

Prevention and Control of Disease. By FRANCIS RAMALEY and CLAY E. GRIFFIN. Copyright by Francis Ramaley, Boulder, Colorado. 1913.

In the preface to the book the authors state the purpose for which it has been written. The work of investigators, physicians and public health officers should be more widely known in order that an intelligent body of citizens may cooperate in its extension. The book is intended for the general public and as a text for college classes. It is not written for medical students or biologists. After discussion of death rate, types of disease and certain hygienic considerations nine chapters, constituting almost half of the book, are given to a concise summary of the "germ theory of disease," the nature, life-history, metabolic activity and distribution of animal and vegetable parasites, the mode of infection and spread of infectious diseases, disinfection, susceptibility and resistance, immunity and specifics in the treatment of disease. One familiar with the complexity of any biological science may doubt the possibility of conveying to the general reader a conception of the nature of the objects or of the phenomena described or in the absence of a clear understanding of the subject the possibility of maintaining his interest. For those who wish this information a satisfactory synopsis is furnished. It is even more doubtful if matter described in this part of the book can be used as the basis of a collegiate course. To appreciate the form and life-history of bacteria and protozoa and the chemical changes caused by them both preliminary biological training and objective demonstration of selected forms may be regarded as essential. Study of the phenomena of immunity including the intricacies of Ehrlich's side-chain theory or of phagocytosis and opsonic action must be relegated to the biological student who wishes to acquire technical training and superficial information may leave the impression of occult mystery in the mind of the general reader. The book contains a large amount of information which the layman should have and it is presented in interesting form. The statements concerning

medical practise are generally accurate, but occasionally an indefinite or erroneous impression is produced. Advice to eat moderately at the beginning of a "cold" may be worth heeding, but its value is not strengthened by the suggestion that side-chain receptors become coupled to toxins when intoxication takes place and the body is unable to assimilate food until new side chains are developed. The cause, dissemination and prevention by personal and governmental precautions of "cold," diphtheria, contagious diseases of childhood, tuberculosis and other diseases are adequately discussed. The value of vaccination and of the serum treatment of diphtheria is emphasized with the purpose of overcoming lingering prejudice. As an illustration of desirable information which may aid the layman to judge his professional attendant may be cited the author's discussion of the importance of surgical cleanliness on the part of dentists. Historical data defining the changes that have occurred in the prevalence of certain diseases or describing the progress of medical discovery add interest and clearness to the book. E. L. OPIE

SPECIAL ARTICLES

ON INDUCING DEVELOPMENT IN THE SEA-URCHIN (*ARBACIA PUNCTULATA*), TOGETHER WITH CONSIDERATIONS ON THE INITIATORY EFFECT OF FERTILIZATION¹

I. THE INITIATION OF DEVELOPMENT WITH DILUTE SEA WATER

In the course of work on the energetics of development, it became necessary to study in detail the question of water absorption at various stages of embryogeny. For certain phases of these studies the eggs of *Arbacia punctulata* proved extremely favorable. In various concentrations of sea water these eggs behave exactly as expected, but in 25 per cent. sea water (25 c.c. sea water + 75 c.c. H₂O dist.) fertilization membranes appear. The process takes place in from one to one and a half minutes at ordinary temperatures. In two minutes many eggs as well as their nuclei

¹ Preliminary communication.

are cytolized, and in three minutes this is true of most of the eggs.

The membrane in question is a true fertilization membrane, and if at the proper moment the eggs are brought back into normal sea water, or better still, hypertonic sea water (50 c.c. sea water + 8 c.c. 2.5 N NaCl), cleavage takes place. Since July 18 I have succeeded in rearing a considerable number of ciliated larvæ.

II. THE INITIATION OF DEVELOPMENT WITH EGG EXTRACT

If fresh ovaries of *Arbacia* are ground up in a mortar with pulverized glass and a small quantity of sea water, the liquid, when filtered, has a color not unlike that of blood serum. This fluid, if allowed to act on ripe eggs contained in an equal quantity of sea water, proves to be an excellent initiatory agent, for if the eggs after one to two hours are placed in normal sea water, many divide, although no fertilization membrane appears.

III. THE THEORY OF INITIATION, PARTHENOGENETIC METHODS AND THE FERTILIZATION MEMBRANE

It is well known that development can be induced in many kinds of eggs by very diverse means—lipoid solvents, increased osmotic pressure of the surrounding medium, electricity, heat, cold, mechanical shock and even pricking the egg surface, have all proved effective in one case or another, but so far as I am aware the use of egg extract from the same species is new, as well as the production of genuine fertilization membranes in *Arbacia punctulata* by means of dilute sea water. In one of the California sea urchins, Loeb² has reported the formation of membranes after the addition of distilled water, but from certain details it seems that the fertilization membrane of at least one of the California urchins resembles that of *Asterias forbesii*, and this differs quite markedly from that of *Arbacia punctulata*.

Loeb,² on the basis of his own investigations

² Loeb, Jacques, "Die chemische Entwicklungs-erregung, etc.," Julius Springer, Berlin, 1909.

and those of others, has formulated a theory on the initiation of development which for normal fertilization and certain of the parthenogenetic methods, postulates (a) an increased permeability of the ovum due to the action of lipoid solvents or hæmolytic agents; (b) the formation of a fertilization membrane in consequence of this superficial cytolysis.

Of an increase in permeability synchronous with the initiation of development there is not the slightest doubt, although the great variety of parthenogenetic methods long ago indicated that permeability is increased, in other ways than by action on surface lipoids. With the employment of some parthenogenetic methods, fertilization membranes appear, with others, not, and even the employment of lipoid solvents themselves may or may not be followed by the appearance of a fertilization membrane. One and the same egg, as in the present case, may be induced to develop with or without the appearance of such a membrane.

IV. EXPERIMENTAL ANALYSIS OF THE FERTILIZATION MEMBRANE

According to Kite's³ dissection, the egg of *Arbacia* has a vitelline membrane tightly glued to its surface. Outside this is a thin jelly. The appearance of the fertilization membrane, according to this description, is due to the swelling of the vitelline membrane, and the formation of a phase boundary between it and the thin outer jelly.

This description I believe to be essentially correct for the following reasons:

1. The fertilization membrane also has an inner visible boundary. In certain localities of the two- and four-cell stage this inner surface of the fertilization membrane is plainly visible, has indeed been often figured and I believe misinterpreted. In the stages in question a narrow perivitelline space can be seen around the egg, but the fertilization membrane adheres to the egg surface here and there by strands. As a consequence, when

³ Kite, G. L., "The Nature of the Fertilization Membrane, etc.," SCIENCE, Vol. XXXVI.

the egg divides, some of these strands are drawn down between the cleavage cells, and as certain portions of the surface of these are further removed from the fertilization membrane than the original egg, the inner limit of this membrane, as well as the perivitelline space itself, becomes visible. The perivitelline space seems to be identical with the so-called "hyaline plasma-layer," and homologous with the perivitelline space of the fertilized starfish egg.

2. By means of hypertonic solutions as well as by extract of themselves, sea-urchin eggs can be induced to divide without the appearance of a fertilization membrane. Development, however, does not proceed normally because the blastomeres fall apart. Since the vitelline membrane is tightly glued to the surface of this egg and a perivitelline space appears after the membrane has swollen, eggs dividing without the formation of this space have the membrane adhering to the resulting blastomeres. In consequence, these cells, instead of being in intimate contact with one another as they normally are, are each enclosed in a separate vitelline membrane. In other words, when the vitelline membrane is not lifted off the egg surface, it divides with the egg, which is what one would expect. If this idea is correct, cleavage cells which have originated by the division of an egg without a "fertilization" membrane should be able to "form" such membranes under suitable conditions, and this I have observed. Immersed in dilute sea water, isolated cleavage cells, derived from ova which have not formed "fertilization" membranes, form them in from one to two minutes.

3. Egg fragments can also be produced by shaking. No fertilization membranes appear in such eggs or their fragments as the result of the mechanical agitation, but when treated with dilute sea water or sperm, membranes appear in some of the fragments, but not in others. Both kinds of fragments have been fertilized with sperm and allowed to develop, some with and some without the membrane. This result can only be understood if we accept Kite's discovery that the fertilization membrane in *Arbacia punctulata* appears

when a preexisting jelly, closely adherent to the surface of the egg, swells and changes its optical properties.

4. From the above experiments one may infer that a fertilization membrane may appear around part of an egg, instead of the whole. If Kite's jelly is ruptured the egg flows partially through the hole in the membrane, and assumes a dumbbell shape. If it is now fertilized with sperm, or treated with dilute sea water, a fertilization membrane appears on one sphere of the dumbbell, but not on the other. Such eggs are capable of development.

5. The appearance of a fertilization membrane in *Arbacia punctulata* is not a function of the living egg, for if the egg is crushed, or even dried completely in a desiccator for days, membranes still appear after proper treatment.

V. WHAT MAKES THE FERTILIZATION MEMBRANE APPEAR NORMALLY?

If the interpretation given to the results outlined is correct for *Arbacia punctulata*, it is easy to see why the fertilization membrane should appear in dilute sea water, or in distilled water. But why does it appear under normal conditions in sea water?

The exact mechanism of the process is not yet clear, but it seems to be a function of the number of sperm present. If one inseminates eggs very carefully so that not more than four or five spermatozoa come into contact with each one, the fertilization membrane does not appear. I have repeated this experiment many times and have controlled it by the most careful observations with different powers on fresh material as well as stained. Such preparations show sperm plainly adhering to Kite's jelly in every egg, but the "membrane" does not appear. Eggs treated in this manner do not develop, although some of the smaller ones may form asters. What it is in the sperm that brings about the swelling of the jelly has not yet been determined. However, beautiful fertilization membranes may be caused to appear in two to three hours by treating the eggs with minute infusoria.

No membranes appear in the controls, nor do the eggs whose membranes have appeared develop when returned to sea water. Three possibilities suggest themselves—an acid effect, a mechanical effect or a heat effect. No decisive experiment has as yet been devised.

These experiments suggest that in *Arbacia punctulata* the membrane swells before the sperm enters the egg, and not after. Experiments also show that when the phase boundary between Kite's jelly and the outer jelly is complete, sperm do not readily penetrate the fertilization membrane. From this it follows that the penetration occurs at the moment when the jelly is softened and begins to swell. Accordingly, eggs whose jelly has been partially softened by heat or infusoria should be capable of fertilization with small doses of sperm. This has actually been observed in a number of instances. The opposite experiment of hardening the jelly with Ca has been performed. Such eggs are extremely difficult and in many cases impossible to fertilize as the sperm do not stick.

VI. THE RELATION BETWEEN FERTILIZATION AND THE FERTILIZATION MEMBRANE

The relation between the initiation of development and the fertilization membrane in *Arbacia punctulata* is one of association rather than "causal," for the membrane may be made to appear without development, and development may be initiated without the appearance of the membrane. In *Asterias forbesii* the association is somewhat different, and so intimate that any method which causes the membrane to appear is at the same time a method of initiation provided the violence is not too great and the egg is in good condition and in a suitable medium. The explanation is simple. In *Asterias* the fertilization membrane does not depend on the swelling of a formed jelly, but instead, the egg peels itself away from the inner surface of a thin pre-existing membrane. This peeling away seems to depend, not upon changes in the fertilization membrane, but upon changes in the surface film of the egg. When this is rendered more permeable, material leaves the egg and

the egg shrinks away from its closely adherent covering which thus becomes visible. The perivitelline space in the starfish egg is homologous with that of the sea urchin egg, but is much larger.

The type of fertilization membrane found in *Arbacia punctulata* may be called hydrophilous, that of the starfish, *Asterias forbesii*, anhydrophilous.

VII. ON THE LOSS OF SUBSTANCES BY THE EGG AND THEIR NATURE

The starfish egg upon peeling off from its anhydrophilous fertilization membrane is markedly smaller in volume than before. The same thing is true of *Arbacia*. Exact measurements will be given when I publish exact details of these experiments. No doubt much of the material lost by the egg is water. F. R. Lillie⁴ in a series of fundamental researches has shown that the fluid over-fertilized eggs may contain at the least two classes of substances, (a) "iso-agglutinins" and (b) a substance having a chemotactic influence on the sperm. From Elder's⁵ investigations as well as certain observations of my own, it appears possible that the chemotactic substance is contained in the outer jelly of the *Arbacia* egg. I have been able to verify the "iso-agglutinin" and its effects as described by Lillie in the case of *Arbacia* and *Asterias*.

Ovarian extract of *Arbacia*, when present in sufficient quantities, retards the development of normally fertilized *Arbacia* eggs. If the extract is added to blastulae which have developed in normal sea water, these are instantly slowed down and absorb water. *Arenicola* larvæ also have their permeability increased by the *Arbacia* extract, as can be very prettily seen by their loss of pigment. They also slow down in their movements and are slightly and reversibly agglutinated.

⁴Lillie, F. R., "Studies of Fertilization," I. and II., *Jour. of Morph.*, Vol. 22; III. and IV., *Jour. of Exp. Zool.*, Vol. 12; V., *Jour. of Exp. Zool.*, Vol. 14.

⁵Elder, J. C., "The Relation of the Zona Pelucida to the Formation of the Fertilization Membrane," *Arch. f. Entwicklungsmechanik*, Vol. 36.

These observations suggest that the ovarian extract, as well as the secretions of the egg on fertilization contain substances that not only influence permeability, but may reduce the oxidations in the cell.

VIII. THE THEORY OF INITIATION

The theory of initiation, as given by Loeb, postulates essentially that initiatory influences place the egg in a condition in which its oxidative processes can proceed, or proceed normally. This is accomplished by increasing the permeability of the egg, and in the case of many parthenogenetic agents, as well as in normal fertilization by sperm, the permeability change may be brought about by lipid solvents. The fertilization membrane may or may not appear after the use of lipid solvents, and when, as in the case of the starfish egg, it does appear, it may also be made to do so with any other method of increasing the permeability of the plasma film. These facts, many of which have been emphasized by Loeb,² R. S. Lillie³ and others, by no means prove that the theory of initiation is wrong. Indeed, they are all in harmony with this view if we remember that an hydrophilous fertilization membrane may or may not appear, depending on circumstances, whereas an anhydrophilous one like that of *Asterias* is certain to appear when, as the result of a permeability change, the egg shrinks away from its enclosing capsule.

How can increased permeability initiate development?

The ovum demonstrably has the necessary mechanism to undergo development of itself. It is a cell with a long metabolic history and before development is initiated its plasma film is relatively impermeable. This may involve the accumulation of "waste" products, and these we may believe to automatically inhibit further metabolic processes. Loeb has shown that these processes are oxidations, and my experiments show that substances can be extracted from the eggs which reduce the rate of development and have a marked effect in de-

creasing the activity of *Arbacia* as well as *Arenicola* larvæ. It does not seem unreasonable to suppose therefore that these materials are active because they reduce oxidations. The mere fact that they also increase cell permeability and are good initiatory agents is beside the point, for increased permeability in *Arenicola* larvæ is also associated with acceleration of movement.

One may extend the theory of initiation and assume that all agencies that initiate development do so because through increased permeability of the plasma film the egg is enabled to loose substances antagonistic to oxidation. By freeing itself of these inhibitors, a chemical equilibrium is disturbed, and oxidation, and with it development, is free to go on.

In this way we can explain why a mature starfish egg, if unfertilized, may oxidize itself to death, for we may suppose that its permeability has been sufficiently increased by maturation to accelerate oxidation, but not enough to initiate development proper. We can also bring all parthenogenetic methods whatsoever, as well as normal fertilization, under a common point of view, for the increased permeability, no matter whether produced by electricity, heat, cold, mechanical shock, specific chemical alteration of the membrane, lipid solvents, or pricking, is all that is necessary to enable the egg to free itself from its accumulated inhibitors. Why the egg should develop after treatment with hypertonic solutions is also clear, for if in such media the plasma film is permeable to the inhibitors, loss of water by the egg would, directly or indirectly, accelerate the loss of antagonists. That these are lost in hypertonic sea water is shown by special experiments.

In conclusion, I must thank my colleague, Dr. W. E. Garrey, who kindly allowed me to demonstrate to him various steps in the investigation, and to whom I am indebted for a number of valuable suggestions and criticisms.

OTTO GLASER

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²Lillie, R. S., "The Physiology of Cell Division," *Jour. of Morph.*, Vol. 22.