

# FERTILIZATION

Lord  
Rothschild

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BY

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LONDON : METHUEN & CO. LTD

NEW YORK : JOHN WILEY & SONS, INC.

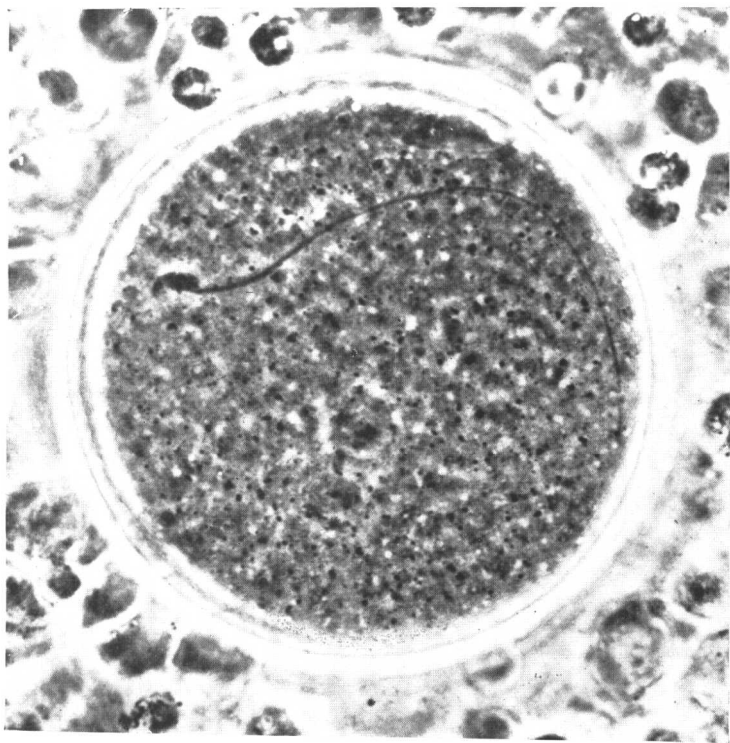


PLATE I

A live fertilized mouse egg, showing the whole spermatozoon in the cytoplasm. Positive phase contrast,  $\times 770$ . Photograph by J. Smiles.

## PREFACE

THIS book is intended for those who have read, or are reading, Gray's *Experimental Cytology*, Heilbrunn's *Outline of General Physiology*, Fruton & Simonds' *General Biochemistry*, Höber's *Physical Chemistry of Cells and Tissues* and similar textbooks. The subject is the life of the egg from the attachment of the fertilizing spermatozoon to the fusion or apposition of the male and female pronuclei. This process, except in mammalian eggs, usually takes a little less than one hour. Even so, several important subjects have had to be omitted: some of these are: (1) *Fertilization in the decapod crustacea and in sponges*. Both of these are too far removed from 'normal' fertilization to be included in a comparatively short book; but there are excellent accounts of them by Bloch (1935) and Tuzet (1950). (2) *Asters and the origin of the first cleavage amphiaster*. Much has been written recently on these, apart from the relevant sections in some of the textbooks mentioned above. (3) *Androgenesis and Gynogenesis*. (4) *Merogony*. (5) *Parthenogenesis*. A comprehensive review of parthenogenesis has been published by Tyler (1941b); but reference to the General Index will show that the subject is occasionally mentioned. (6) *Fertilization in the plant kingdom*. Although two chapters are devoted to this subject, its treatment is far from systematic.

The scope of this book precludes any discussion of cleavage, which is frustrating; not only because cell division is such a dominantly important subject, but also because important papers such as Brachet's *Constitution anormale du noyau et métabolisme de l'embryon chez les Batraciens* (1954) cannot be considered.

*References.* Modern reviews sometimes consist mainly of a list of papers with little or no attempt at evaluation. Even if this practice served some useful purpose it would be inappropriate in a short book. The papers referred to represent a limited selection from the immense number on fertilization written during the last hundred years and, as a rule, I have excluded the following: (1) references to work which has recently been repeated, under more modern conditions. But attention is sometimes called to early papers on subjects in which there has been a revival of interest, such as cortical granules and the effect of calcium on the hardening

of the fertilization membrane, it having been forgotten or ignored that these were first described some forty years ago. (2) References to brief and scrappy papers which have not been followed up. Some exceptions to this rule will be found in chapter 7, Metabolic and Other Changes at Fertilization. (3) References to papers which I do not think good. Where work has been, or might be, wrongly accepted as true, I have drawn attention to the errors in it. But, in general, such papers have not been mentioned.

Every writer of a book on fertilization must be uncomfortably aware of his sins of omission and commission, so great is the labour imposed by the literature on the subject. The author is no exception and proffers his apologies.

*Index of Plants and Animals.* There are three columns in this index. The first gives the name of the organism, some of the familiar synonyms and the English or American names, when known. The second column states the order and class to which the animal or plant belongs. When I was a child, my father expected my sisters and myself to know the Latin names of the plants, bees and butterflies which we had to collect. It was inevitable therefore that an Index of Plants and Animals should figure in this book. But there was a more cogent reason. Reference will be found in several places to the specificity of fertilization, to the alleged specificity of the polysaccharides in egg jelly, and to interspecific, intergeneric and interphyletic cross-fertilization. We cannot think clearly about such subjects, nor describe and compare experiments relating to them, unless we are reasonably sure of the identity of the organisms concerned. Reference to the Echinoid synonyms shows that this is not always easy. A diverting example of the confusion which springs from careless nomenclature is to be found in a paper by Mitchison & Swann (1954b), which is discussed, for other reasons, in chapter 8. These authors measured the elastic modulus of the cortex of the unfertilized egg of the sea-urchin *Arbacia lixula* (Linn.). With the aid of their own and E. N. Harvey's measurements (1931), they calculated the tension at the surface of the unfertilized egg of an 'American species of *Arbacia*' (p. 469), which they refer to as *Arbacia pustulosa*. *Arbacia lixula* (Linn.) and *Arbacia pustulosa* (Leske) are synonyms for the same sea-urchin, although Harvey actually used the eggs of *Arbacia punctulata* (Lamarck) in the experiments in question.

Some Latin names of organisms are abbreviated after they have once been mentioned. If a reader is in doubt, reference to the Index of Plants and Animals will provide the full name. Its preparation was made very much easier by the assistance of Sir Gavin de Beer, F.R.S., and Dr H. W. Parker, of the Natural History Museum, London.

*'Theories' of fertilization and activation.* The desire to formulate a new theory of fertilization seems almost to be an occupational disease of the gametologist. Such theories have been connected, at one time or another, with the names of Boveri, Bataillon, Dalcq, Delage, Heilbrunn, F. R. Lillie and Loeb. At the present time we have gone too far—and yet not far enough—to formulate theories, or even to make an 'Attempt at a Comprehensive View', as Runnström tried to do in 1949. One function of a new theory is to provoke further experiments and, although I have no new theory of fertilization to offer, I hope that this end, at least, will be achieved.

*Acknowledgments.* I am particularly indebted to Professor Sir James Gray, F.R.S., who has been my mentor for twenty-five years; to Dr George Beadle, Chairman of the Biology Division of the California Institute of Technology, for his help and for his hospitality at 'Caltech', where most of this book was written; to Professor Albert Tyler for valuable advice and criticism, not all of which has been taken; and to the Medical Research Council for financial aid. In addition I am glad to record my thanks to the following scientists and non-scientists for their help and advice: Dr R. D. Allen; Dr C. R. Austin; Dr J. Beament; Miss G. Bending; Prof. J. Brachet; Miss M. Brewster; Dr R. R. A. Coombs; Prof. E. G. Cox, F.R.S.; Dr G. Fankhauser; Prof. L. V. Heilbrunn; Prof. A. L. Hodgkin, F.R.S.; Mr A. F. Huxley, F.R.S.; Dr G. W. Kenner; Dr M. E. Krahle; Dr T. R. R. Mann, F.R.S.; Dr R. Markham; Dr R. E. F. Matthews; Dr J. M. Mitchison; Dr F. Moewus; Prof. A. Monroy; Prof. C. Niemann; Prof. L. Pauling; the Hon. Miriam Rothschild; George Rylands; Prof. E. C. Slater; Prof. M. M. Swann; Dr E. Vasseur; and Prof. L. Zechmeister.

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## CHAPTER I

### THE MORPHOLOGY OF FERTILIZATION

FERTILIZATION is the incitement of an egg to development by a spermatozoon, together with the transmission of male hereditary material to the egg. At fertilization the spermatozoon contributes *a*, the stimulus for development; *b*, a set of chromosomes embodying the paternal contribution to the genetic make-up of the zygote; and *c*, a central body which gives rise to, or is concerned with, the machinery for cell division. In some cases the spermatozoon, according to its point of entry into the egg, also determines the plane of bilateral symmetry of the embryo. Fertilization is specific and crosses between different sorts of animals are almost always impossible. Apart from a few exceptional cases to be discussed later, fertilization is irreversible. Once an egg has been fertilized, it cannot be re-fertilized, and once an egg has been stimulated to develop parthenogenetically, fertilization cannot be superimposed on parthenogenesis.

Fertilization can be divided into two phases. The first occurs when the homologous spermatozoon collides with and becomes attached to the egg surface. This is sufficient to set off a train of reactions in the egg which may lead to development. This first phase is called *activation* and one talks about a spermatozoon activating an egg or an egg being activated by a parthenogenetic agent. The spermatozoa of the worm *Rhabditis monohystera* Bütschli activate eggs of the same species so that they develop 'parthenogenetically', without containing any male hereditary material (Peacock, 1944). This phenomenon is known as pseudogamous fertilization and it can be achieved experimentally, by mixing homologous eggs and spermatozoa and separating them after a short time (F. R. Lillie, 1912*b*; Rothschild, 1953), or by heterologous insemination. Bataillon, for example, observed in 1909 that the spermatozoa of *Triturus alpestris* (Laurenti) activated the eggs of *Pelodytes punctatus* (Daudin) pseudogamously and it was this observation which led him to carry out his famous experiments on the parthenogenetic activation of frogs' eggs by puncturing them with fine glass needles. Both Loeb (1913) and



Godlewski (1912) made similar observations following heterologous insemination.

The second phase of fertilization is concerned with the events which take place after the spermatozoon has entered the egg, culminating in the disappearance of the sperm head and the egg nucleus as separate entities. Strictly speaking, therefore, fertilization begins with the sperm-egg collision and ends with syngamy, the fusion or apposition of the germ nuclei, when the spermatozoon loses its individuality. This series of reactions may take less than an hour; but the student of fertilization inevitably finds himself asking questions about the pre-fertilization behaviour of eggs and spermatozoa, the domain of the gametologist, and about the activity of the egg after syngamy, the domain of the embryologist. Examination of the pre-fertilization behaviour of the gametes must accompany any study of fertilization and this may well seduce the student away from his intractable problem. Mention has been made of spermatozoa colliding with eggs; why should they be anywhere near each other? Nature answers this question in bewildering and fantastic ways: the archegonia of plants produce chemicals which attract spermatozoa; dogfish and human beings rely on copulation to ensure sperm-egg collisions. Provided there is no moon, the male *Platynereis megalops* (Verrill) deposits spermatozoa in the mouth of the female, which bursts in about six seconds, liberating fertilized eggs into the sea (Just, 1914). Some further aspects of this problem, the liberation of spermatozoa and eggs in the right place and at the right time, are discussed in later chapters.

*Maturation.* The condition of the egg before fertilization, particularly as regards the stage of maturation it has reached, should always be borne in mind when trying to gain some understanding of fertilization. It has been insufficiently emphasized that echinoderm eggs, on which so many experiments have been carried out, are in an exceptional condition from the point of view of maturation, at the time of fertilization. Fig. 1, which is adapted from Dalcq (1952), explains this point. In sea-weeds, coelenterates, and echinoderms, and not all of them, the egg is fertilized after maturation (Class 4 fertilization). In all vertebrates and *Branchiostoma*, fertilization takes place at the second maturation metaphase (Class 3 fertilization), though there is some evidence that fox, dog and horse eggs may be fertilized in the germinal vesicle stage (Pearson & Enders, 1943; van der Stricht, 1923;

Hamilton & Day, 1945). In the eggs of *Ciona*, *Chaetopterus*, *Cumingia* and *Mytilus*, fertilization occurs at the first maturation metaphase (Class 2); while in sponges, *Nereis*, *Spisula*, *Urechis caupo* Fisher & MacGinitie (Plate II), *Ascaris* and *Sagitta*, the egg

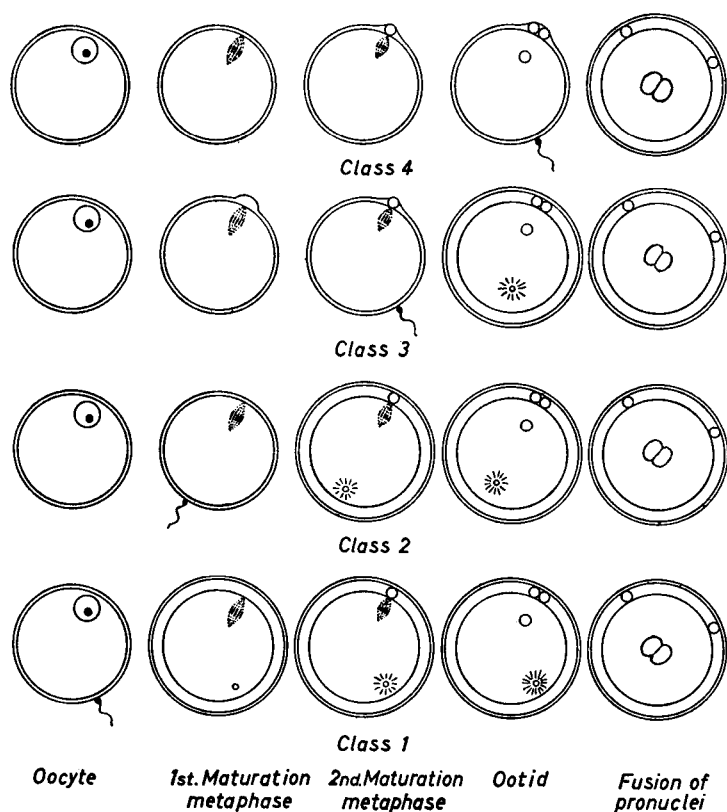


FIG. 1.—The four stages of egg maturation at which fertilization occurs in the animal kingdom, after Dalcq (1952).

is fertilized before the germinal vesicle of the oocyte has broken down, that is before either maturation division (Class 1).<sup>\*</sup> These four classes should be remembered when studying fertilization, as we are sometimes tempted to make generalizations based on

<sup>\*</sup> Needless to say, there are exceptions to this classification, e.g. starfish eggs, which come into Classes 1, 2, 3 and 4.

the behaviour of eggs in one class when those in other classes behave differently. There is, admittedly, a tendency for the sperm nucleus to remain relatively quiescent within the egg until after the formation of the second polar body; but this is not a sufficient reason for ignoring the fact that in the majority of *phyla*, fertilization does not occur at the same time in the life-history of the unfertilized egg as it does in sea-urchins. We shall return to this question when considering the metabolism of fertilized and unfertilized eggs.

*Jelly and membranes.* In some cases, there are what appear to be barriers between the egg and the spermatozoon. In echinoderms and frogs, for example, this barrier takes the form of a gelatinous shell round the unfertilized egg, through which the spermatozoon must bore or burrow to reach the egg surface. The egg of the salmon is surrounded by a rather tough chorion, which is impermeable to spermatozoa except at one point, the micropyle; this is a narrow channel in the chorion, through which spermatozoa must pass to reach the egg. When an unfertilized salmon egg is put into fresh water, the chorion hardens, the micropyle becomes occluded and the egg is unfertilizable. This is one of the reasons why breeders of trout and salmon mix eggs and spermatozoa 'dry', before dilution with fresh water, though not all of them realise that the success of 'dry' insemination is due to the egg micropyles remaining open in the presence of seminal plasma as opposed to fresh water. The other reason for mixing salmon or trout eggs with their respective semen in the 'dry' condition is because the spermatozoa of these fish only live for a few minutes after dilution with fresh water.

Many insect eggs are surrounded by hard and, one would have thought, impermeable egg shells, which contain several so-called micropyles. Insect spermatozoa, however, do not necessarily make use of these structures, which are often too small for the passage of a spermatozoon, and in many species, fertilization occurs before the egg shell is laid down. Insect spermatozoa are sometimes said to contain enzymes capable of dissolving or softening up egg shells. They can penetrate thin wax layers round the egg (Beament, 1946); but a careful perusal of Cragg's interesting paper (1920) on copulation in *Cimex lectularius* Linn. shows that the claim that bed bug spermatozoa can 'burrow' through chitin is less certain than has sometimes been thought. E. B. Wilson (1928) noticed that the

spermatozoa of *Cerebratulus (lacteus* Verrill?) pay no attention to the so-called micropyle present in the membrane round the eggs of this nemertine, and can reach and fertilize the egg at any point on the surface. The unfertilized egg of *Megathura crenulata* (Sowerby) is surrounded by a tough membrane which is distinct from the surface of the egg proper, and the same applies to human eggs, in which the enveloping membrane is called the *zona pellucida*. Spermatozoa get through these barriers with the help of enzymes located in their heads. Finally, most mammalian eggs are surrounded by follicle cells, in two layers; the innermost of these consists of densely packed, radially arranged cells and is known as the *corona radiata*. Outside this there is a layer of sparsely distributed cells, the *cumulus oophorus*. The enzyme hyaluronidase, contained in or on the surfaces of most mammalian spermatozoa, assists in the dissolution or depolymerisation of the intercellular cement, hyaluronic acid, by which the follicle cells are stuck to the unfertilized egg surface.

*Cortical change.* Having got through these 'barriers', the spermatozoon becomes attached to the surface of the unfertilized egg. After attachment, the sperm tail may continue to move quite vigorously, though in other cases, it sticks out from the egg surface, motionless. The first visible reaction of the egg to the attachment of the *fertilizing* spermatozoon—spermatozoa quite often become attached to eggs but fail to fertilize them—is a change in cortical structure, which, starting at the point of sperm attachment, passes completely over the egg surface. The time relationships of this reaction are discussed in chapter 9, Polyspermy. According to J. C. Dan (1950a, p. 402), this change in cortical structure is 'a visible wave which travels around the egg at speeds varying with the species, . . .'. 'In the relatively fluid eggs of *Mespilia (globulus* (Linn.)) the passage of this wave is especially striking; it causes a slight deformation of the surface layers of the egg, which gives the impression that some sort of tension is being progressively released, or that a local band of contraction and expansion is passing around the egg.' This wave of so-called contraction has been observed by numerous students of fertilization, but it is doubtful whether the word 'contraction' is apposite or even desirable, except in special cases such as that of the brook lamprey, *Entosphenus lamottenii* (Lesueur), which does contract after fertilization (Okkelberg, 1914), or in the case of mammalian eggs. The German word

Schrumpfung (wrinkling), roughening, granulation, or simply cortical change are nearer the facts. Moser (1939a) examined this reaction in the eggs of *Arbacia punctulata*. He found that a layer of cortical granules immediately below the plasma membrane, diameter  $0.8\mu$ , disappeared at fertilization, the disappearance starting at the point of attachment of the fertilizing spermatozoon and passing progressively over the egg surface, in about 10 seconds at  $26^{\circ}\text{C}$ . A breakdown of cortical granules in the eggs of *Sabellaria vulgaris* Verrill, 5–10 minutes after fertilization, was described in the same year by Novikoff (1939). Moser's studies were followed up by Endo (1952), who observed that at fertilization, the cortical granules, of which there are about  $0.6/\mu^2$  in the eggs of *Clypeaster japonicus* Döderlein, doubled their diameters and then exploded. Just before they disappear, sea-urchin egg cortical granules, which, according to Monné & Hårde (1951), contain polysaccharides esterified with sulphuric acid residues, exhibit Brownian movement, which suggests that at this time, the cortex becomes more fluid (Allen, 1954). A similar phenomenon occurs when fish eggs and those of the marine worm *Nereis succinea* (Leuckart) are fertilized, though in these, alveoli in the cortex break down progressively after fertilization (Yamamoto, 1944; Kusa, 1953; F. R. Lillie, 1919). In addition, Kusa (1954) has shown that the cortical alveoli in the egg of the dog salmon, *Oncorhynchus keta* (Walbaum), contain mucopolysaccharides esterified with sulphuric acid residues. As regards the cortical response to fertilization, there is, therefore, a marked chemical and morphological resemblance between fish and echinoderm eggs. But, as we shall see later, it would at present be dangerous to ascribe too important or dominating a role to exploding cortical granules or discharging cortical alveoli in fertilization.

There has been some misunderstanding (Allen, 1954), perhaps of a verbal nature, about the disappearance of the cortical granules and the change in the light-scattering properties of the egg surface at fertilization, when viewed with dark-ground illumination. There is no doubt that the cortical granules disappear, but at the same time, the cortex becomes more *granular*, or roughened. Rothschild & Swann (1949) suggested that this granulation, which is associated with an increase in light scattering, might be due to the formation of microscopic or sub-microscopic particles at the egg surface. The appearance of this granulation naturally does not imply that

the cortical granules remain unchanged after fertilization. Both phenomena occur and are intimately related to each other. The disappearing cortical granules are concerned in the formation of a structure which appears round some eggs after fertilization, the Fertilization Membrane (q.v.).

**Fertilization cone.** After attachment of the spermatozoon, a conical hyaline protuberance, the fertilization or entrance cone, appears at the egg surface, Fig. 2. In the eggs of *Psammechinus miliaris* (P. L. S. Müller), the fertilization cone disappears in less

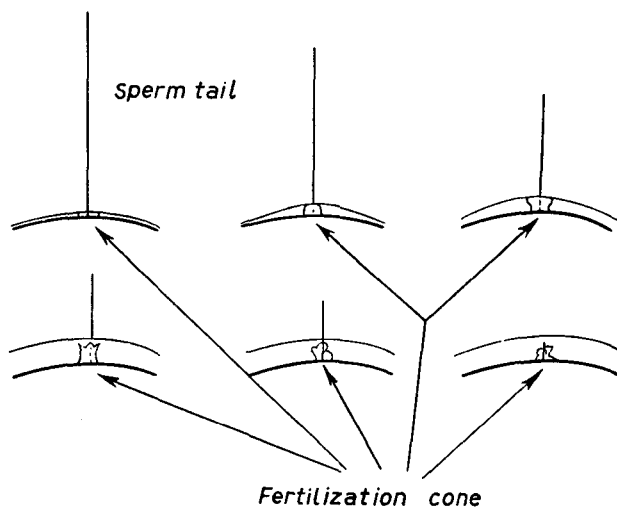


FIG. 2.—Entry of the spermatozoon into the egg of *Patiria pectinifer* (J. Müller & Troschel), after J. C. Dan (1950a).

than 20 seconds at 18° C., but in other eggs it may persist for much longer. In the case of the egg shown in Fig. 2, for example, the fertilization cone is visible until the tail of the spermatozoon has passed into the egg cytoplasm, after which it is more or less reabsorbed into the egg.

**Sperm-egg filaments.** In 1877 Fol reported that the starfish spermatozoon became connected to the surface of the egg by a long, exceedingly thin filament, which he believed was an extension of the fertilization cone. This observation was confirmed by R. Chambers in 1923, contradicted by Just in 1929 and reaffirmed by Hörstadius in 1939. Similar claims, that filaments derived from

the egg pull the spermatozoon towards the egg surface, have been made elsewhere; for example, Colwin & Colwin (1949) reported that a thread-like structure connected the fertilizing spermatozoon to the fertilization cone in the egg of *Saccoglossus kowalewskyi* (A. Agassiz), while Monroy (1948) refers to the fertilizing spermatozoon of *Pomatoceros triqueter* (Linn.) being connected to the egg surface by a thread. In the case of the starfish spermatozoon, J. C. Dan (1954) has shown that in certain circumstances, a thin filament, about  $25\ \mu$  long and  $0.13\ \mu$  in diameter, can be observed protruding from the front end of the head. Although immature eggs respond to insemination, and therefore to sperm-egg collisions, by emitting filament-like structures (E. B. Harvey, 1938), Dan's work leaves little doubt that Fol, R. Chambers and Hörstadius were wrong in thinking that the starfish egg responds to a nearby spermatozoon by emitting a filament which joins the egg to the spermatozoon and pulls the latter towards the egg surface. The presence of long filaments on the front ends of sperm heads may be of wider incidence than has hitherto been realised. Rothschild & Tyler (1955), for example, have reported their occurrence in the spermatozoa of *Echinocardium cordatum* (Pennant), *Mytilus edulis* (Linn.), *Strongylocentrotus purpuratus* (Stimpson) and *Lepidochitona cinerea* (Linn.). There are, however, some spermatozoa, e.g. those of the bull and ram, in which such filaments do not exist. The subject of acrosomal filaments and their role in fertilization is still very much in its infancy. In a recent paper, J. C. Dan (1955) has adduced convincing evidence that the spermatozoa of Japanese sea-urchins eject acrosomal filaments in the presence of sea water in which eggs of the same species have been standing, though the reaction does not occur if the calcium content of the medium is reduced. Do some spermatozoa always have acrosomal filaments on their heads and others only after responding to some stimulus? Further experiments are needed to resolve this interesting and important question, which has been brought into prominence mainly through the work of J. C. Dan.

*Fertilization membrane.* Unfertilized echinoderm eggs are surrounded by a vitelline membrane outside the plasma membrane, Fig. 3. At fertilization and shortly after the cortical change, the vitelline membrane separates from the egg surface, the separation starting at the point of sperm attachment and passing progressively over the egg surface (Kacser, 1955). After this, the vitelline mem-

brane becomes known as the fertilization membrane, which is about 500 Å thick (Mitchison, 1953). As will be seen from an examination of Fig. 3, the cortical granules which disappear at

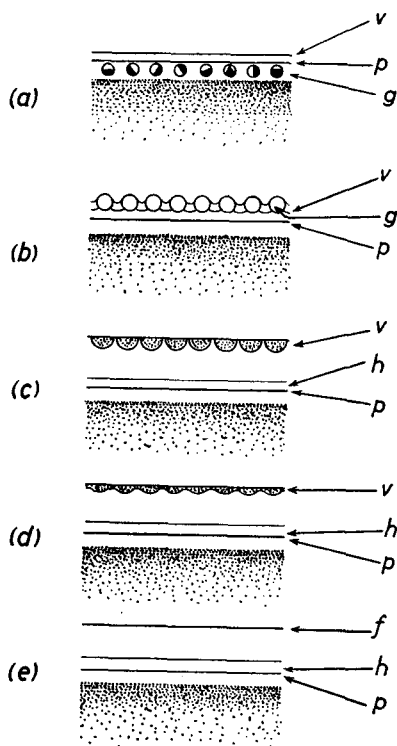


FIG. 3.—Formation of the fertilization membrane in the sea-urchin egg, after Endo (1952). *a*, Unfertilized egg; *b*, extrusion of cortical granules; *c*, adhesion of cortical granules to vitelline membrane; *d*, further transformation of fertilization membrane; *e*, completely transformed fertilization membrane. *v*, vitelline membrane; *p*, plasma membrane; *g*, cortical granules; *h*, hyaline layer; *f*, fertilization membrane. *Note*. Diffraction effects at the surface of a large egg make it extremely difficult to distinguish by optical methods closely apposed layers which are less than 1–2  $\mu$  thick.

fertilization in fact fuse with the inner surface of the vitelline membrane, a phenomenon which was first systematically examined by Motomura (1936, 1941), though Just observed the escape of granules from the cortex, their appearance in the perivitelline space, and possibly their incorporation into the fertilization



membrane, as early as 1919.\* Endo (1952) has published some remarkable photographs of cortical granules adhering separately to the inner surface of the fertilization membrane of *Clypeaster* eggs. The space between the fertilization membrane and the surface of the egg is called the perivitelline space, an unfortunate term as the vitelline membrane is outside this space, not inside it. Globular isotropic cortical granules can sometimes be seen in the perivitelline space, where they may undergo a spontaneous transformation into positively birefringent rod-shaped particles. If unfertilized eggs are treated with trypsin and then fertilized, these rod-shaped particles are clearly visible. The fusion of the transformed cortical granules with the vitelline membrane is responsible for its hardening and transformation into the fertilization membrane, which takes place during the first ten minutes after fertilization. Calcium ions and a third factor which can be extracted from eggs are also concerned in the hardening or 'tanning' of the fertilization membrane (Motomura, 1950, 1954; Runnström, 1951). The properties of the fertilization membrane have been studied in great detail under a variety of environmental conditions by Runnström and his colleagues. A detailed review of this subject will be found in *The Cell Surface in Relation to Fertilization* by Runnström (1952). One interesting property of the fertilization membrane, which has not received sufficient mention, was described in some detail by Pasteels in 1950. He observed that the fertilization membranes of the eggs of *Chaetopterus variopedatus* (Renier), *Nereis succinea*, and of *Spisula solidissima* (Dillwyn), are contractile. More accurately, at certain times after fertilization the membrane 'expands', thereby becoming creased or folded. The effect is soon reversed and the membrane re-assumes its usual smooth (contracted?) and spherical appearance. The region on the fertilization membrane where this folding and unfolding phenomenon first occurs, transiently, 20 minutes after fertilization in *Chaetopterus* eggs, is at the vegetative pole, i.e. 180° away from the point of expulsion of the first polar body. The same happens after the expulsion of the second polar body, 30 minutes after fertilization, while 5 minutes later, the

\* Cortical granules and their behaviour at fertilization are much in the limelight at present; it is therefore only right to mention that some forty-five years ago, E. N. Harvey (1911, p. 523), said that in the eggs of *Arbacia punctulata*, there were 'numerous minute stained granules, quite unmoved by the centrifuge. At the time of fertilization these disappear, apparently going to form the substance which passes out of the egg and hardens to a fertilization membrane'! The stain used was neutral red.