

## RECENT THEORIES OF FERTILIZATION AND PARTHENOGENESIS

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Two main problems of fertilization stand in the foreground of all recent work viz: the problem of the initiation of development and the problem of bi-parental inheritance. The latter problem has received a much more complete and satisfactory analysis than the former which is alone under consideration in the present paper. The problem may be stated thus: Why is the unfertilized egg usually incapable of development? And how does the spermatozoon initiate the developmental processes?

This problem was naturally conceived in the early part of the modern period (1875 to the present) in morphological terms; and Boveri's theory that the egg did not develop because it lacked the "organ of cell-division" (viz: the centrosome) and that the spermatozoon enabled it to develop by supplying the missing organ was long held by most naturalists. But with the advent of physiological analysis of cell life the inadequacy of this point of view soon made itself felt. The discovery of artificial parthenogenesis by Loeb started a new era in this problem. It was shown that the role of the spermatozoon could be dispensed with in initiation of development, and that a change in the chemical constitution of the medium could take its place. In such a case it was obvious that no new cell organ was needed by the egg. Following this epoch-making discovery a great many investigators studied the problem of initiation of development in the eggs of various species of animals, with the result that a great variety of agents was shown to be effective in the production of parthenogenesis in different forms. These can be classified under the head of chemical agents, osmotic changes, temperature changes, radiation, mechanical shock and pricking. In

fact a very considerable proportion of the agents effective in stimulating protoplasm may be efficient agents in inciting parthenogenesis in one or another variety of eggs. It has been argued that, if it were possible to find the common factor of all efficient parthenogenetic agents, the cause of the initiation of development would be found. This attempt has not, however, led to a satisfactory explanation; and it may be doubted whether such a form of analysis would lead to any more satisfactory results in the case of initiation of development than in the initiation and propagation of a nerve impulse, which may be equally well started by a similar set of external changes.

Moreover, while it is obvious that in parthenogenesis, part of mechanism of fertilization must be employed, we need not necessarily gain any direct knowledge of the true fertilization reaction between ovum and spermatozoon. In fact if we were to accept any theory of parthenogenesis we would still be far from understanding many of the phenomena of fertilization associated with initiation of development.

On any such basis (1) We do not understand the nature of the immediate reaction between the ovum and spermatozoon in fertilization; (2) We do not understand the nature of the reaction of the sperm nucleus in the interior of the ovum; (3) We do not understand why fertilized ova become non-fertilizable; (4) Why immature ova are non-fertilizable; (5) We do not understand the variations of affinity within a species, leading to almost absolute sterility in self-fertilization in some cases, or between the members of certain varieties in other cases; (6) We do not understand either the sterility or fertility of different species when bred together.

Not only are these unsolved problems, but we have only the slightest experimental basis for their solution by methods of parthenogenesis. A vague conception that the solution of such problems must lie in the field of chemistry has been entertained, but without any experimental basis whatever until very recently, and that of a slight character.

If these things are true the defect must lie in the methods of investigation because some of the most brilliant students of biology have been concerned with the problems of fertilization. The defect in the methods of artificial parthenogenesis is obvious, because it consciously restricts the field of investigation. Nothing less than an analysis of the process of fertilization itself can give a rounded theory of fertilization. The study of merogeny, of hybrid fertilization, of partial fertiliza-

tion, of self-fertilization, of the antagonistic action of sperm suspensions of different phyla, of the fertilizing effect of injured spermatozoa, etc., are methods that have recently been employed for the direct analysis of fertilization, and which have aided greatly in the definition of problems, though but little in their solution.

I have therefore attempted for some years to make a direct analysis of the fertilization problem, and have developed two new methods of analysis: (1) The use of sperm-suspensions as indicators of reactions. (2) The study of fertilization by inhibitors.

The first method was suggested by the fact that many of the most delicate of biological reactions are determined by the use of living cells as indicators. In the field of immunology no other indicators are known for bodies concerned in such reactions in many cases. The sperm cells of a species might be expected to serve as indicator of substances formed by the egg with which reaction normally takes place in fertilization. As regards the second method, it is obvious that an analysis of the reaction concerned in fertilization might be attempted by agents which would hinder the normal course of events at any place. This would constitute a method of analysis by which the total reaction might be broken up into its elements.

#### I. *Results from the Use of the Sperm Suspensions as Indicators.*

If some of the sea-water from a suspension of eggs of *Arbacia* be added to a milky suspension of the sperm of the same species in sea-water, a very rapid agglutination is obvious to the naked eye, resulting in the formation of detached white masses of agglutinated spermatozoa, leaving the fluid more or less clear, depending on the strength of the reaction. In a few minutes the agglutinated masses break up into their constituent elements and the suspension becomes milky again. The reaction cannot be repeated in the case of the sperm suspension, if it is incomplete in the first instance. In chemical terms it cannot be repeated after saturation. The spermatozoa, however, retain their vitality. We have then a combination of some elements of the spermatozoa with a secretion from the ripe ova, which so alters the physical character of the sperm cells that they adhere in masses. The reaction in question is without prejudice to the life of the spermatozoa.

The agglutinating substance is produced only by ripe eggs of *Arbacia*, and not by immature eggs or by any other tissue of the species; in other words, only fertilizable eggs produce it.

The substance is more or less specific in as much as it will not agglutinate the spermatozoa of *Nereis* for instance. Professor Loeb has since been able to determine a higher degree of specificity, as between related species of sea-urchins

It is possible to study the production of this substance quantitatively, that is to say, a given bulk of ripe eggs may be shown to charge a given amount of sea-water to an extent that may be measured in the following way: The agglutination as noted, is reversible. It is therefore possible to establish a minimum reaction as unit in which reversal takes place in four to five seconds. Then the amount of dilution of a given agglutinating solution necessary to reduce it to unit strength is a measure of concentration of the agglutinating substance.

To give an idea of the quantities involved it may be stated that sea-water which has stood over one quarter its bulk of ripe eggs for half an hour may be capable of 1/800 to 1/3200 dilution, or is of the strength of 800 to 3200 agglutinating units. By another method we may ascertain that a single c.c. of the eggs placed in 8000 c.c. of sea-water will charge the whole body of sea-water with sufficient quantity of the agglutinating substance to be detected. The unfertilized ripe eggs of *Arbacia* produce this substance as long as they live and remain in a fertilizable condition; they may be washed repeatedly in sea-water, and when restored to fresh sea-water this is soon found to be charged with the substance in question. In certain experiments I have carried out thirty-five successive washings of a given lot of eggs during a period of three days without being able entirely to dispose of the agglutinating substance.

Definite quantitative relations in the reaction with the sperm suspension may also be estimated by the number of units of the agglutinating substance which can be neutralized by a given standard suspension of the spermatozoa. For the details, I must refer to previous papers.

On account of the activity of the eggs in producing this substance and the avidity of the sperm for it, it seems reasonable to assume that it plays some part in the normal fertilization reaction.

It was therefore interesting to find that the eggs contain a substance, which they do not normally secrete, which neutralizes the sperm-agglutinating action of the egg-secretion,

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\*These observations must be made under the microscope: a quantity of sperm suspension is mounted on a slide under a raised cover slip, and a drop of the fluid to be tested is injected into the suspension by means of a capillary pipette.

*i. e.*, which renders the combination of the sperm with the agglutinating substance impossible. The presence of this substance may be demonstrated by methods that plasmolyze the egg or extract its internal substances. If for instance, eggs are deprived of the jelly in which they are normally embedded, and which is heavily charged with agglutinating substance, in order to get rid of excess of the agglutinating substance, and are then divided into two equal lots, one of which is placed in a certain quantity of sea-water, and the other in an equal bulk of distilled water, it is found that plasmolysis takes place in the distilled water, and internal substances are extracted from the egg. The supernatant fluid from the distilled water is at first more highly agglutinative than the sea-water, which contains only the normal secretion of the eggs. But there is present in the distilled water extract, a substance which tends to inhibit agglutination, and in a short period of time distilled water extract becomes entirely neutral. This is not merely an effect of distilled water on the agglutinating substance, because the latter may be kept for a long period of time in distilled water without deteriorating, but it is due to the presence of a body which actually destroys the agglutinating substance, or combines with it, in such a way as to prevent its effect.

These results suggest that in normal fertilization the combination between the agglutinating substance and the spermatozoon is essential, and that polyspermy is prevented by a neutralization reaction between the two substances produced by the eggs. I have therefore proposed the term *fertilizin* for the sperm agglutinating substance, and *anti-fertilizin* for the other.

It would follow that eggs in which the fertilizin is removed by any method should be incapable of fertilization. While it has proved extremely difficult to completely dispose of the fertilizin content of eggs, and to test this conclusion, it has nevertheless been possible by repeated washings greatly to reduce the fertilization content of eggs and secure the demonstration that the capacity for fertilization is correspondingly reduced.

Eggs when once fertilized are incapable of fertilization, that is to say the protoplasm of such eggs will no longer react with the spermatozoa. Such eggs should therefore *ex-hypotheses*, be devoid of fertilizin content and this is the case.

To sum up: It is shown that ripe unfertilized eggs of *Arbacia* secrete a substance which produces a more or less specific agglutination reaction with sperm of the same species. Before acquiring this substance, *i. e.*, before ripening, the eggs

are incapable of fertilization; after it is lost, whether by washing or preceding fertilization, the capacity for fertilization is also lost. We therefore conclude that the agglutinating substance is necessary to fertilization.

## II. *Use of Inhibitors.*

### 1. Inhibitors in blood.

It is a fact well known to Embryologists, but hitherto not studied in any systematic fashion, that the plasma of blood or tissue secretions of the species tends to hinder the fertilization reaction.

I have made a somewhat detailed study of this phenomenon in the case of the Sea-Urchin, *Arbacia punctulata*, the detailed results of which are published in volume 16, of the *Journal of Experimental Zoology*. Here it may suffice to say that the addition of filtered serum of *Arbacia* blood to the sea-water in which insemination is to be performed, strongly inhibits fertilization. If a series of blood solutions containing, let us say 10, 20, 30, 40 per cent, etc., up to 100 per cent blood be prepared and equal quantities of ova inseminated with equal quantities of spermatozoa in each member of the series, the per cent of fertilization will usually run from about 100 per cent in fertilization control to zero in the undiluted blood serum. All the sperm are living and active in each member of the series.

The inhibition by the blood is not due to direct harmful action on either reproductive element alone for either eggs or spermatozoa may be exposed to the action of such solutions and after washing be found to possess good capacity for fertilization. It might be supposed that the inhibitor acts like the anti-fertilizin by preventing union between fertilizin and spermatozoa. This is, however, not the case because of a solution of fertilizin in blood is as effective in agglutinating spermatozoa as a similar solution in sea-water.

A second hypothesis would be that the inhibitor might act on a second side-chain of the fertilizin which is active in fertilization by combining with certain constituents of the ovum. If this is the case it would follow that the inhibitor in the blood could be neutralized by first saturating it with fertilizin from other eggs and this is found to be the case: If a given sample of blood be divided into equal parts of which one is saturated by fertilizin from unfertilized eggs, and inseminations be made in graded series of both, the usual inhibition reaction will be found in the first series, but is entirely absent in the second series.

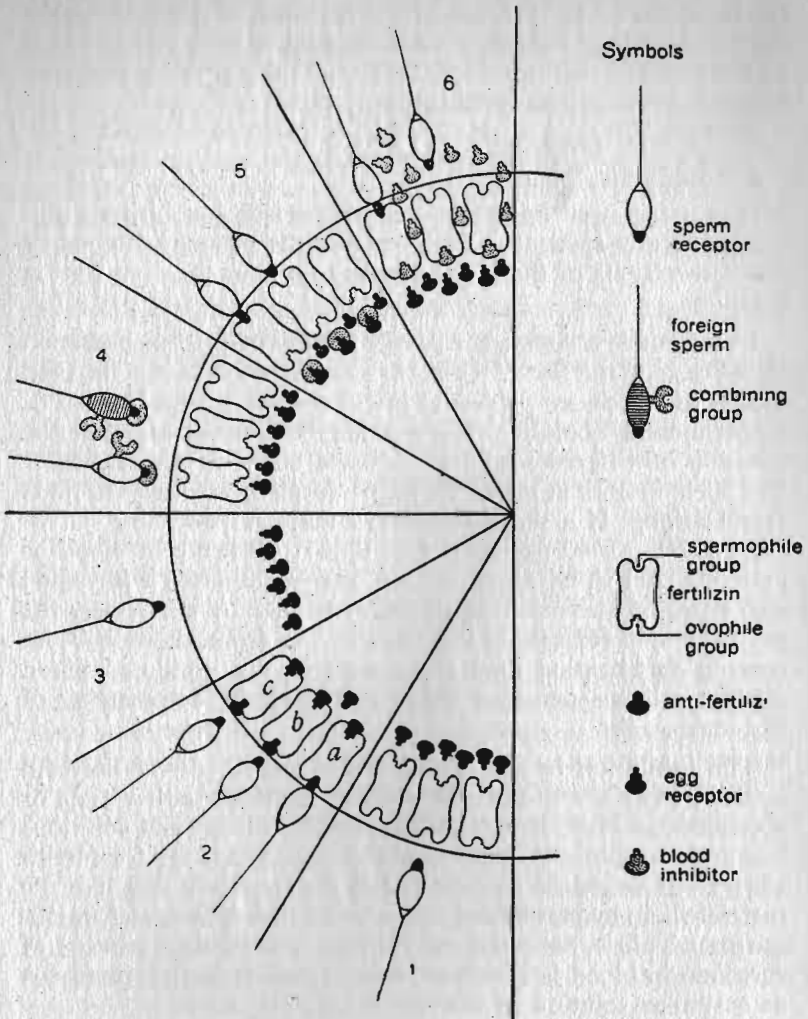


Figure 1

## DESCRIPTION OF FIGURE

Fig. 1. In successive sectors of the egg there are represented the mechanism of fertilization and the blocks to the mechanism, as follows:

Sector 1. The arrangement of substances in the unfertilized egg and in the spermatozoon that are active in fertilization. See explanation of symbols.

Sector 2. The mechanism of normal fertilization. The sperm receptor unites with the spermophile group of the fertilizin and the egg-receptors with the ovophile group of the fertilizin owing to activation of the latter by the sperm (a). Molecules of the anti-

fertilizin combine with the spermophile group of the adjacent fertilizin (b and c) and thus block the way for supernumerary spermatozoa. This is the postulated mechanism for prevention of polyspermy. At the same time molecules b and c of the fertilizin have also united with the egg receptors.

Sector 3. Inhibition of fertilization by loss of the active body, fertilizin.

Sector 4. Theory of antagonistic action of spermatozoa of different phyla. The sperm receptors are occupied by combining groups cast off by the antagonistic spermatozoa.

Sector 5. Fertilization is blocked by occupancy of the egg-receptors. Purely hypothetical.

Sector 6. Theory of inhibitory action of blood of the same species. The ovophile group of the fertilizin is occupied by molecule in the blood (inhibitor) possessing the same combining group as the egg receptors. Molecules of the blood inhibitor also shown in the medium.

The fertilizin is represented in the diagram as occurring only in the cortex of the egg. But it also occurs in high concentration in the jelly surrounding the egg. The spermatozoon must thus normally arrive at the egg-membrane loaded with combined fertilizin. This fact, however, makes no essential difference in the theory, and its representation would complicate the diagram.

We therefore came to the conclusion that the substance which we have been calling fertilizin is the active agent in fertilization. That it possesses two side chains, one of which is spermophile, combining with certain elements of the spermatozoon (sperm receptors), the other of which (the ovophile) combines with certain elements in the egg (egg receptors). Fertilization is therefore not a two-body but a three-body reaction. Of these one is carried by the spermatozoon, the sperm receptors, and one by the egg, the egg receptors. The third, (fertilizin) is produced by the egg and reacts with both the others. The essential reaction in fertilization must be regarded as that of the fertilizin with the egg receptors; and the spermatozoon must be regarded as playing the secondary role of activator. In parthenogenesis the active body is still the fertilizin, and the various parthenogenetic agents, in this case play the role of activators. Thus parthenogenesis and fertilization may be regarded under one point of view.

The diagram represents the foregoing theory with the aid of symbols. Sector one illustrates the relation of the side chains before fertilization. Sector two illustrates normal fertilization. Some of the consequences of the theory are indicated as follows:

1. (Sector 3). If the egg loses its fertilizin it cannot be fertilized. This we have already seen.

2. (Sector 4). If the sperm-receptors are occupied, the spermatozoa cannot fertilize. The *Godlewski phenomenon* (Antagonism of sperm suspensions.)

3. (Sector 5). If the egg receptors are occupied, fertilization cannot take place. *Purely Hypothetical*.

4. (Sector 6). If the ovophile side-chain of the fertilizin be occupied, fertilization cannot take place. *Inhibiting action of blood*.

5. (Sector 2, b and c). If the spermophile side-chain be occupied, fertilization cannot take place. *Action of anti-fertilizin; prevention of polyspermy*.

It is obvious that these five blocks are purely chemical in their conception, but the possibility of other blocks in the fertilization reaction must not only be conceded, but strongly emphasized; as the essential reaction takes place across the egg membrane, the condition of this membrane, whether permeable or impermeable, is an essential factor in the reaction. The reaction must also be subject to the usual environmental conditions of comparable bio-chemic phenomena, such as temperature, ionic constitution of the medium, etc. Modification of these conditions will determine the occurrence or non-occurrence of the fertilization reaction.

This theory transfers the fertilizing power from some hypothetical substance contained in the spermatozoon to a definite substance contained in the egg itself, in relation to which the sperm acts merely as an activator. It is therefore radically opposed to the theory that the sperm carries a lysin or other substance that acts directly on the cortical layer of the egg.

The mechanism which we have been considering concerns the cortical changes in the egg. The question therefore arises, whether the second phase of fertilization which I have shown previously to be dependent on the penetration of the spermatozoon, is likewise a three-body reaction, and if so whether the fertilizin is the intermediate body in this case also? This is a problem that I have not yet fully worked out, but the indications are for a positive answer to both these questions. There are a number of facts of considerable interest in this connection. (1) As is well known for *Arbacia*, spermatozoa may penetrate ovocytes but without fertilizing them; In *Chaetopterus* I have observed the same fact. (2) If the cortical changes are induced mechanically or chemically in the egg of *Nereis*, there is a stage where the spermatozoa may still penetrate but without causing complete fertilization. Miss Allyn demonstrated the same facts for *Chaetopterus*. (3) Dr. Kite

observed that the injection of from 3 to 20 spermatozoa into the interior of the egg of *Asterias* with a microscopic capillary pipette does not result in any fertilization reaction. These facts indicate that when spermatozoa penetrate before the formation of fertilizin (ovocytes) or after its fixation (*Nereis* and *Chaetopterus*), or without the opportunity of reacting with the fertilizin (Dr. Kite's experiment) fertilization fails.

In these experiments we have egg and sperm in most intimate relations, but apparently incapable of reacting in the absence of an intermediate body, which appears to be the same body, viz: the fertilizin, through the mediation of which the cortical changes are induced.

In serum physiology, we have become familiar with many reactions for which living cells alone are adequate indicators; such are agglutination, cytolysis, opsonic reactions and anaphylaxis; the bodies concerned in such reactions viz: agglutinins, cytolysins, opsonins, etc., are not known directly, but only by the use of cell indicators. Their chemical nature is entirely unknown, but they are so definitely known through cell indicators, and so controllable, that many quantitative procedures of the most delicate character, in immunology, are based on this knowledge. The cells themselves are the source of such bodies, yet the site of their cellular origin is unknown, and their roles in normal cell physiology are problematical.

The ordinary chemical analysis of the cell begins by destruction of its more highly organized living constituents; it is obvious that such methods are inadequate for the investigation of the immediate reactions in living protoplasm.

The results of these experiments may then gain a still broader interest if they be taken to indicate a method for the study of such reactions.

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