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The Electrical Conductivity of Echinoderm Eggs, and Its Bearing on the Problems of Fertilisation and Artificial Parthenogenesis

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XI. *The Electrical Conductivity of Echinoderm Eggs, and its Bearing on the Problems of Fertilisation and Artificial Parthenogenesis.*

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PART I.

Introductory.

The precise nature of the change which ushers in the development of the egg has been much discussed. Broadly speaking, the problem may be said to have been considered from two points of view, the chemical and the physical. According to the point of view, the spermatozoon or the parthenogenetic agent has been supposed to start a chemical change within or at the surface of the cell, or to alter the physical properties of the surface. Loeb, for instance, in a recently published book, ascribes development to the introduction of certain substances into the interior of the egg. Lillie, and McLendon, on the other hand, regard an increase in the permeability of

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the egg surface to electrolytes as the precursor of development. The experiments described in this paper favour in general the latter hypothesis; but as they do not support the theories of LILLIE and MCLENDON in detail, it will be well to give at the outset a brief sketch of the work of these investigators.

In 1910 LILLIE showed that the unfertilised eggs of *Arbacia punctulata* lose their pigment when placed in isotonic solutions of various sodium salts. The order of effectiveness of the anions was found to be $Cl<Br<NO_3<CNS<I$; this is also the order in which these ions affect the state of aggregation of colloids. If the eggs are removed from these solutions after an appropriate interval, membranes are formed round the eggs and a certain number develop into larvæ. In the following year he showed that the effect of these sodium salts can be inhibited by the addition of calcium salts to the solutions. Accepting the view of HAMBURGER, HÖBER, and others that the phenomena of cytolysis are primarily due to an increase in the permeability of the cell surface to ions, LILLIE arrived at the following conception of the fundamental processes which attend the development of the unfertilised egg:— Before fertilisation the egg membrane is freely permeable to kations, but only very partially permeable to anions; hence, the egg membrane must be the seat of an electrical charge which is determined by NERNST'S formula

$$E = \frac{u-v}{u+v}\frac{RT}{F}\log\frac{c_2}{c_1},$$

where u is the velocity of the kation and v of the anion; RT/F is constant; c_2 and c_1 the concentration of electrolytes inside and outside the cell. After fertilisation, however, the value of v is increased, so that the surface polarisation is decreased. It is this loss of surface polarisation which is the fundamental factor in the development of the unfertilised egg.

From various general conclusions LILLIE concludes that no cell can withstand a sustained condition of increased permeability, for such a condition would disorganise the electrolytic contents of the cell. Hence, after fertilisation, the permeability must fairly quickly be reduced again to its former value. In the case of eggs which are treated with butyric acid LILLIE holds that the subsequent treatment with hypertonic sea-water effects this decrease in permeability.

LILLIE'S evidence for a change in the permeability of *Arbacia* eggs when treated with isotonic solutions of neutral salts is, however, unsatisfactory. He concludes that because the egg surface becomes permeable to enclosed pigment it must also become permeable to electrolytes. STEWART (52), however, has shown that such a conclusion is quite unwarranted in the case of red blood corpuscles. In these cells hæmoglobin can escape before any of the intracellular electrolytes, and conversely that electrolytes can escape without the loss of hæmoglobin.

MCLENDON'S theory is less complex, but less comprehensive, than that of LILLIE. He regards the essential condition for development to be an increase in the

permeability of the egg to hydroxyl ions and to the ions of carbonic acid. This simple hypothesis is based upon the following observations :—

1. Fertilised eggs shrink more quickly than unfertilised eggs in isotonic sugar solution. Presumably the fertilised eggs are more permeable to the substances exerting the internal osmotic pressure.

2. The electric conductivity of the egg increases about 25 per cent. when it is fertilised or made parthenogenetic with acetic acid, indicating permeability to ions.

3. When placed in an electric field unfertilised eggs show the phenomena of "anodal" disintegration sooner than the fertilised eggs.

This theory resembles that of LILLIE in postulating an increase in the permeability of the egg to kations after fertilisation; the activity of the egg being promoted by the increase in the hydroxyl ion concentration of the egg interior.

The observation that the conductivity of fertilised eggs is distinctly greater than that of unfertilised eggs is at present the most direct evidence in favour of the view that after fertilisation the egg surface is more permeable to ions than before. I therefore undertook a further investigation of this phenomenon.

METHOD OF INVESTIGATION.

A conductivity cell was used such as would fit the holder of a small hand centrifuge. The tube was about 10 cm. long and 1 cm. in diameter at its widest part. The electrodes were two square platinum plates each possessing two equal surfaces of 25 sq. mm. These were fixed about 5 mm. apart and were carried by two silver wires, which were supported by glass and passed through the stopper of the cell (see fig. 1).

In a few of the earlier experiments the volume of the eggs was determined by marking their level with a fine pointed grease pencil, but in the very large majority of the experiments the tube was also graduated.

This form of conductivity cell has two advantages: (1) It fits into the holder of an ordinary centrifuge, (2) the volume of eggs required is small. The latter point is of great importance, quite apart from the difficulty of obtaining large quantities of eggs. If too many eggs are enclosed within a tube, it is not only impossible to ensure a good percentage of fertilisations by the addition of a small quantity of sperm, but the overcrowding of the eggs interferes with their rate of development subsequent to fertilisation (*i.e.*, the rate of division differs very considerably from that of similar eggs in a large bulk of water; in extreme cases the development ceases at an early stage). In all my experiments, except where specially mentioned to the contrary, the eggs developed normally, although in some cases more slowly than the controls.

The temperature at which the experiments were made never differed much from the room temperature so that it was found possible to keep the temperature of the eggs constant to within $\frac{1}{2}^{\circ}$ C. by means of the simple thermostat shown in fig. 2. The calorimeter was surrounded by a worm tube, through which cold tap water was kept in circulation.

The resistance of the eggs was measured by means of a Kohlrausch bridge and a telephone. The electrodes were platinised in the usual way with platinic chloride with a trace of lead acetate. The induction coil was placed outside the room in which the experiments were made, and by keeping the electrodes well platinised it was possible to obtain quite distinct minimal points with an ordinary telephone.

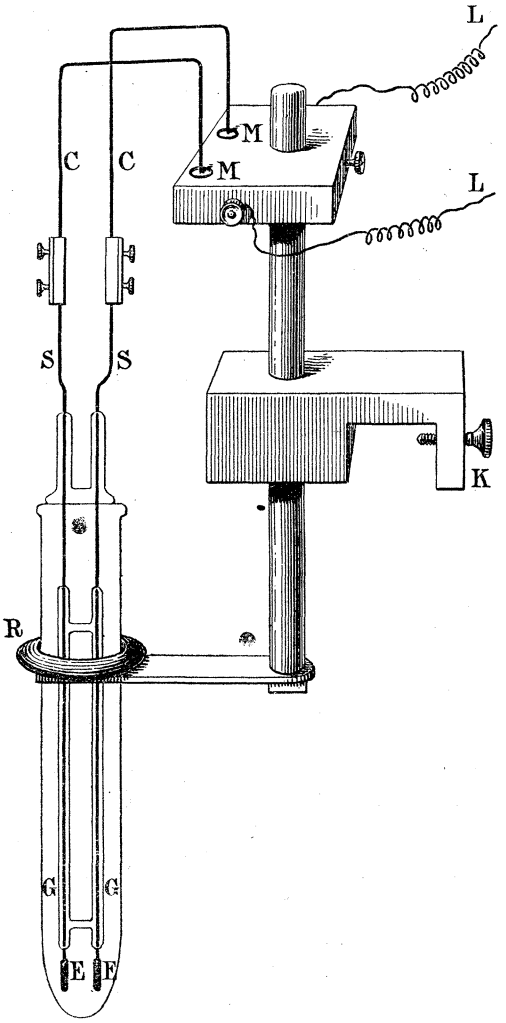

FIG. 1.—Conductivity Tube, showing clamp for attachment to thermostat, and connections to Kohlrausch bridge.

C, stout copper wires; S, silver wires from electrodes; E, electrodes; G, glass supports; R, detachable rubber ring; M, mercury cups; L, leads to bridge terminals; K, clamp for attachment to thermostat.

The procedure adopted during the whole of the experiments was as follows. The ovaries of a perfectly ripe female were shaken into one or more finger bowls containing sea-water. The ovaries were then removed after five or ten minutes, and the sea-water containing the eggs was filtered through a suitable piece of bolting silk. In this way any loose pieces of ovarian tissue were removed from the eggs. The latter were then allowed to settle to the bottom of the bowl. The ripe eggs settled somewhat slowly; but after a short time sufficient eggs for one experiment could be drawn off in a clean pipette and transferred to the conductivity tube. The requisite amount of eggs having been so obtained, the tube was filled up with clean sea-water, corked, and allowed to stand in a bowl of sea-water until the eggs had again settled sufficiently for the bulk of the sea-water to be removed. The eggs were then washed once more in clean sea-water. After two or three such washings all the small fragments of tissue smaller than the eggs were removed, and the tube contained nothing but ripe eggs in clean sea-water. After washing in this way the eggs settled somewhat more readily than when removed from the ovary, owing to the partial removal of the gelatinous ovarian membranes.

In the earlier experiments the conductivity tube containing the eggs was then transferred to the thermostat and left until the eggs had settled to a definite volume, which could be estimated without any difficulty. Great care was taken to make the eggs settle *uniformly* in the tube; if this is not done measurements of the resistance of the same eggs occupying the same volume fail

to agree. It was found possible to collect the eggs in such a way as to obtain uniform readings from repeated observations. In later experiments the eggs were settled by gentle use of a centrifuge; such treatment does not hurt the eggs in any way and greatly simplifies the technique.

Whereas the above technique is sufficient for experiments with the eggs of *Echinus*, it was found to be unsatisfactory for the eggs of those sea urchins which were available at Naples (*Sphærechinus*, *Strongylocentrotus*, and *Arbacia*). If these eggs are settled by gravity it is impossible to obtain resistances of more than 25 ohms owing to the presence of the wide gelatinous membranes which surround the eggs. It was found, however, that if the eggs are washed for a few minutes in slightly acid sea-water (100 c.c. sea-water + 5 c.c. N/10 HCl) the gelatinous membranes are entirely

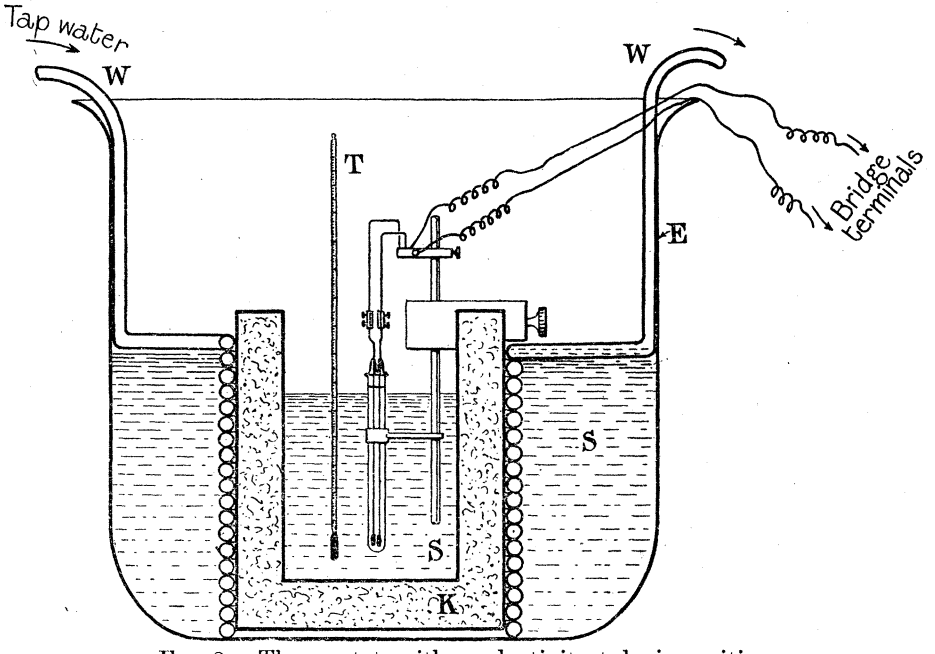

FIG. 2.—Thermostat, with conductivity tube in position.
W, worm tube; S, sea-water; K, kieselguhr; E, large earthenware bowl; T, thermometer.

removed. Such treatment does not injure the eggs in any way, and greatly facilitates their subsequent manipulation for the following reasons:—

1. The eggs settle very much more quickly.

2. They are much closer together when settled and therefore give much higher resistances.

3. After fertilisation, no fertilisation membranes are formed. In the case of *Sphærechinus* the surface of the egg does not appear to change after fertilisation until after the first hour of development. In the case of *Strongylocentrotus* the surface membrane of the egg becomes wrinkled, but no wide fertilisation membrane is formed if the washing with acid sea-water has been sufficient.

4. These eggs always settle quite uniformly in the conductivity tube, and successive determinations of their resistance give remarkably concordant results.

After the volume of the eggs had been accurately determined, some of the sea-water was removed from the tube, the electrodes placed in position and the resistance of the eggs determined. The electrodes were then removed and the tube filled with fresh sea-water (care being taken not to remove any of the eggs with the electrodes*). One or two drops of a dilute emulsion of sperm were then added and the tube inverted so as to distribute the eggs equally in the sea-water. For one minute the tube was allowed to stand in the water of the thermostat. The eggs were then returned to the required volume and the excess of sea-water was again drawn off. The electrodes were then placed in position and the resistance of the fertilised eggs determined. After each determination of the resistance of the eggs, the conductivity tube was filled with fresh sea-water and immersed horizontally in sea-water whose temperature never differed from that of the thermostat by more than 1° C.

Successive determinations of the resistance of the same lot of unfertilised eggs showed that with careful manipulation the variations of the readings never exceeded 3 per cent. of the total resistance in the case of the eggs of *Sphærechinus*, *Strongylocentrotus*, and *Echinus*. With *Arbacia* eggs the readings were not quite so uniform. The experimental error is therefore of an order quite different from the much larger difference between the resistances of fertilised and unfertilised eggs.

Experiments and Results.

The variations in the resistance of the eggs must be due to the egg proper and not merely to the fertilisation membrane, since the changes which follow fertilisation follow the same course in those eggs which do not form such a membrane as it does in those which do.

Eggs from which the ovarian membranes had been removed by treatment for half a minute with faintly acid sea-water do not form fertilisation membranes, although they do develop normally.† The conductivity of such eggs always resembles that of eggs which were fertilised in the ordinary way, that is, without previous treatment with acid. This fact, together with others established by Elder (8) and others, shows clearly that the protrusion of a fertilisation membrane is of secondary importance among the phenomena of fertilisation.

The changes in conductivity cannot be due to the presence of free spermatozoa between the eggs, since the addition of as much sperm as was used in any of the experiments to a volume of sea-water equal to the bulk of the eggs does not change the conductivity of the sea-water.

In a recent paper, Glaser (13) has brought forward evidence to show that the

* When not actually in use, the electrodes were kept immersed in clean sea-water.

† In the case of *Echinus* eggs it was found that prolonged washing of the unfertilised eggs with normal sea-water prevented the membranes of the fertilised eggs being protruded to the usual degree. In such eggs the membranes remained more or less closely applied to the surface of the eggs.

volume of the eggs of *Arbacia* and *Asterias* decreases after fertilisation. As this author points out, this discovery is at variance with the observations of other investigators. There is, however, the possibility that this contradiction is only apparent. Glaser measured the fertilised eggs immediately after fertilisation, and concluded that the amount of sperm present played a *rôle* in the amount of change in the volume of the eggs. Now, when the eggs of *Strongylocentrotus lividus* are fertilised, there is distinct evidence that the egg is compressed by the contents of the perivitteline space, and the phenomenon is very marked if a large amount of sperm is present. Within a few minutes, however, the egg resumes its normal spherical shape, if the excess of sperm is removed. It is impossible to measure the diameter of the eggs when in the contracted state on account of the irregular outline of the egg surface, but all measurements of eggs which have resumed their spherical condition have failed to show any diminution of volume after fertilisation. It is possible that the loss of this contraction or compression of the egg after fertilisation may account for the slight fall in the conductivity which is sometimes observed between 3 and 10 minutes after fertilisation.

That the decrease in the resistance of the eggs after fertilisation is not due to a decrease in their volume is apparent from the following facts :—

(*a*) Microscopical measurements failed to reveal any decrease in the size of *Echinus* eggs after fertilisation. This result was also arrived at independently by Loeb, McLendon (38), and Chevreton and Vles (6), for the eggs of other species.

(*b*) Although the decrease in the volume of *Asterias* eggs is much larger than that of *Arbacia* and other Echinoid eggs (Glaser, 13), the change in the resistance of *Asterias* eggs is much less than that of all the Echinoids investigated.

Direct experiment showed that the currents used to measure the electrical resistance were wholly without effect upon the subsequent course of development.

In order to cover the electrodes about $\frac{1}{2}$ c.c. of eggs is required. The volume of sea-water which the conductivity cell can hold is about 12 c.c. ; the effect of these conditions is that when the eggs are re-distributed in the sea-water after the determination of their resistance they are so crowded as to develop at a different rate to control eggs kept in an abundance of water. The change in rate of development varies with different batches of eggs and with their degree of concentration. Hence it is impossible by this method to determine the conductivity of normal eggs (*i.e.*, eggs developing at a normal rate) at any stated moment. As, however, the development of the eggs in the conductivity tube is only abnormal in respect to time (the cleavage divisions are quite normal but occur at a varying time after those in the control), it may be concluded that the sequence of events in the two cases is the same.

The fact that the resistance can only be determined by concentration of the eggs is an unsatisfactory feature of the method. We may assume that when the eggs are concentrated at the bottom of the tube, their development very quickly becomes

delayed, until the return of normal conditions; hence, it is not possible to take a large number of readings during one experiment.

TABLE I.—Experiments with Eggs of *Echinus miliaris*. Gelatinous membranes were completely removed before fertilisation.

Temperature.	Resistance in ohms of		Time after addition of sperm that R_F was determined.	$R_U - R_F$.	$\frac{(R_U - R_F)\ 100}{R_U}$.
	Unfertilised eggs. (R_U.)	Fertilised eggs. (R_F.)			
12·5°	325	178	5	147	45·2
12·5°	275	152	5	123	44·7
12·5°	285	207	2	78	27·4
12·5°	227	137	4	90	39·6
15·0°	210	164	1	54	25·9
15·0°	238	168	1	70	29·4
15·0°	220	151	1	69	31·4
15·0°	202	150	1	52	25·7
15·0°	215	160	1	55	25·6
15·0°	245	174	1	71	29·0

Average value of $\frac{(R_U - R_F)\ 100}{R_U} = 32·4$.

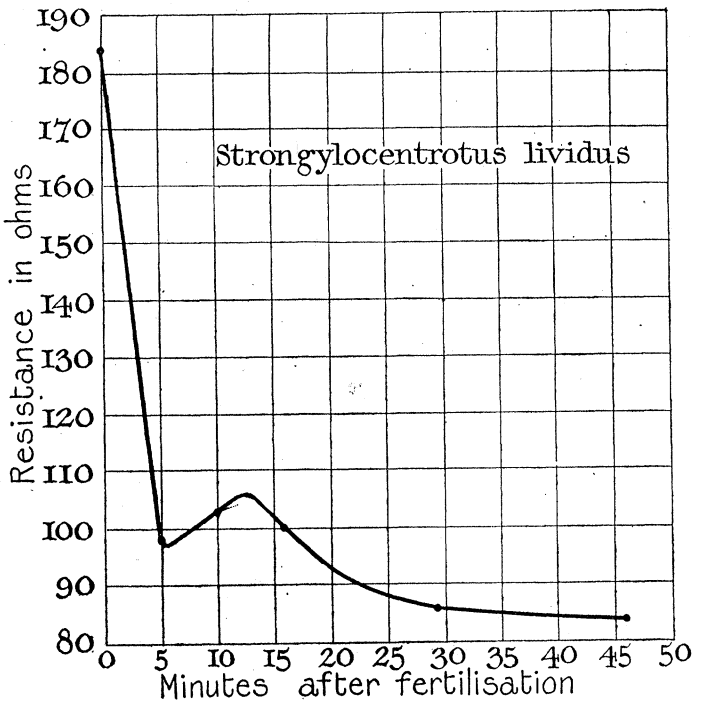

FIG. 3.—Illustrating the effect of fertilisation on the resistance of eggs of *Strongylocentrotus lividus*.

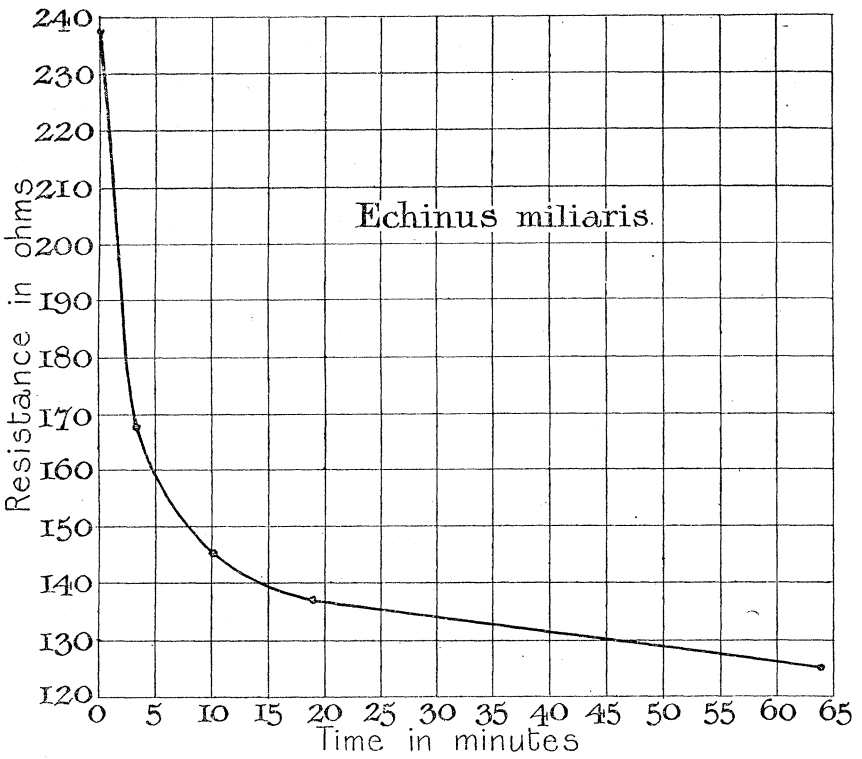

FIG. 4.—Illustrating the effect of fertilisation on the resistance of the eggs of *Echinus miliaris*.

Normal Fertilisation.

As the fall in electrical resistance which follows on normal fertilisation has been already established, it need not detain us long. The curves (figs. 3 and 4) show the general course of the change. The resistance in ohms is plotted against time. The table illustrates the values observed in the case of the eggs of one species. It is obvious that these curves do not support LILLIE'S contention that the increased permeability of the egg, due to fertilisation, lasts only for some 15 minutes after the entrance of the sperm, and then returns to that of the unfertilised egg, if we assume, as LILLIE does, that the electrical conductivity of the egg is a direct measure of its permeability.

The results of a very large number of experiments still leave considerable doubt as to the electrical resistance of the eggs between 3 and 10 minutes after fertilisation. In certain cases there seems to be fair evidence of a slight increase of resistance, which is always much less than the initial decrease, and which is followed by a steady and permanent decrease. On the other hand, other experiments do not exhibit such a break in the conductivity curve, and the resistance of the eggs seems to fall continuously from the moment of fertilisation. It is doubtful whether the experimental method adopted is capable of investigating this point further, owing to the impossibility of obtaining a large number of readings during the first 15 minutes of development.

It is therefore perhaps judicious to limit the conclusions to the following cardinal points :—(*a*) The resistance of eggs is very considerably decreased after fertilisation ; (*b*) During the first hour of development (*i.e.* including the period of the first cleavage division) the resistance of the egg never returns to the value of the same eggs before fertilisation.*

The extent of the rise in the electrical conductivity of eggs after fertilisation differs considerably in different genera of Echinoids. The values for the three Neapolitan genera which were investigated were roughly as follows :—

	Per cent.
Strongylocentrotus lividus	36
Sphærechinus granularis	23
Arbacia pustulosa	15

If we assume that these figures give an approximate estimate of the degree of permeability change which is necessary to allow the eggs to develop, it is interesting to note that, of the three genera, *Arbacia* is by far the most susceptible to parthenogenetic agents ; in other words, it is much more easy to induce the eggs of this genus to form fertilisation membranes than is the case with the eggs of *Sphærechinus* or *Strongylocentrotus*. The artificial parthenogenesis of these two genera has not, unfortunately, been extensively investigated at Naples. Lyon (37), however, mentions that the optimum concentration of hypertonic sea-water which effects the initiation of development of *Sphærechinus* was 100 c.c. sea-water + 10 c.c. $2\frac{1}{2}$ mol. potassium chloride, whereas the optimum concentration for *Strongylocentrotus* was 100 c.c. sea-water + 14 c.c. $2\frac{1}{2}$ mol. potassium chloride. These observations are, however, based upon a comparatively small number of experiments, and, in view of the variability of the eggs of different females to similar reagents, it is perhaps unwise to lay too much emphasis upon Lyon's quantitative results. It must also be remembered that the permeability of the eggs of the three genera may alter at a different rate when exposed to the same treatment. For example, a solution which will cause a change of 10 per cent. in the electrical conductivity of *Strongylocentrotus* eggs, might only cause a change of 5 per cent. in that of the eggs of *Sphærechinus*, and *vice versa*. Later it will be seen that this possibility cannot be left out of account.

* A few of my earlier experiments led me to a different conclusion ('Journ. M.B.A.,' vol. 10, p. 50, 1913), but subsequent work with more satisfactory material has convinced me that the experiments quoted were unreliable.

TABLE II.—Decrease in the Resistance of Fertilised Eggs 30 mins. after Fertilisation (expressed as Percentages of the Resistances of the Unfertilised Eggs).

Echinus miliaris.	*Echinus acutus.*	*Arbacia.*	*Sphærechinus.*	*Strongylocentrotus.*	*Asterias.*
43·3	44·2	22·9	27·3	27·3	16·0
43·7	—	30·8	24·8	24·8	23·1
54·5	—	21·3	28·0	28·0	27·3
46·3	—	20·5	—	—	32·1

ARTIFICIAL PARTHENOGENESIS.

LOEB has shown that the eggs of many genera of Echinoids can be made to develop parthenogenetically in the following different ways :—

A. The unfertilised eggs are immersed in 50 c.c. sea-water + 3 c.c. N/10 butyric acid for $1\frac{1}{2}$–3 minutes. They are then transferred to normal or slightly hyper-alkaline sea-water for about 10 minutes, during which period perfectly normal "fertilisation" membranes are formed round the eggs. These eggs are then placed in 50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ mol. sodium chloride solution. In this solution the eggs are left for about one hour. After treatment with hypertonic sea-water the eggs are transferred to normal sea-water, whereupon a variable proportion develop into healthy plutei, which may eventually metamorphose in the normal manner.

B. By placing unfertilised eggs in a solution of saponin in sea-water. In this solution "fertilisation" membranes are formed, and unless the eggs are then transferred to fresh sea-water total cytolysis occurs. If, however, the eggs are removed to sea-water after membrane formation, and are subsequently treated with a suitable hypertonic solution, normal larvæ may be obtained.

C. By treating unfertilised eggs with a relatively strong hypertonic solution, and subsequent transference to normal sea-water.

These methods considered as physical processes are sufficiently diverse, yet they all agree in producing a fall in the electrical resistance of the eggs. It is clear, therefore, that the cycle of development brought about by artificial means is also accompanied by an increase in electrical conductivity.

MCLENDON (38) gives the figures of four experiments made with the eggs of *Toxopneustes variegatus*. Treatment with acetic acid was found to increase the conductivity of the eggs in sea-water 9 per cent., 6·4 per cent., 5 per cent., and 7 per cent.

Table III shows the fall in electrical resistance of the eggs of *Arbacia* due to treatment with butyric acid.

Butyric Acid.

TABLE III.—Resistance of *Arbacia* Eggs in Sea-water.

I. Before treatment with butyric acid. Ra.	II. Five minutes after treatment with butyric acid. Rb.	III. Ra – Rb.	IV. $\frac{(Ra - Rb)\ 100}{Ra}$.
127	95	32	25·2
105	79	26	24·8
105	85	20	19·0
83	73	10	12·0
73	66·5	6·5	8·9
112	93	19	16·9
118	93	25	21·1
120	99	21	17·5
85	71·5	13·5	15·8
128	100	28	21·9
117	83	34	29·1
93	78	15	16·1
Average value of $\frac{(Ra - Rb)\ 100}{Ra}$ = 19.			

After each of these experiments a large percentage of the eggs began to develop.

Unless the hydroxyl-ion concentration of the sea-water (or van't Hoff's solution) is above a certain value, the extent of the conductivity change does not approximate to that produced in the egg by the entrance of a spermatozoon.

TABLE IV.—Transference of *Sphærechinus* Eggs from Sea-water to Butyric Acid Solution, and thence to Alkaline Sea-water (50 c.c. s-w + 0·25 c.c. N/10 NaOH).

Resistance of unfertilised eggs. R_U.	Resistance of eggs after butyric acid. R_B in 50 c.c. s-w + 0·25 c.c. N/10 NaOH.	Time in minutes after transference to alkaline sea-water.	$R_U - R_B$.	$\frac{(R_U - R_B)\ 100}{R_U}$.
83	65	15	18	21·7
101	68	15	33	32·7
98	84	14	14	14·3
88	74	10	14	16·0
125	95	11	30	24·0
Average increase in conductivity 21·7 per cent.				

TABLE V.—Transference of *Sphærechinus* Eggs from Neutral van't Hoff's Solution to Butyric Acid, and thence to Neutral van't Hoff's Solution.

R_U.	R_B.	Time.	$R_U - R_B$.	$\frac{(R_U - R_B)\ 100}{R_U}$.
100	93	—	7	7
105	103	—	2	1·9
99	92	—	7	7·1
65	60·5	—	4·5	6·9
118	104	—	14	11·9
124	116·5	—	7·5	6·0
Average increase in conductivity 6·8 per cent.				

TABLE VI.—Transference of *Sphærechinus* Eggs from Neutral van't Hoff's Solution to Butyric Acid, and thence to Acid van't Hoff's Solution (50 c.c. van't Hoff +0·25 N/10 HCl).

R_U.	R_B.	Time.	$R_U - R_B$.	$\frac{(R_U - R_B)\ 100}{R_U}$.
120	113	—	7	5·8
99	93	—	6	6·1
133	125	—	8	6·0
Average increase in conductivity 6 per cent.				

In all the above experiments the conditions were kept as uniform as possible; the length of the exposure of the eggs to the butyric acid solution did not vary more than a few seconds. The variation in the quantitative changes are, however, not surprising in view of the great variability which is always observed in the reaction of eggs to parthenogenetic reagents.

Repeated experiments showed that the resistance of the eggs did not change whilst actually in the butyric acid solution.

The results of the experiments are in entire agreement with the observation of LOEB (32) that unless the hydroxyl-ion concentration of the sea-water (or van't Hoff's solution) to which the eggs are transferred after a treatment with butyric acid exceeds a certain value the eggs do not develop but return to the "resting" condition.

Exposure to a solution of saponin in sea-water similarly decreases the electrical resistance.

Saponin.

TABLE VII.

Temp.	R_U.	Resistance of eggs in saponin solution. R_S.	Time after addition of saponin solution.	Strength of saponin solution.	$R_U - R_S$.	$\frac{(R_U - R_S)\ 100}{R_U}$.	Reaction of saponin solution to neutral red.
				1. *Arbacia.*			
°			mins.	per cent.			
18	82	64	17	$\frac{1}{8}$	18	21·9	Same as sea-water.
18	70	58	13	$\frac{1}{8}$	12	17·1	Ditto.
18	78	62·5	15·5	$\frac{1}{8}$	15·5	19·9	Ditto.
18	82	66	15·0	$\frac{1}{8}$	16	18·3	Ditto.
Membranes formed in control after about 15 mins. Cytolysis after about 20 mins.							
				2. *Sphærechinus.*			
°				per cent.			
18	82	76	14	$\frac{1}{8}$	6	7·3	Same as sea-water.
		69	28		13	15·7	
18	103	101	19	$\frac{1}{8}$	2	1·2	Ditto.
		85	36		18	17·5	
18	115	95	10	$\frac{1}{8}$	20	17·4	More alkaline than the above, 50 c.c. saponin solution + ·75 c.c. N/10 NaOH.
		86	30		29	25·2	
18	93·5	88·5	18	$\frac{1}{8}$	5·0	5·3	Saponin solution made acid to phenolpthalein.
		88·0	30		13·5	14·4	
18	115·5	109	10	$\frac{1}{8}$	6·5	5·6	Ditto.
		98	20		17·5	15·2	
13·9	112	96·5	8	$\frac{1}{4}$	15·5	12·9	Same as sea-water.
		75	14		37	33·0	

In $\frac{1}{8}$ per cent. saponin membranes formed in control after 15–20 mins. Cytolysis at about 30 mins.
In $\frac{1}{4}$ „ „ „ „ 10 „ „ „ 12–17 „
The addition of saponin to sea-water has no effect upon the conductivity of the latter.

Hypertonic Solution.

Numerous authors have shown that unfertilised eggs can be made to develop by an exposure to hypertonic solutions of sea-water and subsequent transference to

normal sea-water. The effect of such treatment upon the electrical conductivity of the unfertilised eggs of *Sphærechinus* is shown in the following Tables :—

TABLE VIII.—*Sphærechinus.*

Temperature.	R_U.	R_H (resistance of eggs after hypertonic treatment).	Number of minutes eggs were exposed to hypertonic solution.	$R_U - R_H$.	$\frac{(R_U - R_H)\,100}{R_U}$.
13·3°	138	105	60	32	23·2
14·3°	121	103	45	18	14·9

That the conductivity increases while the eggs are in the hypertonic solution is shown by the following experiment. Eggs were put into the hypertonic solution and their resistance measured at intervals of 10 minutes :—

TABLE IX.—*Sphærechinus.*

Time after addition of hypertonic solution.	Resistance of eggs in ohms.
10	79·5
20	71·5
30	69
45	68

For a reliable investigation of the effect of hypertonic solutions *after* membrane formation, two essential conditions are necessary. (1) All the eggs must respond to the treatment with butyric acid, otherwise the conductivity of the eggs will be affected by the action of the hypertonic solution upon those eggs which are still in the resting stage. (2) After the treatment with the hypertonic solution, a large percentage of the eggs must develop normally : otherwise there is no certainty that the effect of the hypertonic solution is a normal one.

As explained above, it is fairly simple to satisfy the first condition ; in fact, the eggs of most females of *Sphærechinus* give 100 per cent. membranes after exposure to a butyric acid solution for $1\frac{1}{2}$–3 minutes. On the other hand, it is the exception to obtain a large number of larvæ from these eggs after a subsequent treatment with a hypertonic solution. A large number of experiments were performed in which the number of eggs which developed was only about 20 per cent. In the following experiment, however, practically all the eggs developed into blastulæ, the large majority of which were perfectly healthy and normal.

	Resistance of unfertilised eggs of *Sphærechinus*	99·0 ohms.
	Time of exposure to butyric acid solution	2 minutes.
	Resistance of eggs in "alkaline" sea-water after 15 minutes . .	88·0 ohms.
	,, ,, ,, ,, 25 ,, . .	86·0 ,,
	,, ,, ,, ,, 35 ,, . .	81·5 ,,
	,, ,, ,, ,, 46 ,, . .	78·5 ,,
	,, ,, ,, ,, 66 ,, . .	70·0 ,,
4.12 P.M.	,, ,, ,, ,, 77 ,, . .	66·6 ,,
4.13 ,,	Eggs placed in hypertonic sea-water for 52 minutes	—
5.5 ,,	,, ,, pure sea-water	—
5.12 ,,	Resistance of the eggs in sea-water	66·5 ohms.
5.22 ,,	,, ,, ,, ...	64·0 ,,

It seems clear, therefore, that after membrane formation the hypertonic solution does not alter the conductivity of the eggs.

Summary of the Effects of Hypertonic Solutions upon the Conductivity of Unfertilised Eggs.

1. The conductivity of normal unfertilised eggs is distinctly increased by hypertonic sea-water, and this increase takes place while the eggs are actually in the hypertonic solution.

2. After artificial membrane formation, the conductivity of unfertilised eggs is unaltered by treatment with hypertonic solutions.

PART II.

Origin of the Variation in Resistance.

I propose now to present evidence that the variations in the electrical resistance described in Part I are due largely to changes in the surface of the eggs. Changes in the interior of the egg cannot, of course, be eliminated, but in one special case it is possible to prove that the change does not affect the electrical resistance; namely, when the alkalinity of the egg contents is increased. According to McLendon the decreased resistance after fertilisation is due to an increased alkalinity of the egg contents. This can be directly tested in the case of the eggs of *Arbacia*, since the red pigment which they normally contain serves as an indicator. When the pigment is extracted the colour is found to change to dark purple when precipitated by alkali. In normal eggs exposed to exceedingly dilute ammonia the pigment changes to purple and we may, therefore, conclude with certainty that the egg contents are alkaline. In spite of this, as Table X shows, the electrical resistance is unchanged.

It is, therefore, necessary to abandon in its simplest form McLendon's hypothesis that changes in the electrical conductivity of the eggs are due to changes in the alkalinity of their contents.

TABLE X.

Eggs.	Resistance in ohms of eggs in		
	I. Normal sea-water.	II. Sea-water + a trace of NH_4OH. (Egg interior alkaline to indicators.)	III. Sea-water after 10 minutes in NH_4OH.
Sphærechinus . . .	83	80	—
Arbacia	118	—	115
„	71·3	71	70
„	78·5	78	—
„	92	92·5	92
„	66·5	73	67
„	113·5	114	112
„	55	56·5	55

It is known from the work of STEWART, HÖBER (23), and others that the high electrical resistance of red blood corpuscles is due to the impermeability of their surface to ions. The impermeability is broken down by hæmolysins (STEWART, 52 and 53).

In the following Table I put side by side with hæmolysins a list of known parthenogenetic agents. The parallelism is obvious and suggests that the electrical resistance of an egg, like that of the corpuscles, is controlled by the surface.

TABLE XI.

Hæmolytic Agents (after STEWART).	*Parthenogenetic Agents.*
1. Mechanical. Pressure, trituration, shaking.	1. Mechanical. Shaking. MATTHEWS (42).
2. Physical. Freezing and thawing. Heat. Condenser discharges. Water. Drying and subsequent exposure to salt solutions.	2. Physical. Low temperature. GREELY (15). Induction shocks. MCLENDON (38). Hypertonic solutions. LOEB (32). Hypotonic solutions. MCLENDON, and GLASER (12).
3. Chemical. Saponin. Bile salts. Ether. Chloroform. Acids. Alkalies.	3. Chemical. Saponin. Bile salts. Acids. Alkalies.
4. Biological agents. Specific hæmolysins. Bacterial hæmolysins.	4. Biological agents. Spermatozoa. Foreign spermatozoa. Egg extractives. Infusoria. GLASER (12).

The problem may be attacked indirectly. MINES (47) has shown that trivalent ions affect the heart beat of Elasmobranchs by altering the permeability of the surface of the fibres to other electrolytes. I was led by this to investigate the effect of such ions upon the conductivity of eggs.

THE EFFECT OF "POLARISING" IONS UPON THE ELECTRICAL RESISTANCE OF THE EGGS.

According to MINES (47) the relation of ions to the membranes of the Elasmobranch heart permits of their classification into three groups :—

I. "Nomadic" ions, *e.g.*, $Na^{\cdot}$, $K^{\cdot}$, $H^{\cdot}$, Cl', OH'.
II. "Combining" ions, *e.g.*, $Ca^{\cdot\cdot}$, $Sr^{\cdot\cdot}$, $Ba^{\cdot\cdot}$.
III. "Polarising" ions, *e.g.*, $H^{\cdot}$, OH', $Mg^{\cdot\cdot}$, $Ce^{\cdot\cdot\cdot}$, $La^{\cdot\cdot\cdot}$, Cit''', and other trivalent ions.

"The nomadic ions produce their effects by passing from one region to another, carrying their charges, and so setting up differences in potential. The combining ions are those which form chemical compounds with some constituent of the heart muscle. The polarising ions modify the electric charge, and thus the ionic permeability of certain surfaces or membranes in the heart muscle, thus affecting in a differential manner the passage through these membranes of the nomadic ions."

If, therefore, changes in the electrical conductivity of the eggs be due to changes in the permeability of their surface membranes to ions, then the electrical conductivity should be affected by exposing the eggs to substances which are known to be capable of affecting the permeability of artificial membranes; in other words to such substances as cerium chloride, sodium citrate, etc.

The results obtained from a large series of experiments have given very definite results, but it must be confessed that their complete interpretation is difficult. The energetics of diffusion through artificial membranes is far from clear (see Appendix II). It should, however, be clearly understood that definite experimental proof exists to show that the permeability of a membrane is influenced by the presence of polarising ions. The reason for such alterations is the only point that remains doubtful.

As far as I am aware the only other observations on the effect of trivalent ions upon the dynamics of living matter are those of MINES (47), OSTERHOUT (49), and Miss DALE (6A).

As mentioned above MINES has brought forward strong evidence to show that trivalent ions affect the heart muscles of Elasmobranchs and of other animals by virtue of their electrical charges, and he concludes that their effect is to change the permeability of the surface membranes to other electrolytes by altering the degree of aggregation of the surface colloids.

OSTERHOUT (49) has shown that less than 0·01 mol. lanthanum nitrate decreases the electrical conductivity of certain vegetable tissues by 26·5 per cent., and he refers

the change to a decrease in the permeability of the protoplasmic surfaces to other electrolytes.

Miss DALE has advanced evidence in favour of extending MINES' conclusion to the locomotory behaviour of *Paramœcium*.

TABLE XII.—The Effect of Trivalent (Positive) Ions upon the Conductivity of Unfertilised Eggs.

Eggs.	Strength of trivalent ion.	Decrease in conductivity.	After.
		per cent.	mins.
Strongylocentrotus	0·0001 mol. $CeCl_3$	26·7	5
Sphærechinus	0·0001 „	20·0	5
„	0·0001 „	32·0	3
„	0·0001 „	22·4	4
„	0·0001 „	25·4	6
„	0·0002 „	47·0	4
Echinus acutus	0·0008 mol. $La(NO_3)_3$	22·0	3
„ „	0·0008 „ „	16·6	3
„ „	0·0012 „ „	17·6	3
„ „	0·00001 mol. $Nd(NO_3)_3$	10·0	5
„ „	0·00001 „ „	18·6	3
„ „	0·00001 „ „	26·7	4
„ „	0·00001 „ „	9·1	3

In other words, the presence of a very low concentration of simple trivalent positive ions greatly decreases the conductivity of the unfertilised eggs. This primary decrease in conductivity is, however, not a permanent one. Figs. 5 and 6 show the general effect of these ions upon the eggs; it will be observed that the resistance after the initial change gradually falls again, and may ultimately reach the same value as that of the normal eggs. This secondary fall in resistance is not due to the removal of the trivalent ions from the sphere of action, because the addition of more cerium or lanthanum does not affect the conductivity of the eggs. The phenomenon is at present inexplicable, but it is not altogether isolated. The same series of events is described by OSTERHOUT (51) as being the effect of anæsthetics upon the resistance of plant tissues.

Fig. 7 shows the effect of 1 per cent. ether in sea-water upon the resistance of *Laminaria* tissue, and the similarity to figs. 5 and 6 is obvious.

If the concentration of ether is increased, then the maximum increase in resistance is more rapidly attained (being of the same value as with 1 per cent. ether), the subsequent decrease in resistance is correspondingly rapid, and equilibrium is only attained at the death value of the tissue (*cf.* fig. 8). Also, whereas the primary increase in resistance due to the anæsthetic is reversible on removal of the latter, the subsequent fall produced by stronger solutions below the normal resistance of the tissue is quite irreversible (OSTERHOUT).

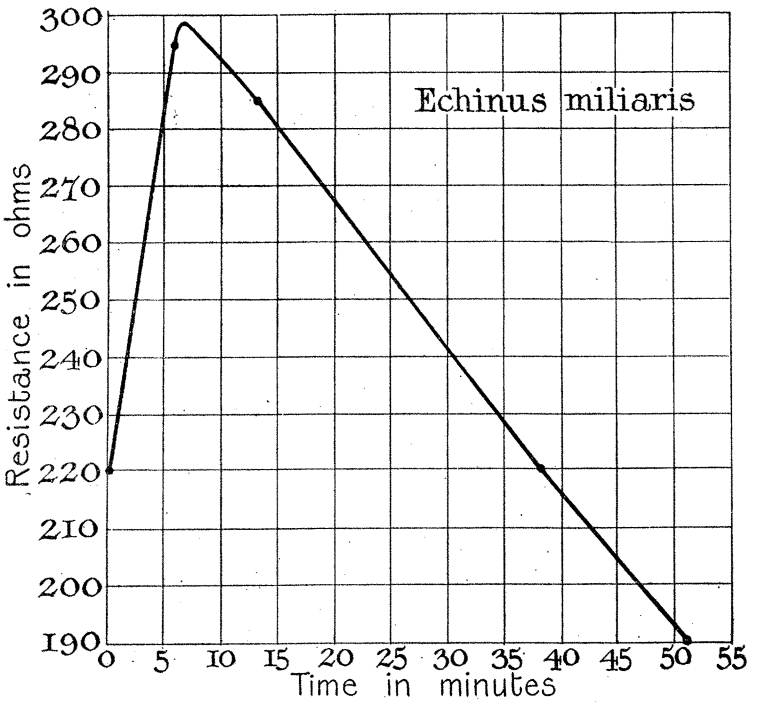

FIG. 5.—Illustrating the effect of trivalent positive ions upon the resistance of unfertilised eggs of *Echinus miliaris*. Ce··· ·0001 mol.

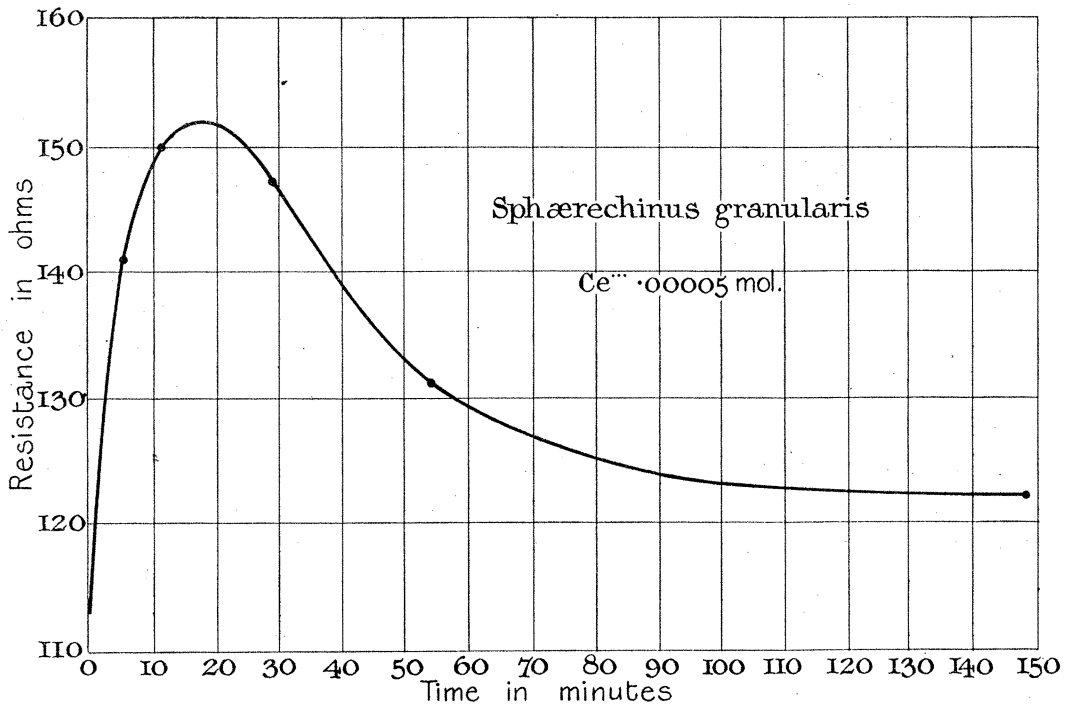

FIG. 6.—Illustrating the effect of trivalent positive ions upon the resistance of unfertilised eggs of *Sphærechinus granularis*.

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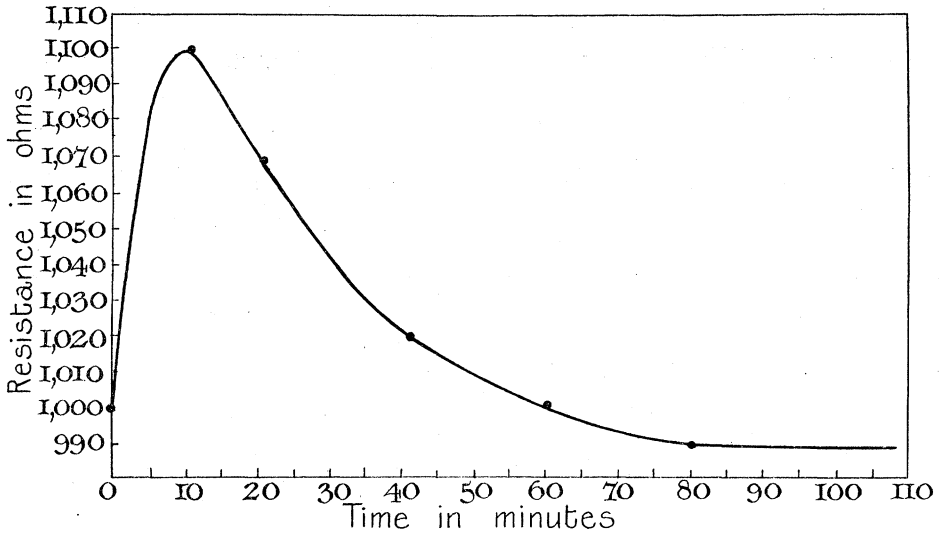

FIG. 7.—Changes in resistance of *Laminaria* tissue produced by 1 per cent. ether in sea-water. (From OSTERHOUT'S data.)

Again, MINES (47) found that after a short period of rest the heart of Pecten may begin to beat strongly in a perfusion fluid containing 0·0002 mol. $Ce^{\cdots}$ (see MINES, p. 501). A. TRÖNDLE (53A) found that the effect of light upon the permeability of certain plant cells is not a progressive change, and the data of this author provide another analogy to the effect of $Ce^{\cdots}$ upon the electrical conductivity of echinoderm eggs.

That the electrical resistance of tissues is increased during anæsthetisation has also been shown by ALCOCK (1). He found that the resistance of the sciatic nerve of the frog is increased by 51 per cent. during anæsthetisation in 50 per cent. ether vapour, and that the change is completely reversed on removal of the anæsthetic.

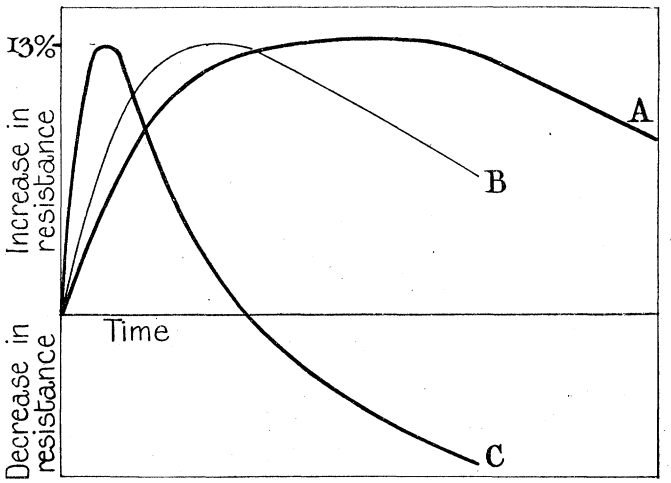

FIG. 8.—Diagrammatic illustration of the effect of various concentrations of anæsthetics upon the resistance of *Laminaria* tissue. (From OSTERHOUT'S data.)

A, with low concentration of anæsthetic; B, with medium concentration of anæsthetic; C, with high concentration of anæsthetic.

The similarity of the effects of anæsthetics and of trivalent positive ions upon the electrical resistance of living tissues is possibly of extreme importance, but further discussion is postponed.

The Effect of Trivalent Negative Ions upon the Conductivity of Unfertilised Eggs.

In order to investigate the effect of such ions upon the eggs, a 20-per-cent. solution of sodium citrate was used.* The following Table shows the results of some of the experiments :—

Table XIII.

Concentration of sodium citrate.	Decrease in resistance of the unfertilised eggs.
mol.	
0·07	8·7 per cent. in 20 mins.
0·14	12·4 „ 16 „
0·175	18·8 „ 13 „

The solutions used in the experiments were made by adding a known volume of a 20-per-cent. solution of sodium citrate to a known volume of sea-water.

As shown by fig. 9, the action of citrate solutions upon the conductivity of the eggs is a continuous process of progressive increase during the first half-hour.

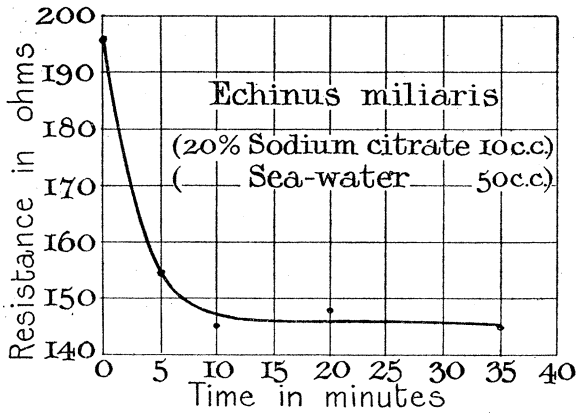

Fig. 9.—Illustrating the effect of trivalent negative ions on the resistance of unfertilised eggs of *Echinus miliaris*.

* A solution of this strength has a conductivity somewhat below that of sea-water $\left(\frac{R\,Na_3Cit}{R\,s\text{-}w} = \frac{153}{149}\right)$. Its freezing point is also somewhat lower, but the difference is not sufficient to introduce purely osmotic effects when diluted with four times its bulk of sea-water. The freezing point of sea-water containing 0·175 mol. sodium citrate, prepared by the addition of the necessary amount of a 20-per-cent. solution of the salt, is only 0·08° C. below that of sea-water. That such a small difference in O.P. does not affect the conductivity of the eggs is readily shown by a comparison between the effects of a short exposure of the eggs to the citrate solution and that of a much longer exposure to the comparatively highly hypertonic solution used in other experiments.

Eventually equilibrium is attained, and remains unchanged as long as the solution in contact with the eggs remains the same.

From these results it is clear (1) that the electrical conductivity of the eggs is increased by the presence of trivalent negative ions in the surrounding sea-water; (2) that the complex trivalent negative ions have a much less powerful effect on the eggs than have the simple trivalent positive ions. Whereas the presence of only 0·0001 mol. cerium ions *increases* the resistance of the eggs about 20 per cent., a corresponding *decrease* in resistance by citranion is only produced by a solution of 0·2 mol. sodium citrate. In other words, the complex anion is 20,000 times less powerful than the simple kation.

It should be noticed that the decrease in the resistances of the eggs produced by sodium citrate and by hypertonic solutions are not altogether identical phenomena. In the presence of sodium citrate the resistance of the eggs attains a definite equilibrium, which is completely reversible in certain cases. The decrease in the resistance of eggs which is due to treatment with hypertonic solutions is not reversible, and there is no evidence of any equilibrium being attained—in fact, the injurious effect on the eggs of prolonged treatment with such solutions suggests that no equilibrium is attained until the cells are dead.

Antagonistic Effects of Positive and Negative Trivalent Ions.

If the action of the trivalent positive ions is produced owing to the production of a positive charge on some constituent of the egg, then the effect of these ions should be removed by exposing these eggs to a solution containing free hydroxyl ions. The following experiments show this to be the case.

Table XIV.

Eggs.	Resistance of normal unfertilised eggs in sea-water.	Resistance of the same eggs in sea-water containing 0·0001 mol. $CeCl_3$.	Resistance of the same eggs in sea-water after exposure of 10 mins. to slightly hyper-alkaline sea-water.
Strongylocentrotus .	187	237	180
Sphærechinus . . .	130	156	125
„ . . .	125	153	124

That the increase in the conductivity of the eggs in the alkaline sea-water is due to a removal of the effect of the cerium ions, and not to the secondary effects of the cerium ions themselves, is shown by the following facts:—

1. The increase of the conductivity in the alkaline solution is much too rapid to be accounted for by the secondary effect of the cerium ions.

Eggs from same Female *Sphærechinus* in Experiments A and B.

A.	Resistance of normal unfertilised eggs in sea-water	125	ohms.	
	Resistance of same unfertilised eggs in 0·0001 Ce··· . . .	153	,,	after 4 mins.
	Resistance of same unfertilised eggs in alkaline sea-water (50 c.c. s-w + 0·75 c.c. N/10 NaOH)	124	,,	,, 12 ,,
B.	Resistance of normal unfertilised eggs in sea-water	112·5	,,	
	,, same ,, ,, 0·0001 Ce··· . . .	141·0	,,	,, 6* ,,
	,, ,, ,, ,, 0·0001 Ce··· . . .	150·0	,,	,, 12* ,,
	,, ,, ,, ,, 0·0001 Ce··· . . .	147·0	,,	,, 29* ,,

* After each reading the Ce··· solution was changed.

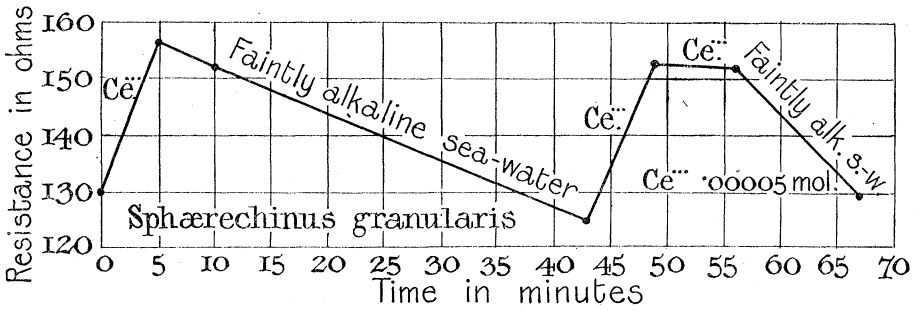

FIG. 10.—Illustrating the antagonistic effects of alkaline sea-water and trivalent positive ions.

2. It has been shown that the secondary increase in the conductivity of eggs in cerium solutions is unchanged by the presence of a fresh cerium solution round the eggs. If, however, the primary effect of a cerium solution is removed by alkali, then, on addition of a fresh cerium solution, the conductivity of the eggs is again decreased. The complete antagonism between cerium ions and alkali is shown by the following experiment :—

Resistance of normal unfertilised eggs (*Sphærechinus*) in sea-water	130	ohms.		
Resistance of same unfertilised eggs (*Sphærechinus*) in—				
0·0001 Ce···	156	,,	after 5 mins.	in sol.
Normal sea-water	152	,,	,, 5	,,
Alkaline sea-water (50 c.c. + 0·5 c.c. N/10 NaOH)	125	,,	,, 33	,,
0·0001 Ce···	152·5	,,	,, 6	,,
Alkaline sea-water (50 c.c. + 0·75 c.c. N/10 NaOH)	129·5	,,	,, 19	,,

In normal sea-water recovery from the effects of trivalent positive ions is much slower and not always so complete as that in alkaline sea-water.

A further proof that the effect of cerium ions upon the conductivity of the eggs is due to their electrical charge is furnished by the antagonistic effect between these ions and the trivalent negative ions of sodium citrate.

1. Resistance of normal unfertilised eggs of *Sphærechinus* in sea-water	117	ohms.			
Resistance of same unfertilised eggs of *Sphærechinus* in—					
0·0002 M Ce···	172	,,	after	4	mins.
0·14 M Cit'''	105	,,	,,	2	,,
0·0002 M Ce···	125	,,	,,	4	,,
2. Resistance of normal unfertilised eggs of *Sphærechinus* in sea-water	115	,,			
Resistance of same unfertilised eggs of *Sphærechinus* in—					
0·14 M Cit'''	103	,,	,,	7	,,
0·14 M Cit'''	95·5	,,	,,	14·5	,,
0·0001 M Ce···	115	,,	,,	5	,,
0·14 M Cit'''	102	,,	,,	11	,,

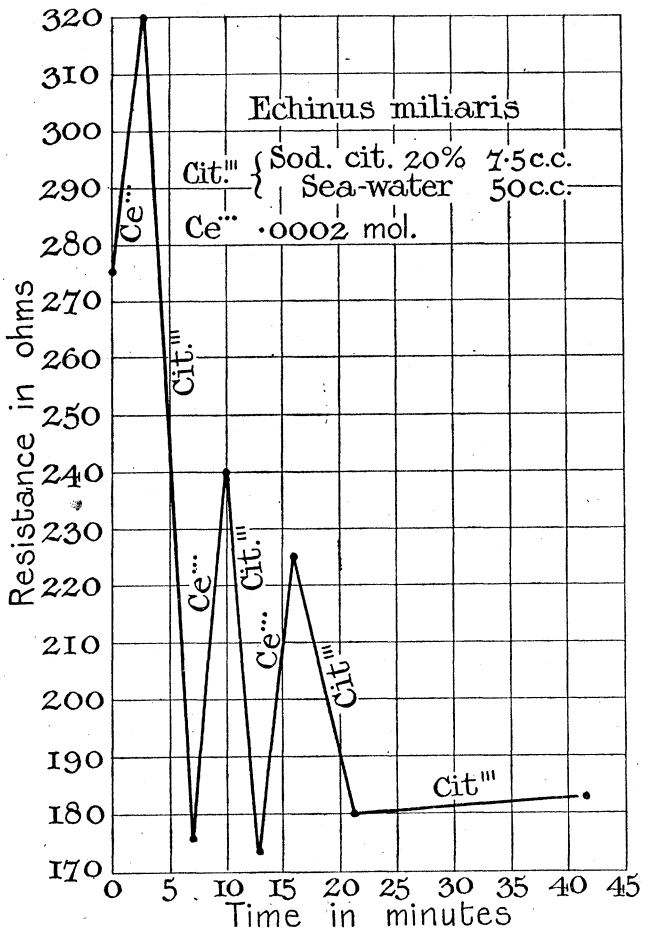

FIG. 11.—Illustrating the antagonistic effects of trivalent positive and negative ions on the resistance of the unfertilised eggs of *Echinus miliaris*.

Before commenting on the above experiments, it is necessary to describe the effect of a prolonged treatment of the eggs with a solution of sodium citrate or alkaline van't Hoff's solution. If unfertilised eggs are treated with an alkaline van't Hoff's solution for about an hour, the conductivity of the eggs is practically unaffected by the presence of cerium ions.

Resistance of normal unfertilised eggs of *Spærechinus* in neutral van't Hoff's solution	145 ohms.		
Resistance of same unfertilised eggs of *Sphærechinus* in—			
Alkaline van't Hoff's (50 c.c. V.H. + 0·25 c.c. N/10 NaOH) solution	140 ,,	after 45 mins.	
,, ,, ,, ,, ,, ,,	130 ,,	,, 58	,,
0·0002 Ce···	134 ,,	,, 15	,,
0·0002 Ce···	132 ,,	,, 25	,,
Five mins. after addition of sperm	88 ,,		

A prolonged treatment with citrate solutions also renders these eggs less sensitive to cerium ions.

TABLE XV.—*Sphærechinus* Eggs.

Previous treatment of unfertilised eggs before return to normal sea-water.	Effect of cerium on the conductivity of the eggs.		
	Strength of cerium solution.	Decrease in the conductivity.	After x minutes.
		per cent.	
Nil	0·0001 mol.	22·4	4
In 0·14 mol. Cit‴ for 12·5 mins.	0·0001 ,,	20·4	5
Nil	0·0002 ,,	47·0	4
In 0·14 mol. Cit‴ for 42 mins.	0·0002 ,,	21·8	8
In alkaline van't Hoff's solution for 60 mins.	0·0002 ,,	4·7	15
Fertilisation	0	33·3	5

These experiments show that the effect of cerium upon unfertilised eggs depends upon the previous treatment to which the eggs have been subjected. It is also clear that the effect of negative ions upon the egg is not altogether reversible by positive ions, if the exposure to the first treatment has been prolonged. The figures given in the above Table are the maximum effects which were observed—later readings always showed a slight decrease in the effect of the cerium ions.

The same phenomena are exhibited by the following experiments with eggs of *Strongylocentrotus* (Table XVI). The eggs of *Echinus* appear, however, to be less sensitive to a prolonged treatment with sodium citrate solutions, as complete recovery usually takes place if trivalent positive ions are applied to eggs which have been exposed to sodium citrate for one hour.

Experiments 1–4 in Table XVI require but little comment, as they show very clearly that the effect of the trivalent positive ions is some inverse function of the time which the eggs have been exposed to a solution containing negative trivalent ions.

Experiment 5, however, exhibited a remarkable phenomenon and is therefore quoted below in full.

After the experiment the eggs were transferred to abundance of clean sea-water and all developed as far as the first mitotic figure and resembled eggs which have been subjected to a butyric acid treatment. Only a few eggs cleaved and these were

TABLE XVI.—*Strongylocentrotus* Eggs.

No. of experiment.	Previous treatment of unfertilised eggs.	Effect of cerium upon conductivity.		
		Strength of cerium solution.	Decrease in the conductivity.	After x minutes.
			per cent.	
1	Nil	0·0001 mol.	26·7	5
2	In 0·14 mol. Cit‴ for 25 mins.	0·0002 ,,	12·8	10*
3	In 0·7 mol. Cit‴ for 40 mins.	0·0002 ,,	−1·3	10 and 20*
4	In alkaline van't Hoff's solution for 66 mins.	0·0001 ,,	−2·9	6
			−9·7	11
5	In alkaline van't Hoff's solution for 57 mins.	0·0001 ,,	−35·3	5

* Subsequent readings showed that the first readings give the maximum effect of the cerium ions.

Details of Experiment 5 *of Table XVI.*

Resistance of normal unfertilised eggs in neutral van't Hoff's solution . 168 ohms.

Resistance of same unfertilised eggs in—

Alkaline van't Hoff's solution (50 c.c. + 25 c.c. N/10 NaOH)	158 ,,	after	3 mins.
,, ,, ,, ,, ,,	158 ,,	,,	19 ,,
,, ,, ,, ,, ,,	155 ,,	,,	39 ,,
,, ,, ,, ,, ,,	150 ,,	,,	57 ,,

Resistance of same unfertilised eggs in—

0·0001 Ce···	108 ,,	,,	5 ,,
,,	97·5 ,,	,,	13 ,,

all irregular. Repetition of the experiment with eggs from other females did not give similar results and only very few eggs showed signs of development. It is probable that the sudden fall in resistance of the eggs on removal to the cerium solution was quite independent of the cerium ions, and was entirely due to the treatment with the alkaline solution, but the sudden decrease in the resistance is at present inexplicable.

That the effect of sodium citrate upon the conductivity is, within certain limits, completely reversible is shown by the following experiment with the eggs of *Echinus acutus*.

P.M.		Ohms.
2.54	Resistance of unfertilised eggs in sea-water	196
2.55	Solution changed to 10 c.c. sea-water + 2 c.c. 20 per cent. sodium citrate.	
3.0	Resistance of eggs in solution	154
3.6	,, ,, ,,	145
3.15	,, ,, ,,	148
3.30	,, ,, ,,	145·5
3.31	Solution changed to normal sea-water.	
4.13	Resistance of eggs in sea-water	182
4.42	,, ,, ,,	188
4.43	Solution changed to 10 c.c. sea-water + 2 c.c. 20 per cent. sodium citrate.	
4.52	Resistance of eggs in solution	150
4.53	Solution changed to 0·0008 mol. La··· in sea-water.	
4.55	Resistance of eggs in solution	188

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A similar experiment was performed with the eggs of *Echinus miliaris*. In this case the recovery in sea-water after treatment with the citrate solution was less complete, so that, whereas the resistance of normal unfertilised eggs of *Echinus miliaris* was practically unchanged by treatment with lanthanum nitrate, after the partial recovery from the effects of the citrate due to the sea-water, the trivalent positive ions rapidly produced a return of the resistance of the eggs to its original value. When treated in this way the eggs are once more highly sensitive to the addition of the citrate solution.

Perhaps the most important conclusion to be drawn from these two experiments is that the effect of trivalent negative ions (*citranion*) *is slowly removed by normal sea-water, but is instantly removed by trivalent positive ions* (e.g., *lanthanum*).

On the whole, prolonged treatment with alkali or citrate is more completely reversible by trivalent positive ions in the case of *Echinus* than in *Strongylocentrotus* or *Sphærechinus*.

In the case of eggs belonging to most of the species used in these experiments, there is very fair agreement between the effects of different reagents on eggs belonging to different females. In the case of *Echinus miliaris*, however, the difference between different batches of eggs is very marked. For example, on June 10, 1914, the resistance of the unfertilised eggs of one female was increased by 34 per cent. in the presence of 0·00008 mol. $La^{\cdots}$. On the following day the eggs of another female, collected on the same day and in the same locality as that used on the previous day, were unaffected by the presence of a similar concentration of $La^{\cdots}$. It should, however, be mentioned that in the above case there is the possibility of a "physiological" difference between the eggs, due to the conditions under which they were obtained. On June 9 the eggs were obtained immediately the female had spawned,* and were therefore absolutely fresh. The eggs used on June 11 were obtained from a female which had been placed in an ice-chest on the previous evening, and which was found to have spawned on examination next morning; the eggs appeared to be in a perfectly healthy state when examined under the microscope.

A similar variation of the eggs belonging to different females was observed in the effects of hypertonic sea-water on the unfertilised eggs of both *Echinus acutus* and *Echinus miliaris*. In the large majority of experiments the effect of one hour's treatment with 50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ mol. NaCl was to increase the conductivity of unfertilised *Echinus acutus* eggs by about 20 per cent. On May 4, 1914, the conductivity of the eggs belonging to a particular female were practically unaffected by such treatment. A similar phenomenon was observed in the case of *Echinus miliaris*. On May 11, 1914, the effect of an hour's treatment with 50 c.c.

* Except in the two experiments mentioned above, the eggs used for experiments were obtained by transferring the ripe gonads to sea-water. In the above two cases, however, all the eggs were shed by the animals themselves.

sea-water + 10 c.c. $2\frac{1}{2}$ mol. NaCl increased the conductivity by 45·7 per cent., whereas on June 10 the eggs of two different females showed practically no change after treatment with 50 c.c. sea-water + 14 c.c. $2\frac{1}{2}$ mol. NaCl for one hour.

Such variation between the reactions of different batches of eggs is not surprising in view of a similar variation which is so frequently found by investigators of the effect of parthenogenetic reagents.* The detection of such differences by means of electrical conductivity determinations provides a quantitative method of determining such variations, which might conceivably be used for a more complete analysis not only of such variations, but also of the various phenomena of artificial parthenogenesis.

The material available for the present experiments has unfortunately not been convenient for such an investigation. The most convenient material would be provided by the eggs of some species which can be made to develop by treatment with a simple hypertonic solution, so that it would be possible to detect any correlation which may exist between the amount of electrical change with the percentage of eggs which develop. The eggs of *Arbacia* or *Strongylocentrotus* might satisfy these requirements, but the present investigations were not sufficiently advanced while the writer was at Naples.

A Comparison of the Effects of Trivalent Ions on the Unfertilised Eggs of Different Genera and Species.

In the following Tables are recorded the results of a number of experiments showing the effect of trivalent ions upon the eggs of *Sphærechinus*, *Strongylocentrotus*, *Echinus acutus*, and *Echinus miliaris*. The available data is somewhat limited, but there is, I think, sufficient evidence to show that the eggs of different genera and species show a distinct difference in their sensitivity to the presence of trivalent ions.

Table XVII.—Showing the Decrease in Resistance produced by 0·14 mol. Sodium Citrate in Sea-water.

Sphærechinus.	*Strongylocentrotus.*	*Echinus acutus.*	*Echinus miliaris.*
per cent.	per cent.	per cent.	per cent.
20·8	12·4	26·0	28·2 21·2 30·9
13·9	—	25·0	20·0 25·0 17·8
—	—	25·5	20·0 24·4 —
Average 17·4	12·4	25·5	23·2

* For example, see Shearer and Lloyd on the reaction of the eggs of *Echinus esculentus* to parthenogenetic agents ('Q.J.M.S.,' vol. 58, p. 523).

TABLE XVIII.—Showing the Increase in Resistance produced by 0·00008 mol. Cerium or Lanthanum.

Sphærechinus.	*Strongylocentrotus.*	*Echinus acutus.*	*Echinus miliaris.*
per cent. 20·0 (Ce···) 32·0 „ 22·4 „ 25·4 „	per cent. 26·7 (Ce···) — — —	per cent. 14·1 (Ce···) 16·6 (La···) 17·6 „ —	per cent. 13 34·1 (3) La··· 5 0 0 „ 26·5 0 0 „ 40·5 0 —
Average 24·7	26·7	16·1	—

As far as these experiments show, the order of sensitivity to trivalent negative ions is roughly the reverse of the order of sensitivity to trivalent positive ions.

Negative.	Positive.
{ *Echinus acutus.*	*Strongylocentrotus.*
{ *Echinus miliaris.*	*Sphærechinus.*
Sphærechinus.	{ *Echinus acutus.*
Strongylocentrotus.	{ *Echinus miliaris?*

THE EFFECT OF THE PRESENCE OF TRIVALENT IONS UPON THE EFFICIENCY OF HYPERTONIC SOLUTIONS.

In view of the fact that hypertonic sea-water and trivalent positive ions have antagonistic effects upon the conductivity of the unfertilised eggs of *Echinus acutus*, a large series of experiments were performed in order to determine whether the effect of hypertonic solutions is inhibited by the presence of lanthanum nitrate. The results as shown in Table XIX are fairly definite, but they cannot, at present, be regarded as being entirely satisfactory. When the eggs are immersed in a hypertonic solution containing lanthanum there is often a tendency for the eggs to behave irregularly after about half an hour's duration in the solution. The hypertonic solution used consisted of 50 c.c. sea-water + 8 or 10 c.c. $2\frac{1}{2}$ mol. NaCl. The action of lanthanum on eggs in such solutions is as follows :—

0·00004 mol. La···	Eggs usually normal, but sometimes injured.
0·00008 „	Eggs tend to adhere to glass, but sometimes injured.
0·00012 „	Eggs adhere to glass, nearly always injured.
0·00016 „	„ „ „ „

A large number of experiments had to be discarded owing to injury or abnormal behaviour of the eggs. In the experiments quoted the strength of lanthanum nitrate used was 0·00004 mol.

TABLE XIX.

Eggs.	Decrease in resistance in sea-water half an hour after transference from hypertonic solution. The eggs were left in the hypertonic solution for one hour.	
	Normal hypertonic solution.	Hypertonic solution containing $La^{\cdots}$.
	per cent.	per cent.
Echinus acutus ♀ A	26·2	7·7
Echinus acutus ♀ B	12·5	0

THE EFFECT OF HYDROGEN AND HYDROXYL IONS UPON THE CONDUCTIVITY OF UNFERTILISED EGGS.

For these experiments a van't Hoff's solution was used, and an examination of fig. 12 shows that the effect of the hydrogen ion upon the conductivity of unfertilised eggs resembles that of the trivalent cerium ion, although its action is less

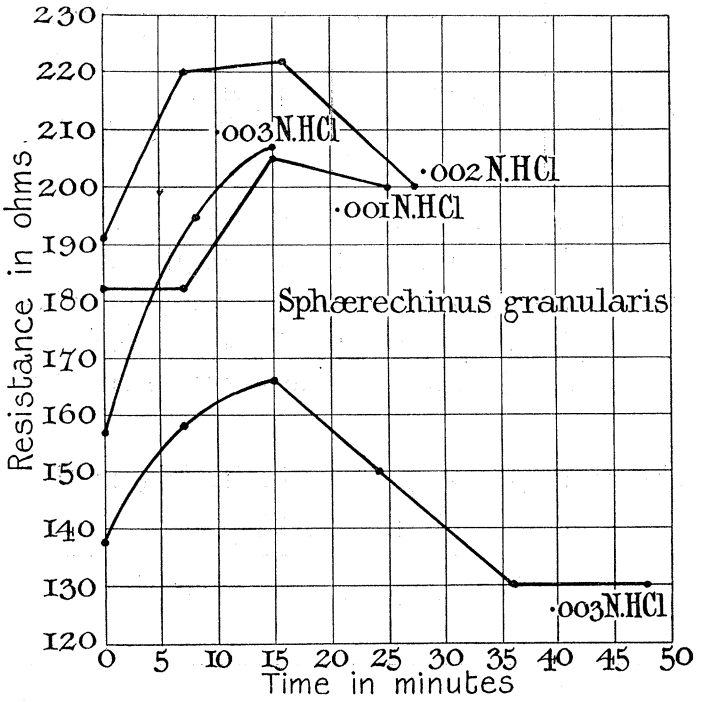

FIG. 12.—Illustrating the effect of hydrogen ions upon the resistance of the unfertilised eggs of *Sphærechinus granularis*.

intense. A concentration of 0·003 N HCl is required in order to obtain the same effect as 0·0001 mol. $Ce^{\cdots}$. The secondary fall in the resistance of the eggs in acid solutions is probably due to injury of the eggs, for after the experiments the eggs were somewhat unhealthy, although in some cases they developed more or less normally when fertilised in clean sea-water.

It is important to note that the action of the cerium ion cannot be attributed to any change in the hydrogen ion concentration of the sea-water, for the addition of 0·5 c.c. N/10 HCl to 50 c.c. of sea-water does not affect the conductivity of the eggs during the period of these experiments.

TABLE XX.—*Sphærechinus.*

Resistance of normal unfertilised eggs.	Resistance in acid van't Hoff's solution after x minutes.		Number of cubic centimetres N/10 HCl in 50 c.c. van't Hoff's solution.
ohms.	ohms.	ohms.	ohms.
184	205	15	0·5
191	220	7	1
191	200	26	0·25
138	166	15	1·5
157	195	8	1·5

TABLE XXI.—*Sphærechinus.*

—	0·0005 N HCl.	0·001 N HCl.	0·002 N HCl.	0·003 N HCl.
Maximum increase in resistance .	per cent. 0	per cent. 12·6	per cent. 16·2	per cent. 32·2 20·3

That acids are unfavourable to growth has been shown by MOORE, ROAF, and WHITNEY (48), who have also shown that alkalies and alkaline salts have a favourable action on cell-division. It is interesting to note that the effect of dilute alkalies upon the fertilised eggs of *Echinus esculentus* (MOORE, etc.) is the same as that of hypertonic solutions (GRAY, 14), viz., the production of chromatic abnormalities and irregular cell-division.

The Effect of the Hydroxyl Ion upon the Conductivity of Unfertilised Eggs.

TABLE XXII.

Eggs.	Strength of alkali.	Decrease in resistance.	After x minutes.
		per cent.	
Sphærechinus . .	50 c.c. van't Hoff's solution + 0·25 c.c. N/10 NaOH	10·3	60
,, . .	,, ,, ,, + 0·25 ,, ,,	1·9	30
,, . .	,, ,, ,, + 0·5 ,, ,,	22·1	44
,, . .	,, ,, ,, + 0·75 ,, ,,	9·4	8
,, . .	,, ,, ,, + 0·75 ,, ,,	23·5	16
Strongylocentrotus	,, ,, ,, + 0·25 ,, ,,	20·9	66
,,	,, ,, ,, + 0·25 ,, ,,	11·8	57

50 c.c. van't Hoff's solution + 0·25 c.c. N/10 NaOH = solution A; 50 c.c. van't Hoff's solution + 0·5 c.c. N/10 NaOH = solution B; 50 c.c. van't Hoff's solution + 0·75 c.c. N/10 NaOH = solution C.

The results given in the above Table are what would be expected by a comparison with the effects of the citrate ions upon the conductivity of the eggs.

Solution A* does not injure the eggs in any way. In solution B, however, the eggs tend to aggregate together; while in solution C this condition is very marked. Solution C, and sometimes solution B, renders the eggs unhealthy after one or two

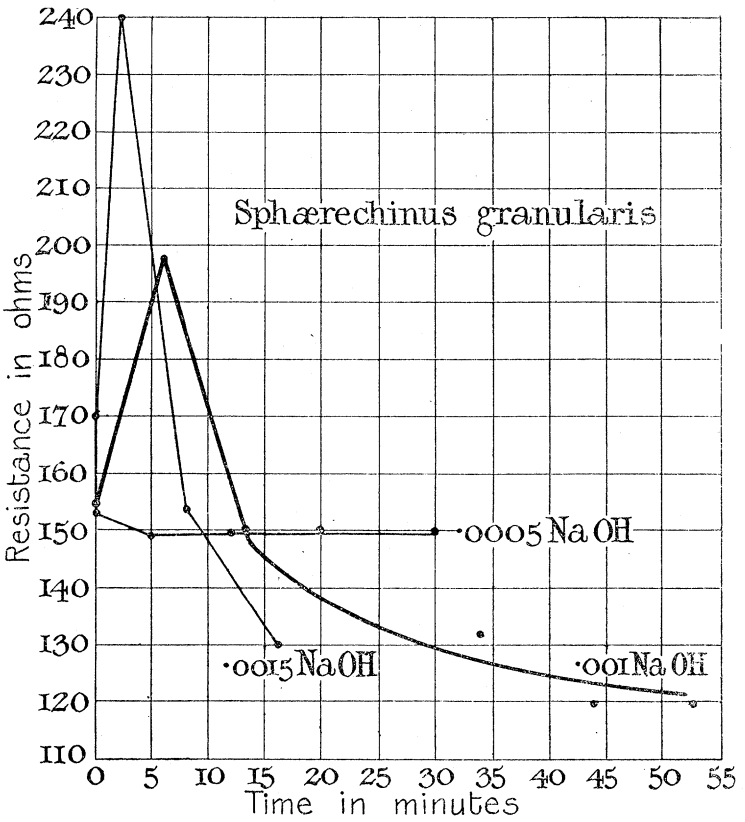

FIG. 13.—Illustrating the effects of hydroxyl ions upon the resistance of the unfertilised eggs of *Sphærechinus granularis*.

hours. The aggregation of the eggs is usually less marked as soon as the eggs are visibly affected by the alkali. Solution A produces a slow continuous fall in resistance. Exposure to solutions B and C induces a very sudden rise in the resistance, which is rapidly followed by a rapid and progressive fall in resistance. The final resistance is always lower than that of the eggs at the beginning of the experiment—which explains the figures given in Table XXII.

* See footnote to Table XXII.

Effect of Trivalent Positive Ions upon the Conductivity of Fertilised Eggs.

Table XXIII.—*Sphærechinus* Eggs.

	Minutes after fertilisation that eggs were put in Ce··· solution.	Strength of Ce··· solution.	Change in resistance.	In x minutes after treatment.
Expt. A. Unfertilised control .	—	0·0002 mol.	per cent. +36·4	3·5
Expt. A. Fertilised eggs . . .	7	0·0002 ,,	−5·1	5
	13	0·0005 ,,	−1·1	3
			+10·1	15
Expt. B. Unfertilised control .	—	0·0001 ,,	+27·7	3
Expt. B. Fertilised eggs . . .	8	0·0001 ,,	+7·9	7
			+2·0	15
Expt. C. Fertilised eggs . . .	6	0·0002 ,,	+20·3	3·4
Expt. D. Fertilised eggs . . .	4·5	0·0005 ,,	+8·2	3·5
			+20·6	6·5

The figures in Column 4 are given as percentages of the resistance of the eggs five minutes after fertilisation. It is therefore apparent that the conductivity of the fertilised eggs is less affected by the presence of cerium than is the conductivity of the unfertilised eggs. This might be due to two causes, either the fertilised eggs are more permeable to Ce··· than are the unfertilised eggs, or the surface of the fertilised egg is less easily polarised than that of the unfertilised egg.

Agglutination Phenomena in the Normal Unfertilised Eggs of* Strongylocentrotus.

During the course of the experiments a certain number of females of *Strongylocentrotus* were found whose eggs showed a distinct tendency to aggregate together in normal sea-water, after the removal of the gelatinous membranes. This agglutination was lost in a solution containing sodium citrate, but occurred again on transference to normal sea-water.

These eggs behaved somewhat differently when treated with cerium and with strong alkali solutions. The presence of 0·0001 mol. Ce··· only lowered the conductivity 5·6 per cent., and the addition of more concentrated cerium solutions did not increase this value. On the other hand, such eggs showed no primary decrease in their conductivity in a strongly alkaline van't Hoff's solution (50 c.c. + 0·5 c.c. N/10 NaOH). In this solution the conductivity was con-

* See p. 520 *seq.*

tinuously increased, and there was apparently no decrease in the state of aggregation of the eggs.

It would therefore seem that, when these particular eggs were in normal sea-water, they were in the same condition as normal eggs (that is, eggs which do not agglutinate in normal sea-water) which have been treated with a cerium solution. In short, these eggs are in a state of maximum impermeability.

If this is the case, one would expect to find that, when such eggs are fertilised, the change in the conductivity would be greater than that of normal eggs. That this is the case is shown by the following experiment :—

	Ohms.
Resistance of unfertilised eggs	184
„ fertilised eggs, 5 mins. after fertilisation	98
„ „ 10 „ „	103
„ „ 17 „ „	89·5
„ „ 29 „ „	86·5

In other words, the increase in the conductivity five minutes after fertilisation was 46·7 per cent.

The General Effects of Trivalent Anions upon the Behaviour of Fertilised and Unfertilised Eggs.

The effect of citrates upon biological phenomena has so far not received much attention. In 1901 Loeb (33) observed that the antitoxic doses of sodium acetate, sulphate, and citrate, are in the ratio 1 : 4 : 16, and came to the conclusion that the poisonous action of these latter salts is due to the negative charges of the anions. In 1903 Spaulding (51A) found that, under certain conditions, an exposure of fertilised Echinoderm eggs to sodium citrate solutions favoured the development of the eggs. More recently, Miss Dale (6A) has shown that sodium citrate affects the behaviour of *Paramœcium* by virtue of the negative charge possessed by the anion.

The Relation between the Effects of Sodium Citrate and Fertilisation upon the Eggs.

It has been shown that the effect of sodium citrate on unfertilised eggs resembles that of fertilisation in that it increases the electrical conductivity of the eggs; also, a few preliminary experiments support the expectation that sodium citrate may, under certain conditions, function as a parthenogenetic agent. But whereas the effect of the spermatozoon on the electrical resistance of the egg is quite irreversible, that of sodium citrate is partially or entirely removed if the eggs are transferred to normal sea-water.

That the two phenomena are related would appear from the fact that exposure to citrate solution, as it were, anticipates a part of the fall in electrical resistance caused by the spermatozoon.

TABLE XXIV.

Eggs.	Resistance.			
	Unfertilised.	In La‴ solution.	In Cit‴ solution.	Fertilised at once in normal sea-water. (Resistance 4 mins. after fertilisation.)
Echinus acutus	225	250	—	130
,, ,, ...	223	—	163	122
,, ,, ...	255	—	—	170

In other words the effect of fertilisation upon—

Normal eggs is to reduce their existence by . . . . 32·9 per cent.
Citrated eggs ,, ,, ,, . . . 25·2 ,,
Lanthanum eggs ,, ,, ,, . . . 45·5 ,,

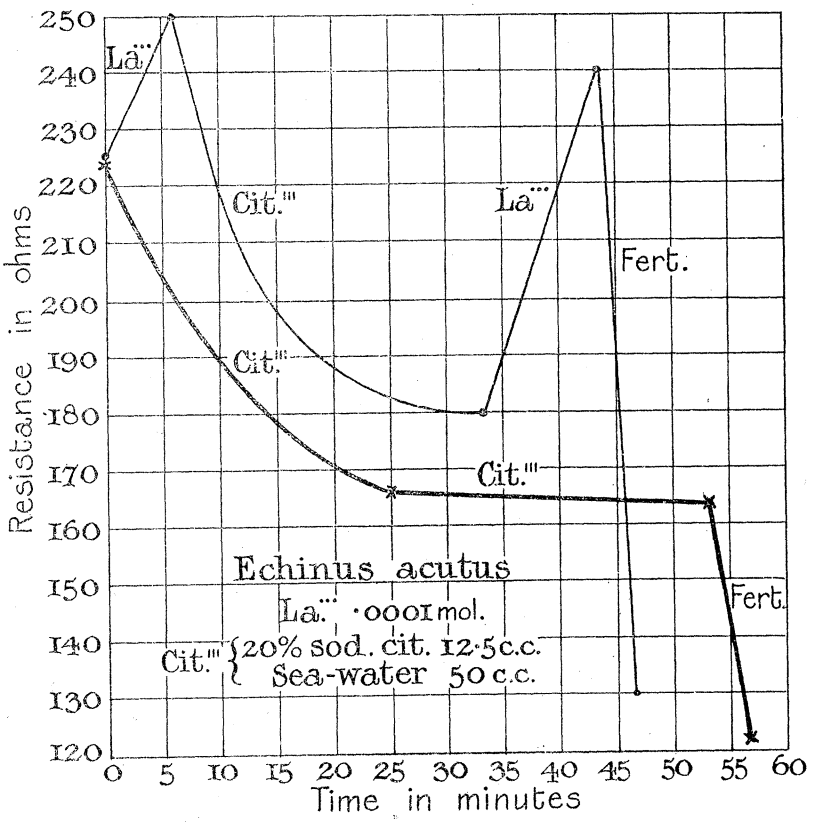

FIG. 14.—Illustrating the effect of fertilisation upon the resistance of eggs of *Echinus acutus* which have been treated with trivalent negative ions and trivalent positive ions respectively.

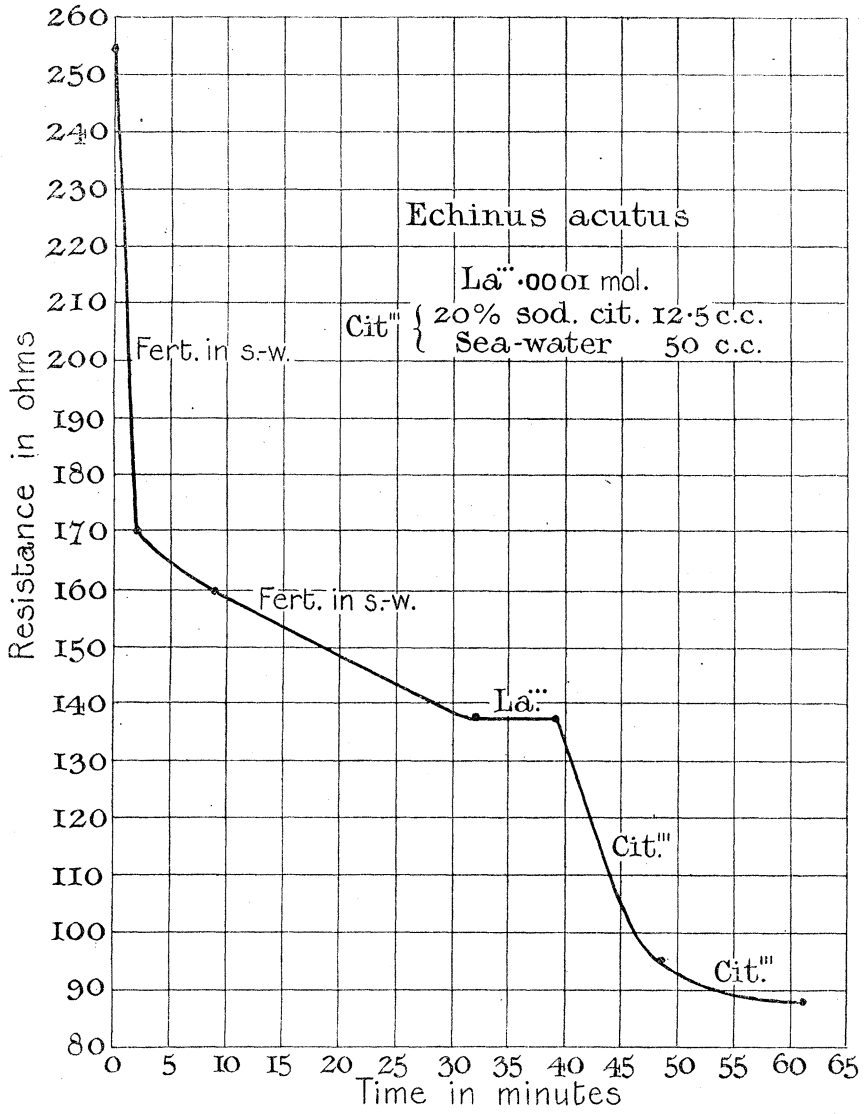

FIG. 15.—Illustrating the effect of trivalent ions on the resistance of fertilised eggs of *Echinus acutus*.

The Effect of Sodium Citrate Solutions upon Fertilised and Unfertilised Eggs.

A. *Fertilised Eggs.*—The general effect of sodium citrate upon the development of normally fertilised eggs of *Echinus miliaris* is shown by the following Table. No evidence was obtained to show that weak solutions of citrate increased the rate of development of the eggs; in stronger solutions the trivalent negative ion is distinctly inhibitive of normal development.

The general effects of these solutions were—

(1) To prevent the formation of the oral processes. The larvæ remained alive in some cases for 10–14 days, but no arms developed.

(2) To cause great irregularities in the skeleton of the plutei. The irregularities were often much more marked on one side of the larva than on the other.

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TABLE XXV.—50 c.c. Sea-Water+x c.c. 20 per cent. NaCit Solution.

Time.	Control (sea-water).	0·25 c.c.	0·5 c.c.	1 c.c.	2 c.c.	3 c.c.	4 c.c.	5 c.c.	7 c.c.	10 c.c.
24 hours	Actively swimming gastrulæ	Same as control	Same as control	Same as control	Same as control, except that many of the larvæ had not risen to the surface, and a few looked unhealthy.	Same as previous solution	Same as previous solution	Considerable amount of cytolysis. Fair number of gastrulæ swimming at bottom of bowl, but surface of larvæ was irregular	A few at surface, but most at bottom of bowl. Abnormal swimming movements.* Did not develop beyond gastrulæ	Swimming at bottom of bowl, but *very* abnormal. Did not develop beyond blastulæ.
48 hours	Early plutei, with oral arms	Same as control	Most same as control, but some stunted	Most same as control but some stunted	Large number dead, the rest stunted	Same as in previous solution	Very irregular and stunted.			
1 week	Normal development	Normal development	Somewhat stunted	Stunted, but swimming	No trace of oral processes still alive	Same as in previous solution	Same as in previous solution	All dead and stunted.		

* Normal blastulæ and gastrulæ swim actively across the field when examined under a microscope, and are in constant motion. When reared in relatively concentrated citrate solutions, the larvæ do not make any forward movement, although the cilia are in active motion; in these solutions the larvæ rotate about their main axis. It is interesting to note that sodium citrate also produces a rotating movement in *Paramœcium*, and which is attended by the loss of forward progression. D. DALE (6A), p. 127.

Transference of the larvæ from the citrate solutions to normal sea-water tended to prolong the life of the larvæ, but the oral arms did not develop, and the skeleton was very irregular.

A few experiments with the fertilised eggs of *Strongylocentrotus lividus* showed that these eggs are killed by a much lower concentration of sodium citrate than the eggs of *Echinus miliaris*.

B. *Unfertilised Eggs.*—Unfertilised eggs of *Echinus miliaris* were placed in the following solutions after the removal of the gelatinous membranes. The appearance of the eggs was noted after 24 hours.

TABLE XXVI.

Solution 100 c.c. sea-water.	60 c.c. sea-water + 40 c.c. 20 per cent. Na_3Cit.	50 c.c. sea-water + 50 c.c. Na_3Cit.	100 c.c. 20 per cent. Na_3Cit.
All unchanged	60 per cent. eggs looked more or less unchanged. 15 per cent. showed cleavages. 25 per cent. were cytolysed. Some had close membranes	Nearly all were black and cytolysed. Many had close membranes. Some irregular cleavages	30 per cent. were black and cytolysed. A few showed signs of division. Some eggs had close membranes.

After about one hour in 50 c.c. sea-water + 50 c.c. 20 per cent. Na_3Cit, some of the eggs in the above experiment were transferred to clean sea-water. The following day 10 per cent. showed signs of cleavage. Repetitions of this experiment with other eggs gave 5–15 per cent. of cleavages—but no larvæ were obtained.

TABLE XXVII.—*Strongylocentrotus* Eggs.

No. of experiment.	Solution.	Time of exposure to solution.	Percentage of eggs which showed signs of cleavage after transference to normal sea-water.
1	25 c.c. sea-water + 4 c.c. $2\frac{1}{2}$ M. NaCl 25 c.c. sea-water + 4 c.c. $2\frac{1}{2}$ M. NaCl + 5 c.c. 20 per cent. Na_3Cit	1 hour 1 hour	3 13
2	25 c.c. sea-water + 4 c.c. $2\frac{1}{2}$ M. NaCl 25 c.c. sea water + 4 c.c. $2\frac{1}{2}$ M. NaCl + 5 c.c. 20 per cent. Na_3Cit	$2\frac{1}{2}$ hours $2\frac{1}{2}$ hours	10 45 (also further advanced than in simple hypertonic solution)

A. *The Effect of Trivalent Positive Ions on Fertilised Eggs.*

Since sea-water contains distinct traces of carbonates, a precipitate is formed when the salts of cerium or lanthanum are added unless the concentrations of the latter are

very small. Thus 0·00025 mol. lanthanum nitrate in sea-water gives the solution a very faint appearance of milkiness, in stronger solutions this appearance is more marked and minute crystals are slowly deposited.

In order to investigate the effects of the salts upon the development of fertilised eggs, it is therefore necessary to employ an artificial sea-water containing no carbonates. Unfortunately the only data available are those derived from the use of weaker solutions of lanthanum and cerium in sea-water.

Time.	A. Fertilised eggs of *Echinus miliaris*.		
	In 0·00005 mol. $La(NO_3)_3$.	In 0·0001 $La(NO_3)_3$.	Control.
hours. 5 24 72	Blastulæ Gastrulæ (Dead)	Blastulæ. Normal and active gastrulæ (Dead)	Blastulæ. Gastrulæ. Prism larvæ.

Time.	B. Fertilised eggs of *Strongylocentrotus lividus*.	
	In 0·00005 mol. $CeCl_3$.	Control.
hours. 2 5 22	Some two-celled stage Some eight-celled stage Swimming blastulæ	Some two-celled stage. Some eight-celled stage. Swimming blastulæ.

This experiment with *Strongylocentrotus* eggs was very surprising, since previous observations had shown that if normally reared blastulæ are put into sea-water containing 0·00005 mol. $CeCl_3$ all movement instantly stops. The above experiment was repeated several times with identical results. It was then repeated in a slightly modified form. A solution containing 0·00005 mol. $CeCl_3$ was made up, and divided into two portions. Into one of them (A) were put fertilised eggs of *Strongylocentrotus*. After 24 hours the larvæ in this solution were quite active, but all movement ceased as soon as they were put into the remainder of the original cerium solution (B). Either the eggs absorb the cerium from the solution, or the salt is precipitated.

B. *Unfertilised Eggs.*

In a 0·0003 mol. solution of cerium chloride in sea-water, unfertilised eggs rapidly agglutinate into clumps, which are only partially resolved on agitating the tube containing the eggs and are re-formed on standing. In a 0·0002 mol. solution, this phenomena is observed to a much less degree, while in a 0·0001 mol. cerium solution,

there is practically no tendency for the eggs to adhere to each other. Now the same phenomenon is observed if eggs are put into an alkaline van't Hoff's solution, whose alkalinity is moderately high, *e.g.*, 50 c.c. van't Hoff's solution + 0·5–0·75 c.c. N/10 NaOH.

The agglutination of the eggs is never observed in acid solutions, though in concentrated acid solutions the eggs have a very decided tendency to adhere to the glass vessel containing them.

The effect of $Ce^{\cdots}$ and strong OH' solutions upon the eggs is exactly similar to their effects upon living spermatozoa.

The change in conductivity is quite independent of the amount of agglutination. Thus 0·0005 mol. $Ce^{\cdots}$ produced very strong agglutination of the fertilised eggs of *Sphærechinus* but the change in the conductivity in one case was only about 10 per cent. On the other hand, the effect of 0·0002 $Ce^{\cdots}$ on unfertilised eggs treated with 0·14 mol. sodium citrate for 42 minutes lowered the conductivity to its original value (a change of 19·6 per cent.) and no agglutination of the eggs occurred.*

Although the conductivity subsequently increases in a cerium solution, there is no sign of the agglutinated condition being lost. In alkaline solutions, however, the agglutinated condition does appear to be lost after about half an hour. It is, however, important to note that if the eggs are removed from the cerium solution to normal sea-water, they all fertilise and develop normally although they remain agglutinated. From alkaline solution, the agglutinated eggs do not develop normally when fertilised in sea-water.

The phenomenon of agglutination is only observed in experiments with 0·0002–0·0003 mol. $Ce^{\cdots}$ and strongly alkaline van't Hoff's solutions. The agglutination caused by cerium is instantly removed by sodium citrate, suggesting that the phenomenon is connected with the electrical charge on the egg-surface.

In 0·00005 mol. $La(NO_3)_3$ solution the eggs of *Sphærechinus* appear to be very little affected although they tend to settle rather more rapidly than in normal sea-water. In 0·0001 mol. solutions of this salt the eggs show a distinct tendency to aggregate together into small clumps, which are partly destroyed by gentle agitation. In stronger solutions agglutination is very marked, and the eggs become unhealthy. Agglutination of the eggs is instantly removed if the eggs are transferred to a solution containing sodium citrate.

The agglutination of the eggs by positive trivalent ions is entirely analogous to the behaviour of the red blood corpuscles of Elasmobranchs under similar conditions (Mines, 44). Mines has shown that the latter phenomenon receives a complete explanation by the assumption that the trivalent ions reduce the repulsion between corpuscles which they normally possess by virtue of the negative charge on the cell surface.

* In the case of *E. miliaris* eggs no agglutination is produced by the concentration of trivalent positive ions used in the investigation of electrical resistances.

From the aggregation of the eggs in the presence of lanthanum or cerium salts it is possible to conclude that the trivalent ions affect the eggs by modifying the electrical charge on their surface.

As far as has been investigated, fertilisation takes place quite normally in solutions containing low concentrations of cerium.

The eggs of *Echinus* require a higher concentration of trivalent positive ions in order to produce agglutination than do the eggs of *Sphærechinus* or *Strongylocentrotus*. In two cases the eggs of the latter species agglutinated in pure sea-water, and behaved towards cerium and to relatively strong alkaline van't Hoff's solution as though they had previously been treated with a trivalent positive radicle (*cf.* p. 514).

It is important to note that a higher concentration of trivalent ions is required in order to agglutinate fertilised eggs than unfertilised eggs; from which it seems legitimate to conclude that the negative charge on the surface of the egg is increased after fertilisation.

The only *direct* evidence of changes in the electric charge of an egg-surface due to fertilisation is given in the experiments of Miss Hyde (25).

By means of a capillary electrometer Miss Hyde investigated the changes in electrical potential at the surface of the eggs of *Fundulus*. She found that definite electromotive changes are induced in the egg by the entrance of a spermatozoon. Before fertilisation the blastodisc is positively charged in respect to the vegetative pole of the egg. After fertilisation, however, the direction of the current is reversed, and the blastodisc becomes relatively negative. This condition lasts for about 15 minutes, after which the blastodisc again becomes positive; it remains thus for about 20 minutes, when the current is again reversed. Finally, the appearance of the first cleavage furrow the blastodisc is again positively charged.

Summary of the General Effects of Trivalent Ions on Unfertilised and Fertilised Eggs.

A. *Fertilised Eggs*—

(i) Trivalent positive ions do not affect the development of the eggs if the salts are present in low concentrations.

(ii) Trivalent negative ions inhibit normal development. The larvæ are stunted and the skeleton is very irregular. The eggs of *Strongylocentrotus* are much more sensitive to these ions than are the eggs of *Echinus miliaris*.

B. *Unfertilised Eggs*—

(i) Trivalent positive ions in low concentrations do not affect unfertilised eggs apart from causing them to agglutinate. This agglutination is instantly removed by sodium citrate, and provides evidence to show that these ions alter the electrical charge on the surface of the egg, and that the negative charge on the surface of the egg is increased by fertilisation.

(ii) Trivalent negative ions in high concentration cause cytolysis, and in a few cases artificial parthenogenesis of the eggs of *Echinus miliaris*.

Summary and Conclusions.

Both natural and artificial fertilisation cause a fall in the electrical resistance of the Echinoderm egg. But whereas an excess of alkali in the interior of the egg does not affect the resistance, exposure to solutions containing trivalent positive or trivalent negative ions, hydrogen ions, or hydroxyl ions, does decidedly change the resistance. Since such solutions are known to polarise membranes, we may, at any rate, provisionally conclude that the variation in the electrical resistance of the eggs is determined by the polarisation of the surface, the surface change defining the permeability of the surfaces to electrolytes.

The purely physical nature of this theory has in some respects distinct advantages over the alternative chemical view advanced by Loeb. It obviates the reference to "membrane-forming substances" and "corrective substances," which are of a purely speculative value. It gives a definite conception of those essential "critical" changes in the egg to which adherents to Loeb's theory refer. These changes are regarded, in short, as changes in the surface energy of the egg (using the term in its widest sense and including changes in permeability).

Support is given to this hypothesis from the following considerations :—

1. The relation of the egg cell to such varied reagents as saponin and trivalent ions is identical with that of the red blood corpuscles of vertebrates.

2. The explanation given to the effects of trivalent ions upon the electrical resistance of the eggs is in entire accord to that which is currently accepted for the effect of these substances upon the heart-beat of Elasmobranchs, etc.

The work has been carried out at the biological stations of Plymouth and Naples, and I take this opportunity of expressing my thanks to all those who have provided me with a constant and abundant supply of material.

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I owe considerable gratitude to many physiologists in Cambridge and elsewhere, and to them I offer my very sincere thanks for much criticism and advice.

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APPENDIX I.

Note on the Effect of Strong and Weak Bases on Animal Tissue.

As is well known, Loeb regards the efficiency of all bases, when used as parthenogenetic agents, as dependent upon the amount of base which diffuses into the egg. He has shown that for certain eggs, weak bases such as ammonia are more efficient than sodium hydroxide or tetramethylammonium hydroxide; Harvey (16), however, finds that the weak bases diffuse into the egg very rapidly, whereas strong bases do not.

Ammonia in weak solutions enters the egg in sufficient quantities to render the interior distinctly alkaline to indicators. In a solution containing NaOH, however, the same amount of alkali does not penetrate the egg until the external solution is sufficiently alkaline to destroy the egg. In weaker solutions of NaOH the interior of the egg is always acid to indicators. If then these alkalies act upon the egg by diffusion into the interior, a solution of ammonia and a solution of NaOH, each of which is just strong enough to render the egg interior alkaline to neutral red, should have the same effect upon the egg. This, however, is far from the case, as such a solution of ammonia does not destroy the egg, whereas the corresponding solution of NaOH causes instant degradation of the cell.*

It is doubtful whether neutral red and other indicators which are absorbed by the egg are actually in solution; it is more likely that they are combined with some protein base.† Nevertheless, neutral red, even in a state of combination, is a very sensitive indicator for changes in hydroxyl ion concentration, and therefore the amount of alkali which penetrates from a solution consisting of 50 c.c. van't Hoff's solution +0·5 c.c. N/10 NaOH must be extraordinarily minute. Now, as Henderson (18) has shown, protoplasm is admirably adapted to maintaining a constant hydroxyl ion concentration, as it must contain carbonate and phosphate radicles, so that any *small* amount of alkali entering the egg fails to change the hydroxyl ion concentration of the cell contents.

The observations of Harvey (17) upon the effect of N/250 NH_4OH and N/250 NaOH upon the muscular and nervous mechanisms of *Cassiopea* afford striking evidence to show that weak and strong bases differ in their action on the cell. When the ectodermal granules of the Medusæ are stained with neutral red, and the animals are placed in the above solutions, muscular and neural responses continue *after* sufficient NH_4OH has entered to affect the colour of the granules, but they cease in N/250 NaOH *before* the granules are affected. This fact points very

* Also an extremely minute trace of ammonia should have the same effect as a solution of 50 c.c. van't Hoff's solution +0·5 c.c. NaOH, which is not the case.

† The pigment of *Arbacia* eggs is certainly not in solution, as when the eggs are treated with ammonia the pigment becomes *purple*, which is the colour characteristic of this pigment when precipitated in the presence of alkali, and not the brownish-red colour of the pigment in alkaline solution.

strongly to the conclusion that these two alkalies differ profoundly in their modes of affecting the cell.

There is, in fact, no evidence whatever that a strong base ever enters a living cell. LOEB's dictum that bases are efficient in the proportion in which they enter the cell is, therefore, opposed to experiment.

As far as I am aware Prof. LOEB has not advanced any other explanation beyond that quoted above. The theory advanced in this paper does, however, give a consistent and practical explanation of all these facts. Free alkali increases the permeability of the egg surface or membrane to other electrolytes, and its action is antagonised by acid or by bivalent metals in exactly the same way as an artificial membrane is affected by these substances (see GIRARD, PERRIN, and MINES).

APPENDIX II.

NOTE ON ENERGETICS OF DIFFUSION.

The diffusion of a salt through a charged membrane is an exceedingly complicated process. The work of BAYLISS (5) and others makes it quite clear that the nature of the membrane itself is a very important factor.

The most definite statements in regard to the energetics of diffusion through membranes are those of GIRARD (11). This author maintains that the rate of diffusion of a salt through a living membrane is dependent upon the E.M.F. which exists between the two sides of the membrane, and he offers both theoretical and experimental evidence in support of his theorem. Without a complete analysis of his argument, it is perhaps worth while to quote one experiment (mentioned by HÖBER (24), p. 309). On one side of an artificial membrane is placed a neutral solution of magnesium chloride, while on the other is pure water; the amount of salt which diffuses through the membrane in half an hour is determined. The salt solution is then replaced by one of exactly similar strength, but of slightly higher hydroxyl ion concentration; on determining the amount of salt which now diffuses through the membrane in half an hour, the quantity is found to be very considerably more than in the previous experiment. On the other hand, if the hydroxyl ion concentration is rendered lower than that of a "neutral" solution, the amount of salt which passes through the membrane is less than 30 per cent. of the amount which diffuses from the neutral solution.

A consideration of GIRARD's work, when combined with the results of PERRIN's experiments upon the polarisation of surface by ions, provides a very attractive interpretation of the experiments described in this paper, and indirectly of many of the phenomena of artificial parthenogenesis. At present, however, it is unwise to lay too much stress upon the rather isolated experiments of GIRARD. The theory of this author is extremely simple, and its application to the phenomena at present under discussion is capable of receiving experimental proof. I hope to apply these tests as soon as possible, and therefore refrain from further comment at present.

BIBLIOGRAPHY.

(1) Alcock, N. H. "Action of Anæsthetics on Living Tissues," 'Proc. Roy. Soc.,' B, vol. 77, p. 267 (1905).

(2) *Idem.* "Action of Anæsthetics on Living Tissues.—Part II," 'Proc. Roy. Soc.,' B, vol. 78, p. 159 (1906).

(2a) Bachman, E. L., and Runnstrom, J. "Der osmotische Druck wahrend der Embro. von Rana temporar," 'Pflüger's Archiv,' vol. 144, p. 287 (1912).

(3) Bachman, E. L. "Die Einwirkung der Befruchtung auf den osmot. Druck der Eier von Bufo vulgaris und Triton cristatus," 'Pflüger's Archiv,' vol. 148, p. 141 (1912).

(4) Bayliss, W. M. 'The Nature of Enzyme Action,' London (1911).

(5) *Idem.* "The Properties of Colloidal Solutions," 'Proc. Roy. Soc.,' B, vol. 84, pp. 81 and 229 (1911).

(6) Chevreton, L., and Vles, F. "La Cinématographie du Développement embryonnaire de l'Oursin (*Paracentrotus lividus*)," 'Archiv. de Zoologie Expér.,' vol. 8, series 5, p. 449 (1911).

(6a) Dale, D. "On the Action of Electrolytes on Paramœcium," 'Journ. of Phys.,' vol. 46, p. 129 (1913).

(7) Doncaster, L., and Gray, J. "Cytological Observations in the Early Stages of Segmentation of Echinus Hybrids," 'Q.J.M.S.,' vol. 58, p. 483 (1913).

(8) Elder, J. C. "The Relation of the Zona pellucida to the Fertilisation Membrane of *Strongylocentrotus*," 'Archiv f. Entwick. Mech.,' vol. 35, p. 145 (1912).

(9) Galeotti, G. "Über die electrische Leitfähigkeit der tierischen Gewebe," 'Zeit. f. Biologie,' vol. 43, p. 289 (1902).

(10) *Idem.* "Neue Untersuchungen über die electr. Leitfähigk. und den osmot. Druck der tierischen Gewebe," 'Zeit. f. Biologie,' vol. 45, p. 64 (1903).

(11) Girard, P. "Sur la Mécanisme Physico-chimique de l'Hemiperméabilité des Cellules Vivantes aux Electrolytes," 'Journ. de Phys. et Path. Gén.,' vol. 12, p. 471 (1912).

(12) Glaser, O. C. "On Inducing Development in the Sea Urchin (*Arbacia punctulata*), together with Considerations on the Initiating Effect of Fertilisation," 'Science,' vol. 38, p. 446 (1913).

(13) *Idem.* "The Change in Volume of *Arbacia* Eggs and *Asterias* Eggs at Fertilisation," 'Biol. Bulletin,' vol. 26, p. 84 (1914).

(14) Gray, J. "The Effects of Hypertonic Solutions on the Fertilised Eggs of Echinus," 'Q.J.M.S.,' vol. 58, p. 447 (1913).

(15) Greeley, A. W. "Artificial Parthenogenesis in Starfish Produced by a Lowering of Temperature," 'Amer. Journ. Phys.,' vol. 6, p. 296 (1912).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(16) Harvey, E. N. "Studies on the Permeability of Cells," 'Journ. Exp. Zool.,' vol. 10, p. 507 (1911).

(17) *Idem.* "A Criticism of the Indicator Method of Determining Cell-Permeability for Alkalies," 'Amer. Journ. Phys.,' vol. 31, p. 335 (1912–3).

(18) Henderson, L. J., and Black, O. F. "Concerning the Neutrality of Protoplasm," 'Amer. Journ. Phys.,' vol. 18, p. 250 (1907).

(19) van Herwerden, M. A. "Sur les Oxydones dans les Organes Génitaux et dans les Larves de *Strongyl. lividus*," 'Archiv. Internat. de Phys.,' vol. 13, p. 359 (1913).

(20) *Idem.* "Sur les Oxydones des Oursins," 'Archiv. Internat. de Phys.,' vol. 14, p. 85 (1913–4).

(21) Höber, R. "Resorption und Kataphorese," 'Pflüger's Archiv,' vol. 101, p. 607 (1904).

(22) *Idem.* "Messungen der Inneren Leitfähigkeit von Zellen," 'Pflüger's Archiv,' vol. 150, p. 15 (1913).

(23) *Idem.* "Ein zweites Verfahren die Leitfäh. im Inneren von Zellen zu messen," 'Pflüger's Archiv,' vol. 148, p. 189 (1911).

(24) *Idem.* 'Physikalische Chemie der Zelle und der Gewebe,' Leipzig (1911).*

(25) Hyde, I. H. "Differences in Electrical Potential in Developing Eggs," 'Amer. Journ. Phys.,' vol. 12, p. 241 (1904).

(26) Lillie, R. S. "The General Biological Significance of Changes in Permeability," 'Biol. Bull.,' vol. 17, p. 188 (1909).*

(27) *Idem.* "Physiology of Cell Division.—II," 'Amer. Journ. Phys.,' vol. 22, p. 106 (1910).

(27A) *Idem.* "Physiology of Cell Division.—III," 'Amer. Journ. Phys.,' vol. 27, p. 289 (1911).

(28) *Idem.* "Physiology of Cell Division.—IV," 'Journ. of Morphology,' vol. 22, p. 695 (1911).

(29) *Idem.* "Physiology of Cell Division.—V," 'Journ. Exp. Zool.,' vol. 15, p. 23 (1913).

(30) *Idem.* "The Relation of Stimulation and Conduction in Irritable Tissues to Changes in the Permeability of the Living Membranes," 'Amer. Journ. Phys.,' vol. 28, p. 197 (1911).*

(31) *Idem.* "Inactivation of Salt Solutions and Hyptertonic Sea-water by Anæsthetics," 'Journ. Exp. Zool.,' vol. 16, p. 591 (1914).*

(32) Loeb, J. 'Artificial Parthenogenesis and Fertilisation,' Chicago (1913).

(33) *Idem.* "The Toxic and Antitoxic Effects of Ions as a Function of their Valency and possibly their Electrical Charge," 'Amer. Journ. Phys.,' vol. 6, p. 411 (1902).

* Further references will be found in Höber (24) and Lillie (26 and 30).

(34) Loeb, J., and Beutner, R. "Über die Potentialdifferenzen an der unversehrten und verletzten Oberfläche pflanzlicher und tierischer Organe," 'Biochem. Zeit.,' vol. 41, p. 1 (1912).

(35) *Idem.* "Über die Bedeutung der Lipoide für die Entstehung von Potentialunterschieden an der Oberfläche tierischer Organe," 'Biochem. Zeit.,' vol. 59, p. 195 (1914).

(36) Loeb, J., and Wasteneys, H. "Die Oxydationsvorgänge im befruchteten und unbefruchteten Seesternei," 'Archiv für Entwick. Mech.,' vol. 35, p. 555 (1912).

(37) Lyon, E. P. 'Amer. Journ. Phys.,' vol. 11, p. 52 (1904).

(38) McLendon, J. F. "On the Dynamics of Cell Division.—II," 'Amer. Journ. Phys.,' vol. 27, p. 240 (1910).

(39) *Idem.* "How could Increase in Permeability allow Development of the Egg?" 'Proc. Soc. Exp. Biol. and Med.,' vol. 8, p. 1 (1910).

(40) *Idem.* "The Permeability of the Frog's Egg," 'Science,' July, 1914.

(41) McLendon, J. F., and Mitchell, P. H. "How do Isotonic Sodium Chloride Solutions and other Parthenogenetic increase Oxidation in Sea Urchin's Egg?" 'Journ. of Biol. Chem.,' vol. 10, p. 459 (1912).

(42) Matthews, A. P. "Artificial Parthenogenesis produced by Mechanical Shock," 'Amer. Journ. Phys., vol. 6, p. 142 (1902).

(43) Meyerhof, O. "Untersuchungen über die Wärmetonung der vitalen Oxydationsvorgänge in Eiern," 'Biochem. Zeits.,' vol. 35, p. 246 (1911).

(44) Mines, G. R. "Der Einfluss gewisser Ionen auf die elektrische Ladung von Oberflächen und ihre Beziehung zu einigen Problemen der Kolloidchemie und Biologie," 'Kolloidchemische Beihefte,' vol. 3, p. 191 (1912).

(45) *Idem.* "The Relation of the Heart Beat to Electrolytes," 'Journ. Mar. Biol. Assoc.,' vol. 9, p. 171 (1911).

(46) *Idem.* "The Action of Beryllium, Lanthanum, etc., Ions on the Frog's Heart," 'Journ. of Phys.,' vol. 40, p. 327 (1910).

(47) *Idem.* "On the Relations to Electrolytes of the Hearts of Different Species of Animals," 'Journ. of Phys.,' vol. 43, p. 467 (1912).

(48) Moore, B., Roaf, H. E., and Whitney, E. "On the Effects of Alkalies and Acids and of Alkaline and Acid Salts upon Growth and Cell Division in the Fertilised Eggs of *Echinus esculentus*," 'Proc. Roy. Soc.,' B, vol. 77, p. 102 (1905–6).

(49) Osterhout, W. J. V. "Reversible Changes in Permeability produced by Electrolytes," 'Science,' vol. 36, p. 350 (1912).

(50) *Idem.* "The Chemical Dynamics of Living Protoplasm," 'Science,' vol. 39, p. 292 (1914).

(51) *Idem.* "The Effect of Anæsthetics upon Permeability," 'Science,' vol. 37, p. 111 (1913).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(51A) Spaulding, E. G. "The Rhythm of Immunity and Susceptibility of Fertilised Sea-Urchin Eggs to Ether, to HCl, and some Salts," 'Biol. Bull.,' vol. 6, p. 224 (1903–4).

(52) Stewart, G. N. "The Behaviour of the Hæmoglobin and Electrolytes of the Coloured Corpuscles when Blood is Laked," 'Journ. of Phys.,' vol. 34, p. 211 (1899).

(53) *Idem.* "Mechanism of Hæmolysis," 'Journ. of Pharm. and Exp. Ther.,' vol. 1, p. 49 (1909).

(53A) Tröndle, A. "Der Einfluss des Lichtes auf die Permeabilität der Plasmahaut," 'Jahrb. f. wiss. Bot.,' vol. 48, pp. 171–280.

(54) Warburg, O. "Beobachtungen über die Oxydationsprozesse in Seeigelei," 'Zeit. physiol. Chem.,' vol. 57, p. 1 (1908).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES