_H of the solution to a value of about 6·5.

In the following experiment the P_H of several samples (20 c.c. each) of the same sperm suspension was lowered to the values shown in column 1; after 20 minutes each sample was divided into two equal portions, and to one portion an equal bulk of sea water was added. To each tube sufficient cerium chloride was added to give a concentration of 0·00125 M $Ce^{\cdots}$. After 15 minutes the degree of agglutination was noted.

Table VII.

I. P_H of original 20 c.c. of acid suspensions.	II. P_H of portions of original solution after dilution with sea water.	I. Degree of agglut. by $Ce^{\cdots}$ in original suspensions.	II. Degree of agglut. by $Ce^{\cdots}$ in diluted suspension.
7·6	7·7	+ + +	+ + +
7·3	7·5	+ + ⊕	+ + +
6·5	7·3	+ ⊕	+ +
6·2	6·9	⊕	+ +
5·9	6·6	0	+
5·6	6·4	0	+
5·3	6·3	0	+

The reversal of the effect of cerium by sodium citrate, and the dependence of the agglutination by cerium upon the hydroxyl ion concentration of the suspension, are exactly paralleled by the effect of these ions upon a suspension of albumen or globulin. In the latter case, there is no cause to doubt the conclusion that the degree of dispersal or agglutination caused by acids, alkalis, or trivalent ions is dependent upon the surface charge on the particle. The point of maximum flocculation coincides with the reduction of the surface charge to a minimum (in other words, at the isoelectric point); in acid solutions the colloid particle is positively charged so that the positively

charged trivalent ions cause no precipitation; in alkaline solutions the particles are negatively charged, and positive trivalent ions cause precipitation by reducing the charge to the isoelectric value.

Finally, it must be mentioned that spermatozoa which have been agglutinated by cerium or lanthanum are dispersed by acid sea water, and the concentration of acid required coincides with the critical values found in such experiments mentioned in Table VII. The addition of further cerium to such acid suspensions causes no agglutination.

This fact is important, as it shows very clearly that the effect of cerium on a normal sperm suspension is of the same nature, but much less intense than that of acids. Acids never agglutinate spermatozoa, but at a critical concentration the surface charge changes with great rapidity from negative to positive, and in each case maximum dispersal exists just as in a weak globulin solution, which contains a high concentration of neutral salts. Cerium or lanthanum, however, have a much less intense action than the hydrogen ion; the trivalent ions reduce the normal negative charge, but they cannot convey a sufficient positive charge to cause dispersion; hence, spermatozoa agglutinated by cerium can be dispersed either by acids or by trivalent negative ions; in the former case dispersion being due to a positive charge and in the latter to a negative charge.

The intense action of the hydrogen ion is paralleled by the experiments of Perrin and Mines, and also by the interesting work of Ellis (4). The latter author has shown that the maximum surface potential on an oil-water interface exists in the region of "neutrality"—the addition of acid very rapidly reduces this potential, while alkalis reduce it somewhat more slowly. This latter point is of interest, when we consider the effects of alkalis upon sperm suspensions. As stated in a previous paper (8), the effect of fairly strong alkaline sea water is to agglutinate the spermatozoa. The action is, however, less complete than that of cerium, and it will be noted that some doubt may exist as to whether the two phenomena are comparable. In the first place, the agglutination of sperm by alkali shows well marked grades of agglutination with increasing P_H —, also a concentration of alkali which is sufficient to strongly agglutinate the vast majority of the spermatozoa allows the minority to continue in active movement for a considerable time. Again, with alkali of varying strength, a complete series of intermediate conditions of agglutination is obtained from complete agglutination to complete dispersal, a phenomenon which is not observed in the case of cerium. Finally, concentrations of alkali which cause marked agglutination injure the cells, which then stain yellow instead of red with neutral red, showing that the *interior* of the cell has been affected by the alkali.

It is therefore possible that agglutination of spermatozoa by alkali is a distinct phenomena from that of agglutination by cerium; at the same time it is interesting to note that Ellis found that alkali *reduces* the potential between an oil-water interface, although its action is less intense than that of acids, so that it is possible that the agglutination of spermatozoa by alkali is due to a reduction in the negative surface charge. It should, however, be mentioned that no evidence was obtained to show that the agglutinative power of cerium was increased by raising the P_H of sea water above the normal.

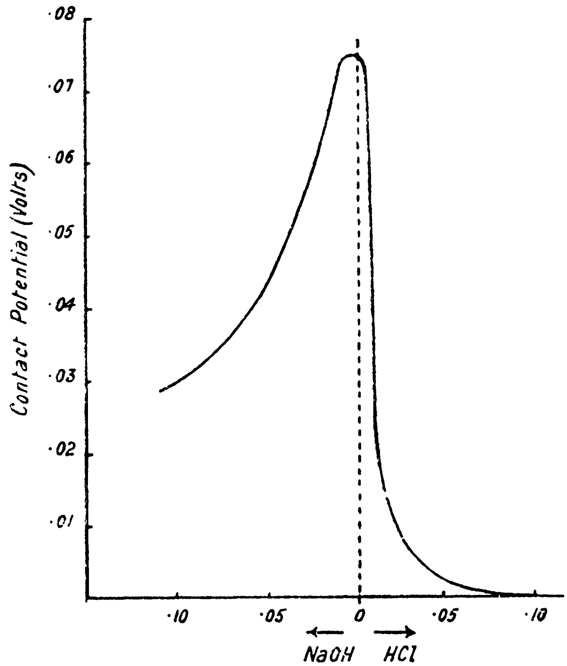

Diagram (after Ellis) showing Relation of Contact Potential of an Oil-Water Interface and Hydrogen Ion Concentration of Water phase.

Summary of Experimental Results.

A suspension of the spermatozoa of *Echinus miliaris* in sea water behaves towards trivalent positive ions in exactly the same way as a suspension of negatively charged particles of such colloids as albumen or globulin. It is only in those solutions which are capable of maintaining the normal negative charge that movement of spermatozoa can take place. Trivalent ions flocculate sperm suspensions by removing the negative charge. The action of the hydrogen ion is very intense and changes the surface charge from negative to positive without any intermediate flocculation.

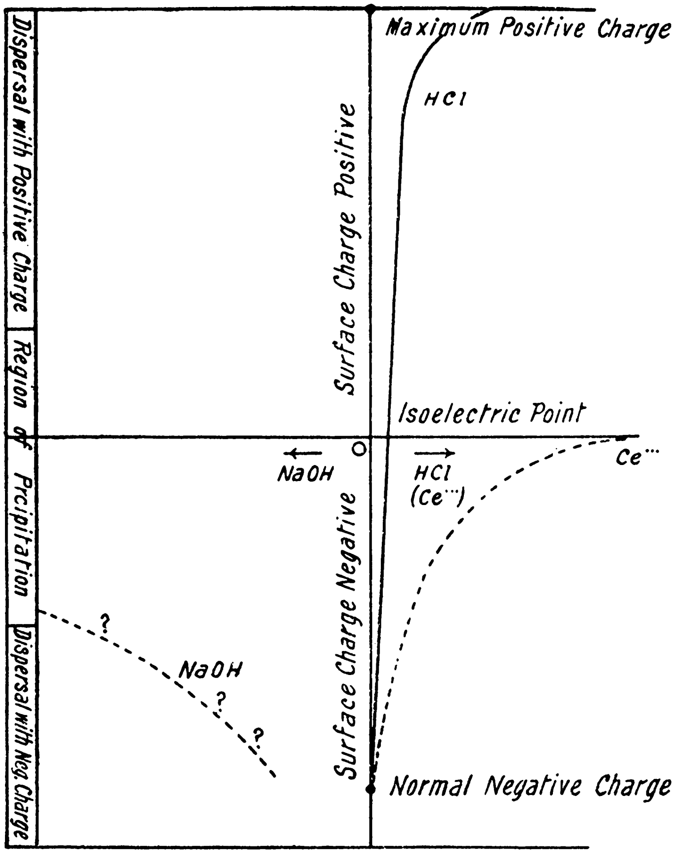

Diagram illustrating effect of Polarising Ions on Surface Charge and Precipitation of Spermatozoa.

Note on Experimental Hybridisation.

In a previous paper (Gray (7)) it was suggested that the action of various electrolytes upon the eggs of echinoderms was to be found in the action of these substances on the surface charge of the eggs, and that this charge played a fundamental *rôle* in the physiological activity of the egg. In the present paper evidence is brought forward to show that the surface charge on the spermatozoa is of fundamental importance to their activity, and that this charge depends upon the nature of the solutions with which the spermatozoa are in contact. Now, just as the particles of different colloids (or membranes of different composition) possess different charges when in contact with the same solution, so the eggs and spermatozoa of different species may have different surface charges when in sea water of the same composition. If, therefore, the possibility of fertilisation of the egg depends partly on the

mutual relationship between the surface charge of the egg and that of the spermatozoon, it is possible that many cases of artificial hybridisation may find a simple solution. One fact is quite certain, namely, that when fertilisation is effected in sea water to which a certain amount of acid has been added, the surface charge of the spermatozoon has been definitely reduced below the normal.

It should be remembered that when both eggs and sperm are exposed to a solution of a definite P_H both gametes will be affected to a certain extent, but the spermatozoa will be very much more affected than the eggs, owing to their much smaller size; also, the egg can react much more strongly to the abnormal conditions, and by an adjustment of its normal metabolism can, conceivably, counteract the effect of an excess of hydrogen ion on its surface charge.*

If eggs of one species and spermatozoa of another are placed in such abnormal conditions, there will be a differential action on the two units, because the surfaces of one species will be altered to a different extent to those of another.

Again, the antagonistic action of mixtures of sperm of two different species may quite likely be due to the changes induced on the surface of the spermatozoa by the changes produced in the hydrogen ion concentration of the suspension by the evolution of CO_2.

In the near future it is proposed to investigate the surface charges of the spermatozoa of different species, with a view to determining whether the possession of a critical surface charge controls the fertilising power of the sperm for eggs of the same and of different species. Since performing the above experiments I have become acquainted with the work of Girard and others (5 and 6) on red blood corpuscles and on bacteria. Girard has shown that very low concentrations of lanthanum decrease the rate at which bacteria migrate towards the anode in an electric field. Further, red blood corpuscles, when suspended in isotonic Ringer solution, behave as negatively charged particles. If, however, they are suspended in acidified saccharose solution, their surface charge is reversed and they become positive. In Ringer solution, however, it is much more difficult to reverse the surface charge by means of acid.

The effect of the hydrogen ion upon the activity of spermatozoa forms the subject of an interesting paper by Cohn (3), which has just come to my notice.

* The relatively greater sensitivity of spermatozoa to changes in hydroxyl ion concentration has been deduced from other facts by Moore and other workers.

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A Further Study of Chromosome Dimensions.

By C. F. U. Meek.

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(Plates 2 and 3.)

Introduction.

In 1912 I published a paper giving measurements of the chromosomes composing the complexes of species belonging to both closely allied and widely separated divisions of the animal kingdom. The material studied consisted chiefly of the spermatogenetic cell generations: and these investigations, so far as I am aware, constituted the first serious attempt to measure chromosomes and to compare chromosome dimensions throughout the animal kingdom. The results obtained proved certain generalisations, and suggested others.

The first generalisation with which I intend to deal here was that the degree of somatic complexity of an animal cannot be correlated with the