ON THE VITELLINE MEMBRANE OF THE EGG OF *PSAMMECHINUS MILIARIS* AND OF *TEREDO NORVEGICA*

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(*Received 8th September*, 1931.)

(With Eight Text-figures.)

It has been assumed very generally that the unfertilised eggs of many animals are surrounded by a clear surface layer, discontinuous with the general protoplasm, which is known as the vitelline membrane. In marine forms this layer is commonly supposed to be separated from the true surface of the egg at the time of, or soon after, fertilisation and then becomes the fertilisation membrane.

There is no doubt that the eggs of very many, if not all, animals become enclosed after they have been fertilised in one or more layers or membranes which were not present, at any rate, in the same condition, before fertilisation. In some cases these, such as the ectoplasm or hyaline plasma layer of the Echinoderm egg, are formed relatively slowly and their development is not an immediate consequence of fertilisation. Other types, such as the fertilisation membrane of the Echinoderm egg, appear almost as soon as fertilisation is accomplished and form one of the most reliable criteria of this event.

The type of structure to which the term "fertilisation membrane" is generally applied is a membrane usually separated by a considerable space from the egg surface. It can as a rule be detected very soon after fertilisation, although the rapidity of its formation in the sea-urchin egg is by no means typical. In the Echinoderms there is a well-developed mechanism for pushing the membrane away from the egg surface (Hobson, 1927). In some other eggs, however, such a mechanism is not present unless it is in an imperfect form. This is seen in *Thalassema* in which the fertilisation membrane, although separated from the egg surface, is frequently not spherical. In *Teredo* the fertilisation membrane is usually fairly spherical but often lies close to the surface of the egg and is not easily distinguishable until the polar bodies are formed.

In the Echinoderms there has been for a long time much controversy concerning the origin of the fertilisation membrane. The majority of workers are of the opinion that it is present on the surface of the unfertilised egg as the vitelline membrane, and that this is merely pushed away from the surface of the egg when

fertilisation takes place. Others (*e.g.* Harvey, 1910; Gray, 1922) have supported the view that the fertilisation membrane is produced by the precipitation of a substance secreted by the egg. It is not necessary to discuss here in detail the evidence for these views, as this has already been done in an earlier paper (Hobson, 1927). It is sufficient to note that the conclusion was reached that the evidence available was not sufficient to warrant unqualified acceptance of either opinion.

It is evident that the hypothesis that the fertilisation membrane of the Echinoderm egg is present before fertilisation depends entirely on the demonstration of the presence of a vitelline membrane. The existence of this structure has been generally assumed, and it has been figured on several occasions as a distinct clear layer on the surface of the unfertilised egg.

It is necessary to consider briefly the possible structure of the surface region of an egg such as that of the sea urchin. There appear to be four possibilities: (1) The granular fluid cytoplasm extends to the surface of the egg which is bounded by an extremely thin membrane which may be fluid, *i.e.* a surface tension "membrane," or may be solid. (2) The granular cytoplasm may extend to the surface of the egg but may be solid at, and for some undefined distance below, the surface. (3) The surface may be bounded by a layer of solid material, free from granules, which has no definite inner limit and is continuous with the fluid ground substance of the cytoplasm. (4) The surface may be bounded by a membrane which has a clearly defined inner surface and is therefore discontinuous with the general cytoplasm.

The recognition of these possibilities is a matter of considerable importance in the study of living cells in general, and there appear to have been very few critical studies of the detailed morphology of the cell surface.

In view of these possibilities examination has been made of the surface structure of the eggs of two species of marine animals, *Psammechinus miliaris* and *Teredo norvegica*. In both of these a fertilisation membrane is formed. In the former it is separated by a considerable distance from the egg. In the latter it lies typically close to the egg surface from which it cannot be distinguished readily until the polar bodies are formed. These push out the fertilisation membrane over a small area so that it becomes clearly visible.

THE UNFERTILISED EGG OF *PSAMMECHINUS MILIARIS*.

In this form the cytoplasm appears to be composed of a hyaline ground substance in which are granules. These granules are arranged irregularly, sometimes isolated, but generally in more or less well-marked clumps or rows. The grouping of the granules gives to the cytoplasm an ill-defined alveolar appearance. Near the surface of the cytoplasm the granules are more numerous and more closely pressed together. The spaces or "alveoli" are constantly changing in size and shape owing to the continual slow movements of the granules. I have not, however, been able to detect the alveolar spheres described and figured by Wilson (1899, 1926) and other authors in the eggs of other species of Echinoderms. The appearance of the cytoplasm at the surface of the egg is represented in Fig. 1.

The surface of a cell is a peculiarly difficult object for study by direct observation with the high powers of the microscope[1]. Even when care is taken to avoid diffraction as far as possible, it is difficult to be certain that some of the apparent structures seen under a 1/12 in. objective are not due to this cause. In view of experiences with the egg of *Psammechinus miliaris* and especially with that of *Teredo*, where a vitelline membrane is certainly present, the suspicion cannot be avoided that the conspicuous vitelline membranes figured by certain authors are diffraction rings.

The observations of Carter (1924) on *Sphaerechinus granularis* are of interest in this connection. He figures the vitelline membrane as a conspicuous zone free from granules on the surface of the egg. No definite inner surface to the vitelline membrane is shown and this point is not mentioned in the text. His illustrations

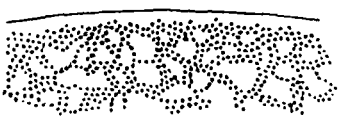

Fig. 1.

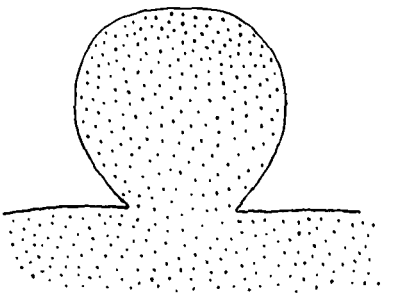

Fig. 2.

Fig. 1. *Psammechinus miliaris*. Semi-diagrammatic view of surface region of living unfertilised egg as seen with 1/12 in. oil immersion objective and × 12 compensating ocular. Note the irregular arrangement of micromeres between which are clear spaces of various shapes and sizes presumably corresponding to the macromeres of other authors. Note also the clear zone at the surface.

Fig. 2. *Psammechinus miliaris*. Unfertilised egg. Surface slightly torn with microdissection needle. Note bulging out of cytoplasm. New surface forms acute angle with old. Stippling is diagrammatic and does not represent true distribution and size of granules.

of the surface of the unfertilised egg agree with my own observations, except that the clear surface layer appears to be much thicker in *Sphaerechinus* than in *Psammechinus*. Carter figures and describes the fertilisation membrane arising outside the vitelline membrane. In *Psammechinus* I have never been able to satisfy myself by direct observation whether or not this is so. The delicacy and similarity in optical properties of the two structures in question is such that, in *Psammechinus*, it has so far proved impossible to distinguish them from one another even in eggs in which the fertilisation membrane is only partially formed.

That there is a solid layer at the surface of the unfertilised sea-urchin egg cannot be doubted. The wrinkling of the surface in hypertonic solutions could scarcely be produced in any other way, unless the whole egg sets to a jelly. That this is not the case is shown by the fact that, when the plasmolysis is slight, slow movements of granules can still be seen in the cytoplasm.

[1] In all the investigations recorded in this paper all high-power observations were made on eggs suspended in a hanging drop in order to avoid as far as possible any effects due to compression.

Prof. Chambers and the writer collaborated during the summer of 1930 in an endeavour to determine the structure of the surface of the unfertilised egg of *Psammechinus miliaris* with the aid of the microdissection apparatus. After repeated examination with 1/12 in. objective (Leitz) and × 12 compensating eyepiece it was concluded that it was impossible to demonstrate with certainty the existence of a definite vitelline membrane. Under the best optical conditions which we could obtain, when diffraction was at a minimum, the granules could be seen to approach the surface of the egg very closely. There remained, however, a thin superficial zone from which granules were absent (Fig. 1). The inner limit of this zone was not smooth or sharply marked. The appearance was not that of a differentiated membrane such as is implied by the term "vitelline membrane," but rather of a part of the clear matrix of the protoplasm from which granules were absent. This view was supported by experiments made with the microdissection needle. Pressing the needle against the surface of the egg in the plane of optical section failed to dispel the appearance of a very thin, clear surface layer. The tip of the needle remained separated by a short distance from the nearest cytoplasmic granules. When the needle was inserted immediately below the surface of the egg and then

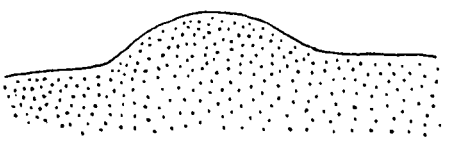

Fig. 3. *Psammechinus miliaris*. Unfertilised egg. Surface damaged, but not torn, with microdissection needle. Damaged region of surface pressed out slightly, forming obtuse angle with normal surface. Stippling is diagrammatic and does not represent true distribution and size of granules.

pulled outwards a cone of gelatinous material was drawn out, but there was no sign of the separation of a membrane as has been described by Chambers (1921, 1930).

If the surface of the egg is slightly torn with the microdissection needle, the cytoplasm exudes and forms a bulge with a smooth surface attached to the main part of the egg by means of a relatively narrow neck (Fig. 2). If the damage has not been too great, the exuded portion of the cytoplasm is gradually withdrawn and the egg presents once more a spherical contour. If the damage caused by the needle is greater, the whole of the exuded cytoplasm may not be incorporated in the egg again. Whenever exudation of cytoplasm takes place, the undamaged surface of the egg contracts so that it forms an acute angle with the surface of the outflowing protoplasm. If, on the other hand, the egg surface is slightly damaged and not actually torn, a rounded elevation develops whose junction with the normal egg surface forms an obtuse angle (Fig. 3).

The results of damaging the egg surface with the microdissection needle show clearly the presence of a solid surface layer. This layer is elastic, as is shown, not only by the spherical form of the normal egg, but also by the way in which it contracts when torn, forcing out part of the egg contents, and by the sharp angle

formed with the newly exposed cytoplasm. Local damage without rupture weakens the solid surface layer so that a slight protuberance is formed. In such a case the strength of the damaged surface layer is still sufficient to prevent the formation of a true exudation of cytoplasm.

The action of hypertonic and hypotonic solutions does not throw much light on the structure of the surface of the unfertilised egg. As these will be considered in more detail in a succeeding paper, they will be mentioned only briefly here.

When the eggs are placed in hypertonic solution shrinkage occurs. This process is divisible into two distinct phases. Firstly the egg contracts with a perfectly smooth surface. Later the surface becomes covered with innumerable fine wrinkles. These become more marked as time goes on and vary, of course, with the strength of the solution employed. The egg shrinks uniformly and remains approximately spherical.

The observed effects can be explained on the view that the egg is surrounded by an elastic, solid layer which is normally in a state of tension. When the volume of the egg is reduced by removal of water this layer contracts until it is completely relaxed. After this, further diminution of the volume of the egg merely results in throwing the attached surface layer into folds.

The response of the egg to a hypotonic solution such as tap water is characteristic. The egg swells and cytolyses. A slight bulge with a faintly irregular surface appears on one side of the egg. There is no outflow of the egg contents, but the cytoplasm becomes clear and only slightly granular. When cytolysis is complete the surface of the egg is smooth, sharply defined and forms a perfect sphere. The appearance strongly suggests the presence of a solid membrane which is not destroyed by the swelling of the egg and which persists, surrounding that part of the contents which has been unable to diffuse through it into the surrounding medium.

THE UNFERTILISED EGG OF *TEREDO NORVEGICA*.

The microscopic examination of the surface of the egg of *Teredo norvegica* presents difficulties similar to those encountered in that of *Psammechinus*. The effects of hypertonic and hypotonic solutions, however, enable a definite conclusion to be reached concerning the existence of a vitelline membrane on the unfertilised egg. The results obtained serve to emphasise the need for great caution in describing the structure of the surface in the sea-urchin egg.

The egg of *Teredo norvegica* is about 50μ in diameter. When shed into sea water by an animal removed from the wood in which it lives, the nucleus is intact, sometimes spherical, but frequently more or less wrinkled. Maturation does not begin until fertilisation takes place or some method of artificial activation is employed.

Under the high power of the microscope an optical section of the surface region of this egg presents an appearance somewhat similar to that of *Psammechinus miliaris*. The granules of the cytoplasm are slightly larger and seem to be rather less numerous in the peripheral part of the egg than in the zone round the nucleus.

Surrounding the egg is a narrow, clear layer somewhat wider than that found in the egg of *Psammechinus*. A distinct inner surface to this layer could not be made out. It appeared to be directly continuous with the granular cytoplasm.

A few attempts to separate the clear layer from the rest of the egg with the microdissection needle failed. The only result was to draw out a cone of clear substance like that which has been described for *Psammechinus*.

When the egg of *Teredo* is fertilised no immediate visible change takes place in the structure of the surface. The fertilisation membrane is not separated from the rest of the egg as in the Echinoderms. The clear layer may be a little thicker, but it is difficult to be certain of this.

When maturation takes place, however, the fertilisation membrane can be seen distinctly at the point where it is stretched over the polar bodies (Fig. 4). It then appears as a clear, rather thick membrane which is, except in this part, so closely adherent to the surface of the egg as to be almost indistinguishable as a separate structure.

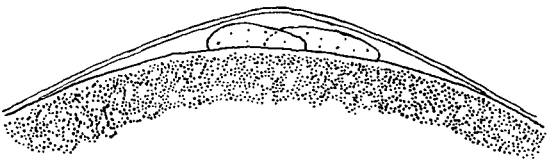

Fig. 4. *Teredo norvegica*. Surface of fertilised egg showing two polar bodies causing local elevation of vitelline (fertilisation) membrane.

The existence of a true vitelline membrane on the egg before fertilisation has been accomplished or maturation begun can be demonstrated by the action of either hypotonic or hypertonic solutions.

The hypotonic solution used was tap water. The Plymouth tap water is collected from the granite district of Dartmoor and is almost free from dissolved salts. When unfertilised eggs are placed in this medium they cytolyse in about 2 min. as a rule. When cytolysis takes place the egg bursts at one point on its surface. A little jet of granular material emerges from the aperture and gradually disperses in the surrounding water (Fig. 5*a*). Then the nucleus, which is now spherical and turgid is forced through the opening which becomes much enlarged by its passage (Fig. 5*b*). Finally the greater part of the granular material of the cytoplasm also passes out of the opening (Fig. 5*c*). All that remains of the egg is a collapsed and much crumpled, clear membrane to which are still adhering a few scattered granules (Fig. 5*d*).

The hypertonic solutions employed were made either by evaporating sea water to about 35 per cent. of its original volume or by the addition of 2·4 *M* NaCl to normal sea water.

If unfertilised eggs are put into a hypertonic medium they assume a very characteristic form. The original spherical shape is lost. The surface becomes indented in one or more places and the appearance is that of a ball of plastic substance, such as clay, squeezed between the finger tips. This shape is similar to the

unfertilised eggs of some other animals, such as *Thalassema*, in normal sea water. It may be called "polyhedral," although it must of course be remembered that the facets of the "polyhedron" are not flat. In the plasmolysed egg of *Teredo* the nucleus is distorted in the same way as the whole egg (Fig. 6*a* and *b*).

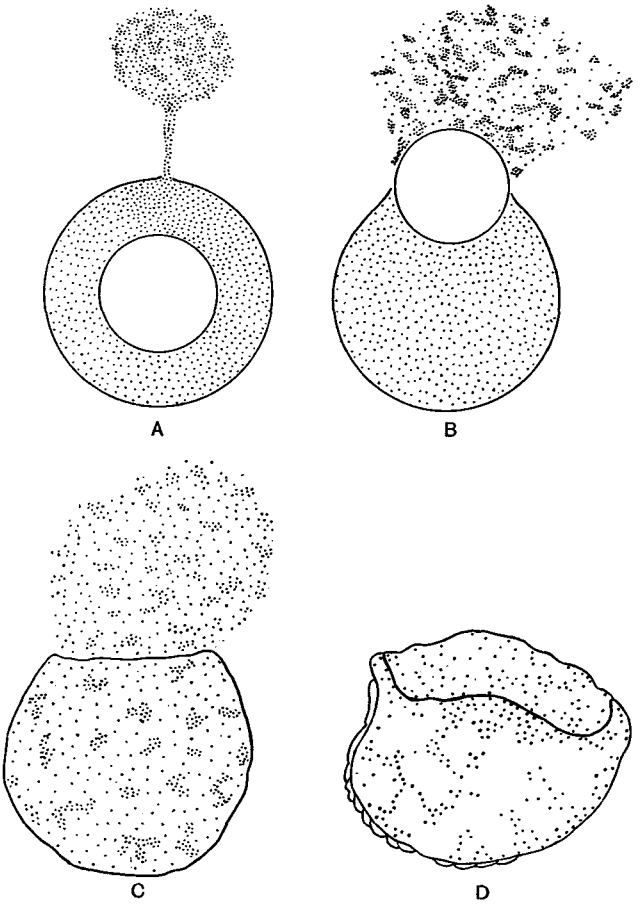

Fig. 5. *Teredo norvegica*. Successive stages in cytolysis of unfertilised egg in tap water. *a*, cytoplasm shooting out through small rupture in surface and dispersing; *b*, nucleus squeezing out and enlarging rupture; *c*, cytolysis almost complete. Nearly the whole of the cell contents has passed out through the large aperture in the vitelline membrane; *d*, cytolysis complete. All that remains is the crumpled vitelline membrane with a few granules adhering to it. Stippling of cytoplasm in Figs. 5*a–c* is diagrammatic and is merely intended to represent relative changes in granulation. Fig. 5*d* is a camera lucida sketch.

If the eggs are put into strong hypertonic solution, such as full strength evaporated sea water or a mixture of equal volumes of normal sea water and 2·4 *M* NaCl, the first visible change is a distortion of the whole egg as described above. After a time varying from 5 to as much as 20 min. a clear membrane begins to separate from the surface of the egg over the indented regions. The separation takes place rapidly, but generally does not extend beyond the concave parts of the surface.

The result is that, at first, the egg is irregular in shape and is enclosed within a spherical membrane. Gradually the protoplasmic surface becomes smooth and rounded, so that finally the egg is more or less spherical but is still attached to the membrane over part of its surface. Occasionally the separation of the membrane is complete.

These facts can be explained on the assumption that a tough elastic membrane surrounds the unfertilised egg. This membrane is firmly attached to the surface of the protoplasm but is not directly continuous with it. The strain in the membrane is greatest in the concave areas where it is most distorted, and here it will separate most readily and, once separated, it will assume its natural spherical form.

The firmness with which the membrane is attached to the surface of the protoplasm can be seen by examining the process of separation. As the membrane draws itself away from the surface of the egg, strands of protoplasm are pulled out with it. These may be slender threads or broad, flame-shaped processes. This is followed by slow retraction of these strands which seem as though composed of a

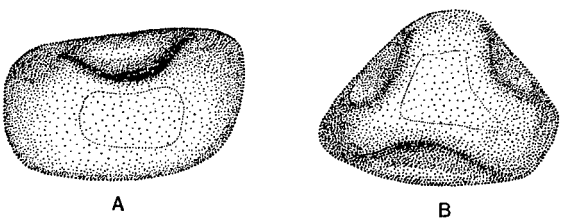

Fig. 6. *Teredo norvegica.* Two unfertilised eggs in hypertonic solution showing the type of plasmolysis. The indentations are often more numerous and complicated than in these examples.

viscous fluid. During retraction a strand often breaks in the middle, and in this case each half contracts rapidly, the one to form a small cone which gradually disappears on the surface of the egg, and the other persists as a globule of cytoplasm attached to the inner surface of the separated membrane.

This type of behaviour is of particular interest, as it shows clearly that even intense plasmolysis is not accompanied by gelation of the protoplasm. The appearance described could hardly be produced unless the consistency of the cytoplasm was that of a somewhat viscous fluid. This is supported by the fact already mentioned that slow movement of granules can be seen both in the normal and in the plasmolysed egg. The polyhedral form of the egg must therefore be due to the properties of the vitelline membrane and not to the gelation of the protoplasm.

After fertilisation and even after cleavage the attachment of the membrane to the surface of the egg or the blastomeres is hardly less firm than in the unfertilised egg. Exactly the same phenomena occur in hypertonic solutions, although the separation of the membrane takes place more rapidly.

Figs. 7 and 8 illustrate typical examples of the cytoplasmic processes described above and their withdrawal.

DISCUSSION.

The results of the observations described above show that there are points in common between the eggs of *Psammechinus miliaris* and *Teredo norvegica*, although they are in many respects profoundly different.

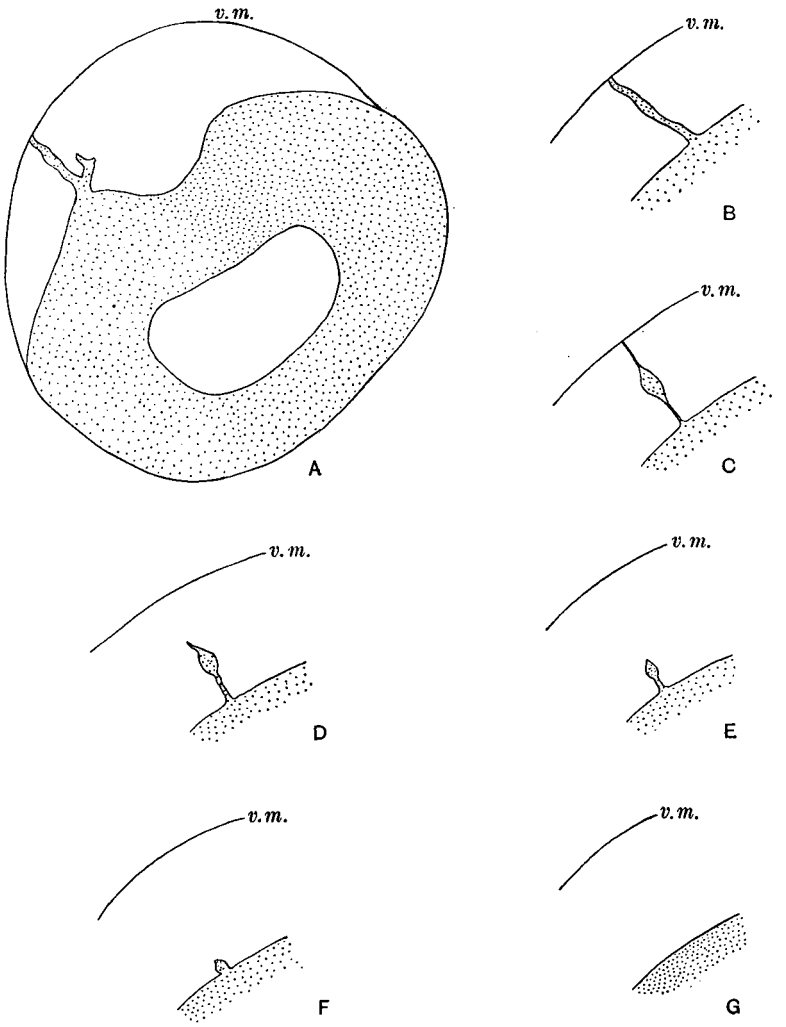

Fig. 7. *Teredo norvegica*. Plasmolysed unfertilised egg showing the separated vitelline membrane (*v.m.*) and successive stages in the withdrawal of a protoplasmic filament attached to the vitelline membrane. Stippling is diagrammatic and does not represent true distribution and size of granules.

In neither case does direct observation of the unfertilised egg under normal conditions throw much light on the structure of its surface. All that can be distinguished is the granular cytoplasm covered externally by a thin layer of hyaline material. No definite inner limit to this hyaline layer can be made out. Either it is

continuous with the general cytoplasm or is so closely attached to the true cytoplasmic surface that it cannot be distinguished from it. The behaviour of the eggs of *Teredo* in hypertonic solutions strongly suggests the latter possibility. Tap water serves as an agent by means of which the solid, hyaline surface layer may be separated from the rest of the egg.

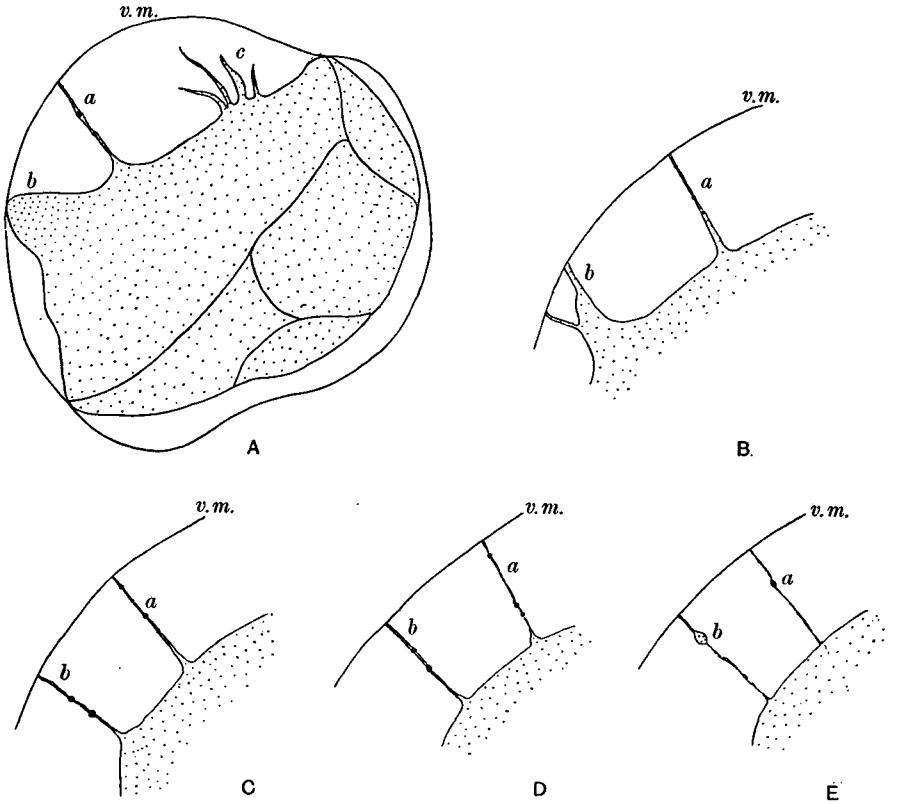

Fig. 8. *Teredo norvegica*. Plasmolysis of an egg which has been fertilised and has undergone cleavage, showing successive stages in the withdrawal of two protoplasmic filaments, *a* and *b*, attached to the vitelline membrane (*v.m.*). The filaments at *c* were attached to the upper part of the vitelline membrane. Filament *b* eventually broke in the middle and the outer part formed a small globule of protoplasm attached to the inner surface of the vitelline membrane. Note that the vitelline (fertilisation) membrane is more completely detached from the surface of the egg than in the unfertilised egg. Stippling is diagrammatic and does not represent true distribution and size of granules.

The eggs of *Psammechinus* are such that they do not lend themselves to an equally clear demonstration of a separate surface layer. Chambers (1921) has described the removal of the vitelline membrane from Echinoderm eggs by means of the microdissection needle. Lack of success in similar experiments on the egg of *Psammechinus* does not necessarily mean that such a membrane does not exist, since, as has been described above, the egg of *Teredo* also resisted efforts to dissect away the vitelline membrane. It merely serves to emphasise the firmness with which the membrane, if present, is attached.

The behaviour of the egg of *Psammechinus* in hypertonic solutions and in tap

water lends support to the view that the surface is covered by a clear, elastic layer. It is difficult to account for the smooth, spherical condition of the egg when cytolysed in tap water in any other way.

If we assume, as a working hypothesis, the existence of a distinct vitelline membrane on the surface of the eggs of these two forms, it becomes possible to suggest a tentative explanation of certain difficulties in the problem of the origin of the fertilisation membrane.

In the Echinoderms the origin of the fertilisation membrane has for long been a controversial matter. The most recent general discussions are by Hobson (1927) and Runnström (1928). While some consider that the fertilisation membrane is a new structure formed as the result of fertilisation, it is more generally held that the vitelline membrane is merely lifted from the surface of the egg. It has been objected by the present writer among others that the fertilisation membrane is so different in its properties from the vitelline membrane as to render it difficult of identification with the latter.

The first step in this identification is obviously to establish the existence of a differentiated vitelline membrane on the unfertilised egg. As has already been shown, this is by no means an easy matter and, although the evidence is in favour of it, its existence cannot be considered a certainty in *Psammechinus*.

The Echinoderm fertilisation membrane has properties which are certainly different from those which can be ascribed to the hyaline surface layer of the unfertilised egg. It is relatively thick, rigid and almost inelastic. Before the fertilisation membrane has reached its final condition, *i.e.* during the process of raising it from the surface of the egg, it is elastic and extensible, thus resembling the hyaline surface layer. The change in the properties of the fertilisation membrane after it has left the surface of the egg is dependent on the presence of calcium in the sea water. If eggs are fertilised in normal sea water and transferred within 1 min. to Ca-free sea water the fertilisation membranes arise as usual. Gradually, however, they sink back on to the surface of the egg so that they can scarcely be distinguished with the 1/12 in. objective. The process of sinking back may take up to 30 min. to complete, although, as a rule, it is accomplished more rapidly. Prof. Chambers and the writer found that if, after the fertilisation membrane had sunk back again, the surface of the egg was punctured in calcium-free sea water so as to cause outflow of cytoplasm, some of the material penetrated below the fertilisation membrane and caused it to rise up once more.

The explanation seems to be simple. Elevation of the fertilisation membrane is accomplished by means of osmotic pressure due to the presence of an amphoteric electrolyte released at fertilisation. This substance can probably diffuse slowly through the membrane, as has been shown by Garrey (1919) for peptone. Unless the membrane is hardened by the sea water it will collapse when the osmotically active substance is removed. When a further supply of similar material appears beneath the membrane, as when the egg is torn, it rises once more. This view is also supported by the fact that removal of eggs with collapsed membranes to normal sea water does not cause a second elevation.

The mechanical properties of the fertilisation membrane when it first appears are somewhat similar to those we have postulated for the hyaline surface layer or vitelline membrane. These properties are maintained for an indefinite period so long as calcium is absent from the surrounding medium.

It is well known that hypertonic sea water is effective in causing parthenogenesis in the eggs of many species of animals. Its action appears to be twofold. Firstly it causes the formation of asters. Secondly it is a membrane-forming agent. In forms such as *Teredo* where the vitelline membrane is comparatively loosely attached to the surface of the egg and in which there is no mechanism for elevating it as the fertilisation membrane, the latter is formed in the hypertonic solution. In the sea-urchin egg, on the other hand, there is a well-developed mechanism for elevating the fertilisation membrane, and if we accept the identification of this structure with the vitelline membrane we may perhaps regard the action of hypertonic solutions in artificial parthenogenesis as loosening the attachment of the membrane to the surface of the egg. Elevation of the fertilisation membrane does not take place until the egg is removed to normal sea water, since the increased concentration of salts in the hypertonic solution lowers the osmotic pressure due to the protein within the membrane (Hobson, 1927).

SUMMARY.

1. Direct microscopic examination of the unfertilised eggs of *Psammechinus miliaris* and *Teredo norvegica* merely shows the existence of a thin, granule-free zone covering the surface. Whether this is continuous with the general cytoplasm or not cannot be made out with certainty by direct observation.

2. A cone of clear material can be drawn out from the surface of the unfertilised egg of both species by means of the microdissection needle. A definite membrane cannot be separated in this way.

3. Hypertonic solutions cause the egg of *Psammechinus* to shrink smoothly at first and later to become wrinkled. This is consistent with the view that the egg is surrounded by an elastic, solid layer which is normally in a state of tension.

4. Cytolysis of the egg of *Psammechinus* in tap water is not accompanied by bursting. The egg swells and is perfectly smooth and spherical when cytolysis is completed. This points to the existence of an elastic, solid surface layer.

5. Plasmolysis of the egg of *Teredo* is of the type here called "polyhedral." The irregular shape of the egg in the hypertonic solution is only temporary, as a clear membrane separates from the concave surfaces and the egg then becomes more or less spherical.

6. The protoplasm of the plasmolysed egg of *Teredo* behaves as a viscous fluid.

7. Cytolysis of the egg of *Teredo* in tap water is accompanied by bursting and dispersion of the entire cell contents. A crumpled membrane alone remains.

8. It is concluded that the unfertilised egg of both *Teredo norvegica* and *Psammechinus miliaris* is surrounded by an elastic vitelline membrane which is much

thicker in the former than in the latter. The vitelline membrane in both cases is tightly attached to the egg surface.

9. In calcium-free sea water the fertilisation membrane is elevated normally in *Psammechinus miliaris*. It does not harden, however, and gradually sinks back on to the surface of the egg owing, apparently, to the loss by diffusion of the osmotically active substance in the perivitelline space. It can be elevated a second time by puncturing the surface of the egg and allowing some of the cell contents to penetrate into the perivitelline space.

10. It is suggested that one action of hypertonic solutions in inducing artificial parthenogenesis may be to cause a loosening of the attachment of the vitelline membrane to the egg surface.

This work has been done at the Laboratory of the Marine Biological Association at Plymouth, partly during the tenure of the Ray Lankester Investigatorship, to the trustees of which I am very grateful. I wish to express my thanks also to Dr E. J. Allen, F.R.S., and to the staff of the laboratory for their interest and assistance in many ways. I am indebted to the Royal Society and the British Association for the use of tables, during the tenure of which part of this work was done. Part of the expenses were defrayed by a grant from the Earl of Moray Endowment of the University of Edinburgh.

THE FOLLOWING NOTE IS ADDED BY PROF. R. CHAMBERS (*August*, 1931).

The question as to whether the fertilisation membrane pre-exists as some sort of a membrane on the surface of an unfertilised Echinoderm egg is a difficult one to answer.

In the case of the starfish common at Woods Hole (*Asterias rubens*) a vitelline membrane undoubtedly exists on the unfertilised egg, and it is this membrane which separates off to form the fertilisation membrane (Chambers, 1921). The difference in consistency of the membrane before and after separating can be demonstrated by injecting a small amount of sea water under the membrane, and then fertilising the egg. The injection causes the elevation of a local blister, the contour of the egg remaining uniform. A pronounced adhesion of the membrane to the surface of the egg is indicated by the fact that the blister remains localised with a sharp angle at the edges of the blister. When the egg is fertilised the separation of the membrane spreads over the egg and it is important to note that, with insemination of the egg, the membrane stiffens and the localised blister flattens out with a corresponding sinking in of the surface of the egg at the region where the blister had been previously. The indentation in the egg gradually diminishes and the egg returns to its spherical shape with a more or less uniformly contoured fertilisation membrane surrounding it.

In the case of the unfertilised Arbacia egg it is far more difficult to demonstrate the existence of a vitelline membrane. This can be done, however, by centrifuging

the egg at a rate of 8000 revolutions per sec., for 2 to 3 min. The forces elongate the egg into a cylindroid. At the heavier pole of the cylindroid it is possible to pull off, with microneedles, the remains of a wrinkled and tenuous membrane. Apparently the centrifugal force has ripped the membrane and carried it as a collapsed sheet to the heavier pole of the egg. These eggs undergo fertilisation with no development of a fertilisation membrane. In the case of an unfertilised Arbacia egg it has been impossible, by means of microneedles, to pick off any such membrane. In this respect these eggs resemble those examined by Mr Hobson and myself at Plymouth. However, by pricking the surface of the Arbacia egg at several spots, or by pushing the egg about as it adheres to the glass coverslip, the egg so treated will, when inseminated, develop an incomplete and collapsed fertilisation membrane.

If we are to conclude that the fertilisation membrane has as its precursor a vitelline membrane the fact remains that this precursor is far weaker and more delicate than the membrane which results after insemination.

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