Zoology. — *The influence of an electric field on the eggs of Limnaea stagnalis L.* By CHR. P. RAVEN. (Zoological Laboratory, University of Utrecht.)

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Previous experiments have shown that a polar gradient field plays an important part in the development of the eggs of *Limnaea* (e.g. RAVEN 1947). The experiments of DE GROOT (1948) indicate that in this gradient field two groups of factors are involved, governing the evolution of the nuclei and the distribution of the cytoplasmic substances, respectively. Nothing is known, however, on the nature of these factors. Therefore, an attempt was made to get some insight into this problem of the physical nature of the gradient field.

It seemed likely that electric forces might play some part in the movements of the constituents of the cell. As a matter of fact, such forces have often been put forward for the explanation of the process of mitosis. It has been shown experimentally that various constituents of the cell have different electric charges (e.g. PENTIMALLI 1909, MC CLENDON 1910, HARDY 1913, MEIER 1921, ZEIDLER 1925, BOTTA 1932, VON LEHOTZKY 1936, CHURNEY and KLEIN 1937).

The presumption that electric forces might be responsible for some of the movements of cell constituents obtained further support from observations of RUITER and BUNGENBERG DE JONG (1947) on the behaviour of coacervate drops in an electric field. In these drops, streaming movements were observed very similar to the shift of cytoplasmic substances during the early development of *Limnaea*.

A direct measure of the electric potential at different points of the *Limnaea* egg did not seem possible, on account of its minute size. Therefore, an attempt was made to study the electric properties of the egg indirectly, by exposing it to a strong electric field. It was supposed that such an external field would interfere in some way or other with the presumed intracellular electric fields within the egg, thereby causing a deviation of some of the constituents of the cell, which might permit some conclusion on the existence and action of these intracellular fields.

First, a problem had to be solved. The eggs had to be submitted to the action of the electric field while lying in water. Though, by using distilled water, the electric current can be made very small, still some electrolysis, with formation of acids and bases at the electrodes, will take place. As I intended to use rather long exposure times, precautions had to be taken against the actions on the eggs of these products formed at the electrodes.

In literature, some dispositions have been described for the exposure of

cells and tissues to electric fields while preventing the injurious action of ions (e.g. PENTIMALLI 1909, GUILLIERMOND and CHOUCROUN 1936). However, none of them appeared to be adapted to our present purpose. Therefore, a new apparatus was constructed preventing with certainty any influence on the eggs of the substances formed at the electrodes [1]).

Methods.

A rectangular cuvette (length 80 mm, width 27 mm, height 19 mm) is divided by 3 transverse sills into 4 compartments (fig. 1). The middle sill S_1 has a breadth of 18 mm, the other sills S_2 and S_3 are 3,5 mm in

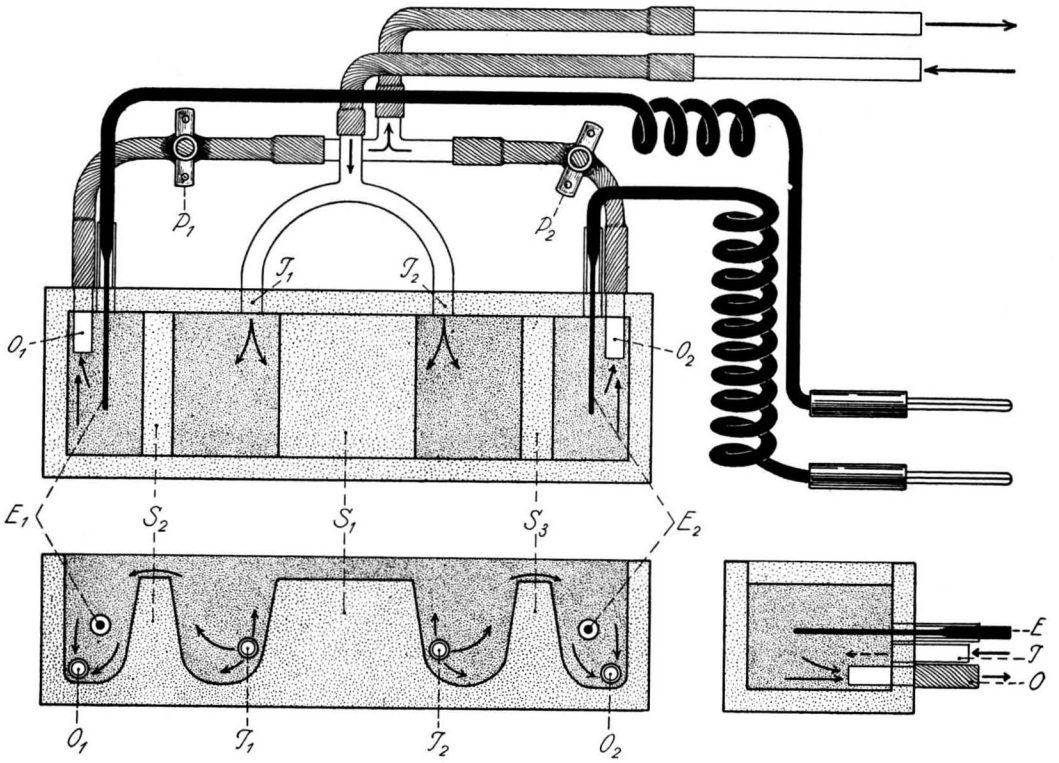

Fig. 1. Electrophoresis cell with removal of products formed at the electrodes. Description cf. text.

breadth. They reach from the bottom till 3 mm under the top. The cuvette with its sills is made of plastics ("Kallodent" 333, made by I.C.I.) and has been cast, as a piece, in a plaster of Paris mould. The platinum electrodes E_1 and E_2 are inserted into the outer compartments by bores into one of their side-walls. Nearer the bottom of these compartments,

1) The author is indebted to prof. H. G. BUNGENBERG DE JONG for his valuable advice in the construction of the apparatus.

small glass tubes (O_1, O_2) pierce the walls, forming a pair of outlets. The inlets of the water-supply I_1 and I_2 are in the inner compartments bordering on the middle sill S_1.

The water-supply is fed by two bottles, which are connected by rubber tubing to a T-piece; these conduits can be closed by means of pressing-screws. In this way, the composition of the medium flowing through the cuvette can be changed e.g. in the vital staining experiments. The main is divided by another T-piece leading to the inlets I_1 and I_2.

The outlets O_1 and O_2 are connected by rubber tubing to a T-piece which communicates with the escape-pipe. This consists of a glass tube bent at right angles, which can be slid up and down through a bore of a cork stopper (fig. 2).

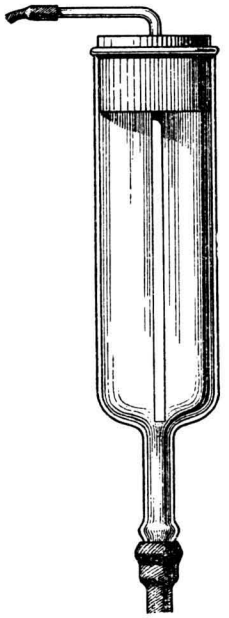

Fig. 2. Water discharge with adjustable level of outflow.

Before the beginning of an experiment, the cuvette is filled with distilled water from the water-supply. The eggs are put on top of the middle sill S_1 and brought into focus under a binocular microscope. Then the cuvette is covered with a large cover-slip, care being taken that no air-bubbles are enclosed; as the water against the plastic brim forms a convex surface, this can be accomplished without any difficulty. Then the water-supply and -discharge are set into action and, by shifting the levels of store-bottles and escape-pipe, adjusted in such a way that neither any leakage occurs nor air-bubbles are drawn in under the margin of the coverslip. By focussing on small particles in the water, brought in accidentally with the eggs, the direction of water current over S_1 is determined; by lightly turning the pressing-screws P_1 and P_2 (fig. 1), the pressures at both sides of S_1 are equalized so that no water current over S_1 remains. Now, a

regular current of water passes from the inlets I to the outlets O over the sills S_2 and S_3. When the electric current is switched in, the products of electrolysis formed at the electrodes (except small gas bubbles accumulating beneath the cover slip) are carried off immediately.

The efficacy of this disposition has been tested by means of indicator solutions. When the electric current passes through a diluted neutralred solution, yellow clouds are formed at the kathode; they remain confined to the kathode compartment, however, and do not surpass the sill which borders the latter. With a cresolred solution (yellow), purple streaks arise at the kathode and are carried away through the outlet; in the same way, in bromothymolblue solution (yellow) blue clouds are formed at the kathode and immediately carried away. We may be sure, therefore, that no acids and bases formed at the electrodes reach the eggs.

Fig. 3 shows the circuit. The direct current of the town-net (220 V) passes through a potentiometer P, from which a voltage varying between 0 and 200 V can be tapped off. The micro-ampèremeter measures the current passing through the cuvette.

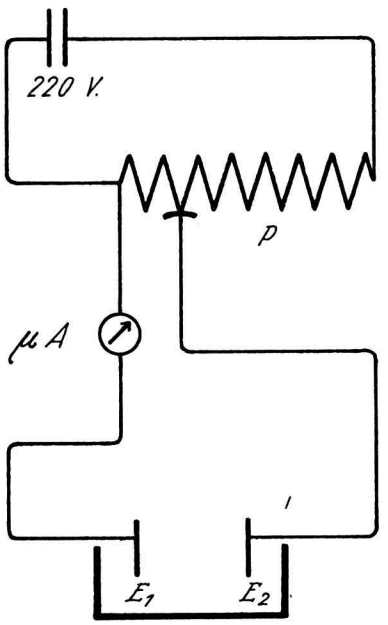

Fig. 3. Electric circuit for electrophoresis experiments.

The eggs are situated in the narrow space between the surface of sill S_1 and the cover-slip, 3 mm in height, 18 mm in breadth and about 20 mm in width. The lines of force of the electric field are passing from one side to the other through this space; the field will be practically homogeneous in this region. The resistance wil be highest at the level of the sills, whereas the deep troughs between them will offer only little resistance to the current. Therefore, for the computation of the potential gradient these troughs have been neglected, the distance of the electrodes being taken as the sum of the breadths of the sills, i.e. 25 mm. Hence, with a potential

difference between the electrodes of 75 Volt, the field strength is 30 Volt/cm.

The electric current has only been small; when distilled water is used, as in most of our experiments, with a potential difference of 200 Volt the current strength does not exceed 50—60 μAmp. (current density = 85—100 μAmp./cm^2).

Results.

1. *Egg capsules.*

In a first series of experiments, whole egg capsules, freed as much as possible from the outer jelly, have been studied.

As soon as the circuit is switched in, the egg capsules begin to move in the direction of the anode. In most cases, they are sticking to the bottom by rests of the jelly so that only a twitching movement occurs; sometimes, however, they move on in the direction of the anode and drop into the trough which borders the sill at this side. This movement is rather quick; though no precise measurements have been made, the estimated electrophoretic mobility (μ/sec/Volt/cm) lies in the neighbourhood of 10.

These observations seem to prove that the egg capsules have a negative charge. However, another explanation has to be considered. Under the influence of the potential difference an electro-osmotic movement of the water will occur, and it might be supposed that the resulting water current accounts for the movement of the egg capsules (cf. ABRAMSON 1934). As a matter of fact, the presence of electro-osmotic currents is indicated by a displacement of small particles, directed to the kathode, right along the bottom of the container. It may be assumed that a similar current exists at the under side of the cover-slip. As the electrophoresis cell is open at both ends, the existence of a counter-current through the middle of the space containing the eggs does not seem very likely in these circumstances, however. Anyhow, such a counter-current would be far too weak to account for the observed anodal movement of the egg capsules. We may conclude, therefore, that these capsules have a negative charge.

The eggs within the capsules do not exhibit, as a rule, any visible movement when the circuit is switched in. However, after the lapse of some minutes they are found in most of the capsules in the half facing the anode. Hence, their electrophoretic movement is very slow; it has to be considered, however, that they are moving through the rather viscous egg capsule fluid. The eggs show no orientation with respect to the electric field, their animal poles pointing in various directions.

After about 10 minutes (at 50—80 V/cm), in the egg capsule fluid a distinct stratification appears. At the anodal side a dense yellow-coloured substance is accumulated, whereas the kathodal side is occupied by a very clear transparent fluid. Both substances are separated by a sharp boundary. The egg cell is lying in the yellow part. When the circuit is

broken, this stratification disappears within some minutes, the components of the egg capsule fluid mingle again into a homogeneous liquid. When the egg capsules are left in the field (80 V/cm) for 20—30 minutes, however, at the boundary between the dense and the transparent part of the egg capsule fluid a kind of flocculation occurs giving rise to the formation of a turbid zone; after breaking the circuit, this zone remains visible for hours, but on the next day the egg capsule fluid has got a homogeneous appearance again. In some cases, egg capsules have been observed to burst and collapse in the electric field.

As to the development of the eggs exposed to the electric field, the following observations have been made. In some cases, eggs have been observed to cleave in a normal way and simultaneously with untreated controls, after being exposed to a field of 70 Volt/cm for 40 minutes. 3 Eggs exposed immediately after the extrusion of the 1st polar body to a field of 50 V/cm for 15 minutes, then another 5 minutes to 70 V/cm, afterwards cultured on agar bottom in the usual way, developed to normal hippo-stage embryos. 16 Eggs treated for 55 minutes (50 V/cm for the first 20 minutes, then increased to 80 V/cm), beginning immediately after laying and ending 20 minutes after the extrusion of the 1st polar body, yielded 5 normal hippo-stage embryos. In another case, of 9 eggs treated between first and second polar body formation with 80 Volt/cm for 45 minutes, 4 developed to normal trochophores. As a matter of fact, mortality in the treated cultures is somewhat higher than in the controls; since a partial flocculation of the egg capsule fluid had taken place in these capsules at the end of exposure, this is not astonishing. The fact, however, that a large percentage of the treated eggs showed a normal development proves that even so strong an electric field does not seriously interfere with the developmental processes in the uncleaved egg when this is surrounded by the egg capsule.

2. *Decapsulated eggs.*

Decapsulated eggs, studied in distilled water, as a rule show no reaction when the circuit is switched in: no displacement or rotation of the eggs takes place. In some cases, however, the eggs showed a slight movement towards the kathode. As these eggs are resting upon the bottom of the electrophoresis cell, it seems probable that this displacement is caused by the electro-osmotic flow of water along the bottom. No orientation of the eggs in the electric field takes place, the animal poles pointing in all directions, but mostly upwards.

Whereas the eggs, when surrounded by the egg capsules, can endure even an exposure to 80 Volt/cm for a longer time without immediate detrimental effects, decapsulated eggs in distilled water soon cytolyse under the influence of stronger electric fields. In one case, of 11 eggs belonging to one batch, in a field of 70 Volt/cm 9 cytolysed within 5 minutes; in

another, 4 out of 11 eggs cytolysed within 10 minutes in 80 Volt/cm. The incidence of cytolysis is dependent on the strength of the field; on an average, after 45 minute exposure 15 % of the eggs have cytolysed in 30 Volt/cm, 27 % in 40 Volt/cm, 44 % in 50 Volt/cm. However, different batches show great differences in their resistance against cytolysis.

Cytolysis proceeds very rapidly; therefore, it is not easy to study its course. As far as could be made out, as a rule it begins with an outflow of protoplasm at the side of the egg facing the anode; at first, the vitelline membrane remains intact, the outflowing cytoplasm spreads under the membrane to the other side of the egg. After some seconds, the membrane bursts, mostly at the anodal side, and the whole contents of the egg are dispersed, forming a cloud in the water. Immediately afterwards, in most cases this "cloud" of protoplasm begins to move to the anode rather quickly, and eventually drops into the trough at this side. The whole process, as a rule, does not take longer than 20 seconds.

Not always cytolysis proceeds in this way. Occasionally, the vitelline membrane remains intact, but is distended considerably by its cytolysed contents; these "swollen" cytolysed eggs do not move to the anode as do those which have burst. In some cases, at the moment of cytolysis the eggs show an indistinct stratification, the anodal side being more orange, whereas the kathodal side is light yellow; furthermore, the pole of the egg facing the anode often shows a conical or somewhat nipple-shaped evagination.

According to VON LEHOTZKY (1936), when an electric current passes through the cells of the onion, their protoplasm shows an acid reaction at the anodal and an alkaline reaction at the kathodal side. I have investigated by means of vital staining experiments if the same holds true in the case of *Limnaea* eggs. Strongly diluted solutions of neutralred, nile blue hydrochloride, cresolred and bromothymolblue have been used; they were flowing through the electric cuvette in the ordinary way.

With neutralred, the intact eggs did not stain perceptibly during the experiment in the weak solutions used. However, at the moment of cytolysis they became deep red at the side facing the anode, whereas the rest of the egg remained yellow.

With nile blue hydrochloride, the same phenomena were observed: The eggs did not stain while they were intact. As soon as cytolysis occurred, however, the anodal side became deep blue, whereas the rest of the egg remained yellow. This was even the case in extremely diluted solutions which had no visible colour.

In cresolred, neither intact nor cytolysed eggs showed a distinct differential colour; in bromothymolblue intact eggs remained colourless, whereas the "swollen" cytolysed eggs showed a light greenish colour all over.

We may conclude from these observations that intact eggs are relatively impermeable to the dyes used. As soon as the egg cortex is destroyed at

the anodal side with beginning cytolysis, the outflowing cytoplasm stains heavily with neutralred and nile blue hydrochloride.

These observations gave no definite answer as regards the occurrence of pH differences in the egg. Therefore, in some experiments another procedure was followed. Egg capsules were placed, immediately after laying, in somewhat stronger solutions of neutralred or nile blue hydrochloride. After some hours, the heavily-stained eggs were decapsulated and exposed to the electric field.

Eggs treated in this way with neutralred are deep red except the area surrounding the animal pole which is orange brown. In distilled water, the eggs give off a part of the absorbed dye substance, which forms reddish clouds in the water; at the same time, they become paler, more brownish red, with yellow-brown animal pole area; a red band of granules remains in the neighbourhood of the equator. No colour differences related to the direction of the electric field, even in a field of 60 V/cm, could be observed. The eggs cytolyse in the usual way; the vitelline membrane bursts, perhaps, less often at the anodal side than in unstained eggs. No regular colour differences in cytolysing or cytolysed eggs have been observed.

After staining in nile blue hydrochloride, similar observations have been made. The eggs are deep blue. Cytolysis occurs in the usual way, but also in this case the point at which the membrane bursts shows no clear relation to the direction of the field. No colour differences in intact or cytolysing eggs can be observed; only after cytolysis is complete, the anodal side of the eggs shows, perhaps, a somewhat heavier staining.

We must conclude, therefore, that our observations do not point to the occurrence of pH differences in the cytoplasm of eggs under the influence of the electric field.

In another experiment, VON LEHOTZKY (1936) treated the onion cells after exposure to the electric field with a mixture of eosin and methylen blue, and observed that the original kathodal side of the cells stained red, the anodal side blue. This observation proves, according to him, that both sides of the cell have got an opposite electric charge.

In my experiments, methylen blue gave the same results as neutralred and nile blue hydrochloride: intact eggs remained colourless, in cytolysing eggs the outflowing cytoplasm at the anodal side stained immediately. In eosin intact eggs remained colourless, cytolysed eggs stained uniformly red after some minutes.

In many instances it has been observed that the decapsulated eggs in a field of 40—50 V/cm pursued their development synchronously with the controls; both the extrusion of first and second polar body, and first and second cleavage have been observed to occur in the electric field. However, in some cases where the eggs had been exposed to the field for about 2 hours, cleavage took place with some delay and only in part of the eggs. No abnormalities in the situation of polar bodies or the direction of cleavages have been observed.

In order to study with greater accuracy the influence of the field on the structure of the eggs, in a number of cases the eggs have been fixed at the end of the experiment. In order to obtain a maximum effect, the treatment was continued until part of the eggs began to cytolyse. At this moment they were sucked up in a pipette, either immediately after or without previous breaking of the circuit, and transferred to Bouin's fluid; the whole procedure took no more than 2—3 seconds. In this way, is was hoped to preserve eventual distortions in the structure of the egg caused by the electric field. At the same time, it would permit to study more accurately the course of cytolysis. In order to fix a greater number of the eggs in the act of cytolysing, in some instances they were subjected to a field of 30 V/cm for at least half an hour, then the field was increased to 40—50 V/cm. Shortly afterwards, many of the eggs began to cytolyse simultaneously.

Table I summarizes these experiments.

TABLE I.

Exp.	Treatment	Stage at fixation	Number of eggs		
			Total	Intact	Cytolysing
RB I	80 V/cm, 20 min.	1st. matur. spindle	4	2	2
RK I	30 V/cm, 30 min.; then 40 V/cm	Formation of 1st polar body	13	10	3
RC I	40 V/cm, 30 min.	2d matur. spindle	5	5	—
RB II	40 V/cm, 60 min.	2d pol. b. just formed	15	15	—
RK II	30 V/cm, 35 min.; then 50 V/cm	Pronuclei	12	10	2
RJ I	30 V/cm, 80 min.; then 40 V/cm, 5 min.	1st cleavage spindle	3	3	—
RK III	30 V/cm, 35 min.; then 40 V/cm, 5 min.	1st cleavage spindle	10	9	1
RJ II	30 V/cm, 35 min.	Late 2 cell stage	7	5	2
			69	59	10

The study of the intact eggs reveals that they show an entirely normal structure. They have pursued their development synchronously with the controls, and resemble, in every respect, normal eggs of the same stage of development. Even the intact eggs of RB I, which had been exposed to a field of 80 V/cm, show nothing abnormal. The only exception to this rule form two eggs of RB II. In one of these eggs, which had been fixed just after the extrusion of the 2d polar body, the first polar body is still connected with the egg; in the other, the first polar body has not been extruded, but remained as a conical projection of the egg surface, whereas the second maturation spindle, which is in early telophase, has remained "submerged" in the interior of the egg. In 4 other eggs of the same batch, there is an indication that the second polar body has been displaced slightly to one side, so that the 2d maturation spindle is somewhat

distorted; this is not very conspicuous, however. In no other case any effect of the electric field on the situation and structure of chromosomes, spindles, pronuclei or cytoplasmic substances has been detected.

The eggs fixed during the course of cytolysis offer many interesting aspects. Fortunately, various phases of the process, from its very first beginning to the final stage of complete cytolysis, are present in my material. This permits us to obtain a survey of the events which follow one another quite rapidly once the process has begun. These changes are most conspicuous in sectioned eggs, which after fixation in Bouin's fluid have been stained with azan; the following description refers to such eggs.

The first indications of beginning cytolysis have been found in 2 eggs of RK II. In both, at two diametrically opposite points of the egg surface, there is a small area where the cytoplasm exhibits distinct signs of disintegration, which are not quite identical on both sides of the egg, however. On one side, probably that of the kathode (*K*), there is some confluence of cytoplasmic vacuoles; the surface layer of the egg has remained intact, but the egg surface is somewhat irregular. On the opposite side (*A*), on the contrary, in a restricted area the protoplasmic structure has been entirely destroyed; a liquefied space has been formed, which is filled, in the sections, with a fine coagulum. The egg cortex is also involved in this process of liquefaction, but the vitelline membrane has remained intact and stands out clearly and sharply (fig. 4*a*). At the same time, a remarkable change in the colourability of the eggs has occurred. Whereas in the normal eggs the red colour of azocarmine is predominant, in those with beginning cytolysis the colour in the central part of the egg has changed to bluish. Moreover, the vacuoles in this part are enlarged and the cytoplasmic meshes between them are narrower: evidently, this central part of the egg is strongly hydrated.

Further stages of the process of cytolysis have been found in RK I. One of them corresponds in the main to those just described, but the irregular contour of the egg at the presumed kathodal side is somewhat more conspicuous. The other two cytolysing eggs of this batch represent a further phase. They are distinguished from normal eggs already at low magnifications by their blue colour, which has now spread over the whole egg except only a narrow zone on one side. On the opposite side (*A*), where in the previous stage the egg cortex had been destroyed by liquefaction, now the outflowing of the cytoplasm under the vitelline membrane has begun; a comparison with our previous observations on the process of cytolysis makes it clear that this is, probably, the anodal side. In one of the eggs, this outflowing cytoplasm is still restricted to a small part of the circumference of the egg (fig. 4*b*); in the other one, it has flown beneath the vitelline membrane till the opposite side of the egg (fig. 4*c*). It contains both β- and γ-granules of the proteid yolk. The hydrated zone, which occupied the centre of the egg in the previous stage,

now has been displaced toward the kathodal half; by further swelling and confluence of the vacuoles a clear fluid space has been formed; the original cytoplasmic meshes have been reduced to an irregular network of fine threads. On the kathodal side a rather narrow superficial layer (*K*) has been formed which differs from the rest of the egg by its denser appearance and its red violet colour; it covers about half of the circumference of the egg. In the middle of this region, the contour of the egg is unsharp and irregular, the vitelline membrane is not clearly visible (fig. 4*b*). The

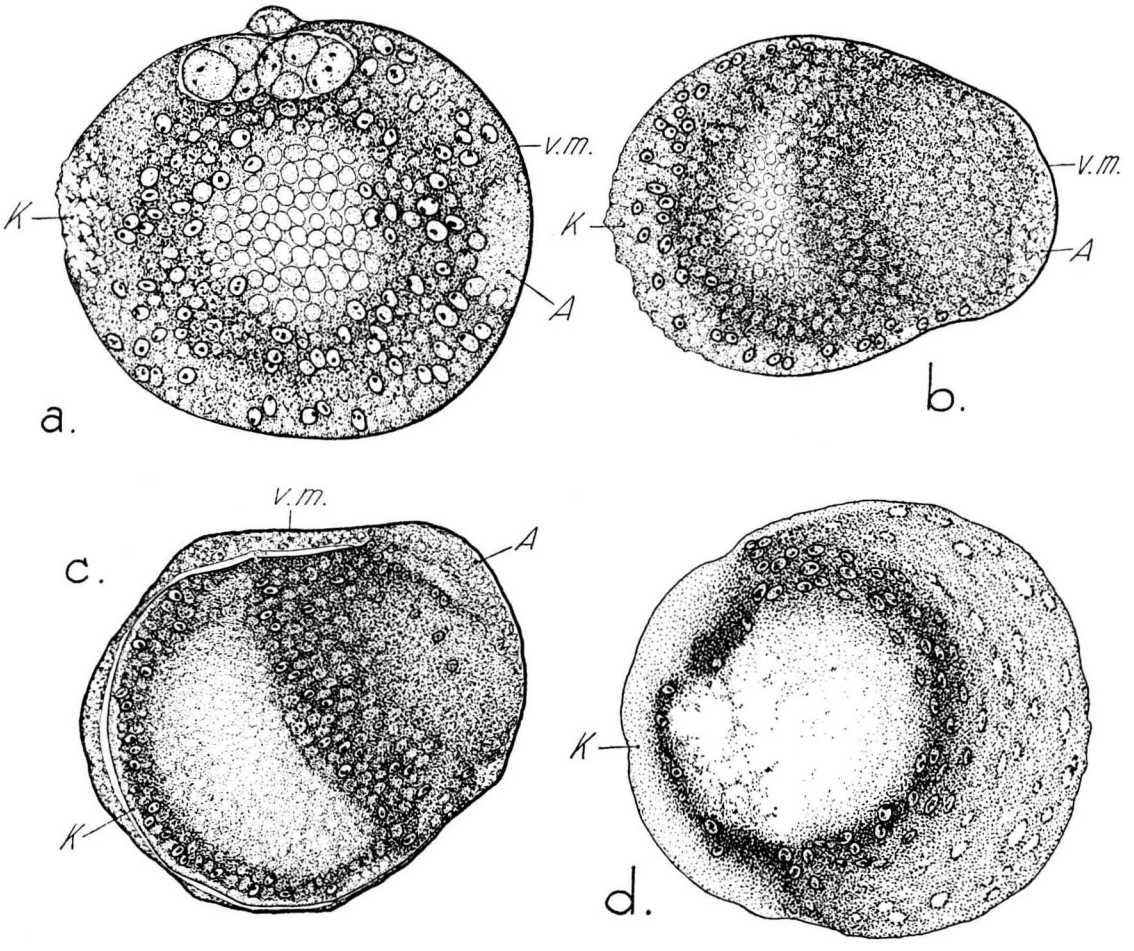

Fig. 4. Cytolysis of *Limnaea* eggs in electric field. *a*. Disintegration at anodal and kathodal pole. Hydration of egg centre. *b*. Beginning outflow of cytoplasm at anodal side. *c*. Outflowing cytoplasm spreading beneath vitelline membrane (v. m.). *d*. Stratification of cytolysed egg.

maturation spindle with its chromosomes is still clearly visible, but in one of the eggs, in which it is situated near the disintegrating region it is somewhat distorted.

Finally, an egg of RB I represents an advanced stage of cytolysis. The egg is now clearly stratified, and the opposite sides are distinguished by striking colour differences (fig. 4*d*). On one side, the dense superficial layer of the previous stage has condensed still more and now has a deep red colour in azan-stained sections (*K*). It is separated by a narrow blue layer from the hydrated area, which has enlarged, but is still situated nearer the kathodal side of the egg; the fine cytoplasmic network pervading it is still more reduced. The rest of the egg is blue and contains many vacuolar spaces; its structure is quite different from that of a normal egg. A somewhat denser layer surrounds the hydrated area. By the extension of the latter, the maturation spindle has been pushed aside and distorted.

Discussion.

1. The electrophoretic movements observed in the electric field show clearly that the egg capsules of *Limnaea* in distilled water have a negative electric charge. Presumably, the same holds true of the egg cells in the capsules, though their displacement is less conspicuous and might be caused by the electrophoresis of the substances of the egg capsule fluid. When the egg cells are decapsulated and transferred to distilled water, no electrophoretic movement or, at the utmost, a weak displacement towards the kathode takes place. Cytolysed eggs, however, immediately get a strong negative charge and move towards the anode. As a matter of fact, this refers only to those eggs bursting in the process of cytolysis; when the vitelline membrane remains intact, no such electrophoretic movement has been observed.

Neither in the egg capsule fluid nor in distilled water do the eggs orient with respect to the direction of the electric field; the animal pole may point in all directions.

2. Both in the capsules and in distilled water the eggs in an electric field pursue their development in a normal way synchronously with the controls. Eggs exposed in the capsules to a field of 80 V/cm for as long as 45 minutes may develop to normal trochophore or hippo-stage embryos. With decapsulated eggs the corresponding experiment could not be made, in consequence of the technical difficulties of rearing the eggs once they are out of their capsules. However, the study of eggs fixed after treatment has shown that such eggs exhibit no abnormalities in structure as long as cytolysis has not yet begun. No displacements of egg components, even in a field of 80 V/cm, have been found, with the exception of one batch, in which some eggs showed disturbances in the extrusion of polar bodies, or small lateral displacements of the 2nd polar body at the time of its extrusion. In another batch (RK I), in which the extrusion of the 1st polar body took place at the moment of fixation, no such displacements have been observed, however. Hence, we may say that in general the structure

of *Limnaea* eggs is not disturbed by the influence of an electric field, as long as they are intact. In this respect, our results correspond to those of GUILLIERMOND and CHOUCROUN (1936) who found that a field of the same order of magnitude did not influence plant cells as long as they were in the living state. To explain this fact, the authors mention two possibilities: either could the cell be surrounded by a protecting conductive layer, or its material could become polarized, so that the electric charges thereby produced at its surface keep in equilibrium the action of the external field. The latter explanation seems more probable.

3. Contrary to the eggs in the capsules, those in distilled water cytolyse in stronger electric fields. Great differences in susceptibility between different batches and individual eggs exist, however. Cytolysis does not occur at once, but after a certain latency period; this period is the shorter, on an average, the stronger the field is. During this latency period no visible changes of the egg can be observed. However, from the observation that, after a prolonged stay in a weak field, many eggs begin to cytolyse immediately after the intensity of the field has been moderately increased, we may conclude that during this latency period certain changes are going on in the eggs preparing the way for cytolysis. The vital staining experiments seem to show, however, that these changes do not consist in the appearance of pH differences in the eggs.

Once initiated, cytolysis takes a rapid and characteristic course. The first changes observed are a hydration in the centre of the egg, combined with a disintegration of cytoplasmic structure both at the anodal and kathodal pole of the egg. On the side of the anode, this leads to a total destruction of a circumscribed part of the egg cortex. At the same time, the colourability of the cytoplasm with azan (after Bouin fixation) changes from red to blue, beginning in the hydrated centre and spreading soon over most of the cytoplasm, with the exception of a narrow peripheral zone at the side of the kathode. At a certain moment, the cytoplasm begins to flow out through the gap at the anodal pole, spreading beneath the vitelline membrane, which may remain intact but in most cases soon bursts. The hydrated zone exhibits a further swelling and is displaced somewhat to the kathodal side. The differences in colourability of the egg substances intensify; the end of the process shows a total stratification of the egg. The whole process, from the beginning outflow of cytoplasm to a full stratification and disintegration of the egg, takes only a few seconds.

4. We may conclude from these observations that the egg substances resist eventual displacing forces due to the electric field as long as the egg cortex is intact. Once the integrity of the cortex is broken, these forces have free scope and lead to a rapid stratification of egg substances. The same has been observed by GUILLIERMOND and CHOUCROUN (1936) for plant cells; as soon as the cells are killed by a prolonged action of the field, electrophoresis begins and the elements of the cell are displaced according to the charge they have at that moment.

Summary.

1. The influence of an electric field on eggs of *Limnaea stagnalis* has been studied by means of an electric cell, in which the products formed at the electrodes were prevented to reach the eggs.

2. The egg capsules have a negative electric charge. The same holds true, perhaps, of the egg cells in the capsules. Decapsulated eggs in distilled water have no charge at all or only a weak positive charge. Immediately after cytolysis, however, they get a strong negative charge.

3. Neither in the egg capsule fluid nor in distilled water do the eggs orient with respect to the direction of the electric field.

4. Both in the capsules and in distilled water the eggs in an electric field may pursue their development in a normal way synchronously with the controls. Eggs treated in the capsules may develop to normal embryos. In general, no displacements of egg components occur, even in a field of 80 V/cm, as long as the eggs remain intact.

5. Decapsulated eggs in distilled water cytolyse in stronger electric fields. Cytolysis takes a rapid and characteristic course and leads in a short time to a total stratification of the egg contents and a disintegration of the egg.

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