A Critical Study of the Facts of Artificial Fertilization and Normal Fertilization.

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With 1 Text-figure.

Facts concerning the process of fertilization and of artificial parthenogenesis have steadily accumulated during the past thirty years, and although numerous suggestions have been put forward as partial explanations of this imposing mass of experimental evidence, yet there are only two theories which claim to give an adequate picture of even a majority of the known facts. These theories we owe to J. Loeb (**24**) and to F. R. Lillie (**20**). According to Loeb, the activation of an unfertilized egg is effected by the introduction into the egg of two substances, (i) a specific cytolysin, which brings about the destruction of the surface layer of the egg, and (ii) a substance which limits or controls the destructive influence of the cytolysin. On the other hand, Lillie holds that the union of the egg and spermatozoon is only possible in the presence of a specific substance or fertilizin which is secreted by the unfertilized egg; if all three elements are present fertilization and normal development take place.

Loeb's theory is based upon the facts of artificial parthenogenesis; Lillie's theory is based upon the behaviour of the normal gametes. It is not surprising to find that each theory encounters its chief difficulties when confronted with the facts which constitute the main argument of its rival. Both theories are essentially chemical, although the door is left open, at rare intervals, to the intervention of physical factors. In 1915 (**8**) I suggested that although the theories urged by R. S. Lillie (**21**)

and by McClendon (**25**) were inadequate, yet the facts appeared to indicate that the activation of the egg, by a spermatozoon, or by artificial parthenogenetic agents, was essentially a physical rather than a chemical process. It now seems possible to put forward a more comprehensive scheme.

The activation of a resting cell, by contact with another cell in a state of activity, is not limited to the reproductive cells. All contractile cells exhibit the same phenomenon ; if localized fibres at the surface of a large muscle are stimulated, the whole of the muscle is rapidly thrown into a state of activity ; the ciliated combs of Pleurobrachia illustrate the same fact (Gray, **10**) ; also cells in contact with each other usually divide at the same moment. There can be no doubt that such co-ordination of activity is due to the responsive cells themselves, and is not due to any nervous or controlling influence. Thus spermatozoa in contact with each other rapidly acquire a synchronous rhythm ; similar examples are readily found in the case of ciliary or muscular elements.

There can be but little doubt that the influence of one cell upon the activities of its neighbours has a very profound bearing on the behaviour of the animal as a whole. There is, however, no reason to regard such co-ordination as essentially vital, since a ready parallel is found in inorganic systems. Ostwald (**27**) found that when a strip of chromium was placed in hydrochloric acid the hydrogen was evolved at regular periods ; each period of activation was followed by a period of inactivity. This periodic condition of activity and inactivity was quite regular for each strip of metal : different strips of metal were, however, characterized by periods of different length. If several such strips are placed in a bulk of hydrochloric acid, the periodicity of each strip exhibits itself ; if, however, the strips are in contact with each other then all the strips exhibit the same uniform periodicity. The activation of a passive strip of iron by contact with an active piece of the same metal has been discussed by R. S. Lillie (**23**), and has a close bearing on the present problem.

The activation of a passive cell or metal by contact with an

active unit is invariably accompanied by an electrical disturbance; and there seems good evidence for the belief that the electrical change is the essential condition of activity. When an inactive unit comes into contact with an active unit, an electro-motive force is established between the two; the active unit is electro-negative to the inactive unit, and if activation of the latter occurs, the state of negativity is not restricted to the region of contact but spreads from it all over the originally inactive surface. Such facts are, of course, well known in the case of fibres in the same muscle, but Kühne (**18**) showed that the action current of one muscle could stimulate another muscle if the two were in close electrical contact. Now the E.M.F. set up between two cells in contact depends on, and is an expression of, the difference in the activity of the two units; the greater the difference in activity the greater is the E.M.F. set up on contact.

In the opinion of the writer an application of the above principles to the problem of fertilization is not without value. In the unfertilized egg metabolic activity is reduced to a minimum, and unless fertilization takes place the cell dies without any recovery from its inert condition. The spermatozoon, on the other hand, is radically different: it is exceedingly active and metabolism proceeds at a rapid rate (Cohn, **3**). When the two cells come into contact it seems legitimate to conclude that an E.M.F. will be set up between the two, and if the conditions be right it is to be expected that some form of activity will be induced in the inert egg. If the process of activation be analogous to that of other cells, then the egg will be activated whenever the E.M.F set up by contact with a spermatozoon reaches a certain minimum value in a minimum time. It is, therefore, not surprising to find that a certain minimum of activity on the part of a spermatozoon is necessary for fertilization. Mobility and proximity of the egg are not sufficient—a fact difficult to explain on any chemical conception; there must be a definite and rather high degree of activity on the part of the sperm, and this degree of activity differs between individual spermatozoa. Glaser (**7**) observed

that the eggs of Arbacia punctulata can be activated by means of highly active minute Infusoria. It is, therefore, the degree of activity of the sperm which determines one condition of fertilization and not its structure or chemical constitution.

Whereas the normal activity of a spermatozoon is usually adequate for the activation of eggs of the same species, yet it requires to be increased to an abnormal degree to fertilize the eggs of another species. Now the activity of spermatozoa can readily be controlled by the hydrogen-ion concentration of the medium (Gray, **9**), and correspondingly it is found that the addition of hydroxyl ions removes the block which normally exists between the eggs of Strongylocentrotus and the sperm of Sphaerechinus. These and similar facts are difficult to explain on the basis that fertilization depends on the existence of specific chemical substances in the egg or sperm (see Loeb, p. 204). They appear to the writer to be less formidable when subjected to physical arguments. According to the present physical hypothesis, if it be mechanically possible for the sperm of one species to gain contact with the egg of another, then activation will take place if the E.M.F. set up between the two cells reaches a certain critical value within a minimum time. Consider two species, A and B. When normal fertilization of egg A is effected by sperm A, let the E.M.F. be E_1 and let it be developed in unit time; similarly when egg B is fertilized by sperm B let the E.M.F. at contact be E_2. Let $E_1 > E_2$; then when egg B comes into contact with sperm A, the E.M.F. will probably be more than enough to activate the egg; when egg A comes into contact with sperm B, no activation will occur unless the activity of the sperm is artificially increased, since otherwise the requisite E.M.F. will not be reached. Such irreciprocal hybridizations have already been mentioned and are by no means uncommon [see Vernon (**28**), Doncaster (**5**)]. Further, the conditions which affect the ease with which hybridization can occur are such as support the view that some such physical factors are involved, e. g. seasonal variation of gametes, degree of maturity, staleness or freshness of gametes,

hydroxyl ions, dilution of sea-water, &c. It is exceedingly difficult to apply the chemical conceptions of Loeb or of Lillie to such facts. It is obvious that the self-sterility of the gametes of Ciona can also be analysed by a similar physical argument to hybridization.

Under normal conditions only one spermatozoon enters an egg. In view of the very large number of spermatozoa which may be in the immediate vicinity of the egg-surface at the moment of fertilization, it is almost inconceivable that any chemical change could be set up, and carried to a conclusion between the time that two successive spermatozoa touch the egg. Neither Lillie nor Loeb has offered what would seem to be a reasonable explanation of monospermic fertilization. Once more, the facts appear to be amenable to physical treatment. Assuming that the rate at which an electrical change can travel round the egg is approximately that at which it travels along a piece of smooth muscle then within 0·00001 sec. after the effective spermatozoon has made its contact with the egg, no other spermatozoon will have any effect: if, however, the eggs are treated in such a way as to reduce the rate of propagation of an electrical disturbance, e. g. by incomplete anaesthesia (cf. nerve or muscle), then the wave will not pass completely over the egg before other spermatozoa can effect contact with unaffected portions of the egg-surface, and polyspermy will result. Hertwig (**17**) showed that unfertilized eggs treated with chloral hydrate and other anaesthetics were markedly polyspermic.

The first visible sign that fertilization has occurred, is at the surface of the egg. In the case of the echinoderm or annelid egg, fertilization is attended by the formation of a 'fertilization membrane'. It must be remembered, however, that the essential change at the egg-surface is completed long before any visible change is possible. What is the nature of the fertilization membrane? In the case of the egg of Nereis it seems certain (Lillie, **20**) that this membrane is the vitelline membrane of the unfertilized egg, which is pushed away from the egg-surface by the disintegration and hydration of the

egg-surface immediately under the vitelline membrane. In the case of the echinoderm egg it seems probable that essentially the same change takes place.

Many years ago Loeb (**25**) showed that the fertilization membranes collapsed when placed in sea-water containing albumen, and that on transference to normal sea-water the membrane regained its normal spherical shape. He concluded

TEXT-FIG. 1.

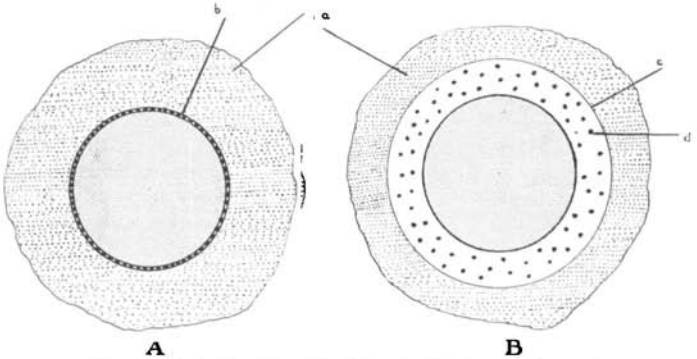

Diagram illustrating the origin of the fertilization membrane of Echinus miliaris. A. Unfertilized egg. B. Fertilized egg.
a, Zona pellucida containing an electro-positive colloid.
b, Vitelline membrane of unfertilized egg.
c, Fertilization membrane formed by the interaction of *a*, and an electro-negative colloid, *d*, which is set free when *b* is emulsified.

that the extrusion of the membrane was therefore due to the existence of an osmotically active colloid (proteid) within the fertilization membrane. Now the osmotic properties of such a colloid are markedly affected by the presence of hydrogen and hydroxyl ions; hence, if Loeb's conclusion be correct, the degree to which the fertilization membrane is extruded should be altered by altering the acidity or alkalinity of the sea-water. This is actually the case. If the water be made acid

the membrane remains close to the egg; the higher the alkalinity the more water is absorbed and the farther out is the membrane pushed.

Ph.	*Relative Extrusion of Membrane.*
9·2	121
7·6 (normal) . . .	100
7·3	44
6·9	16

It seems tolerably clear, therefore, that the extrusion of the fertilization membrane is due to the existence of an osmotically active electro-negative colloid between the egg-surface and the fertilization membrane.

The origin of the fertilization membrane and of the enclosed colloid is, however, more difficult to determine.

The relative impermeability of the unfertilized egg to water and to all substances not soluble in oils or fats leads to the suggestion that, like the protoplasmic surfaces of many cells, the vitelline membrane contains a continuous lipoid phase. Such a conclusion cannot be substantiated by direct experimental evidence, but, if it be correct, then many facts receive a reasonable explanation. It is, of course, well known that the formation of the fertilization membrane can be brought about by a great variety of artificial agents. Of these, the simplest and most efficient are saponin, benzol, fatty acids, esters, and soaps. All these substances are essentially emulsifying agents for a lipoid surface in contact with water.[1]

Since the use of fatty acids is most commonly adopted as a means of artificial membrane formation, it is interesting to analyse their action in detail. The process is as follows:

(i) Unfertilized eggs are placed in 50 c.c. sea-water + 1·5 c.c. N/10 Butyric Acid for 1½ mins.

(ii) They are then transferred to sea-water, containing a minimum concentration of hydroxyl ions.

[1] And for this reason these substances in higher concentrations destroy the normal protoplasmic surface of most cells: in other words, they are also 'cytolytic' agents.

Whilst in the acid solution no change, either visible or physiological, takes place, apart from the fact that the acid rapidly penetrates into the egg and can be detected by means of indicators (Gray, **8**). It may be noted that in such a solution the activity of normally fertilized eggs is completely stopped.

No. of c.c. N/10 Butyric Acid in 50 c.c. s.w.	*Approx. Ph.*	*No. of Fertilized Eggs of Echinus miliaris which Segmented.*
c.c.		Per cent.
0	7·9	100
0·4	7·2	100
0·7	6·8	10
0·9	6·3	0
1·0	6·1	0
1·5	5·0	0

It is only when the unfertilized eggs are removed from the butyric-acid solution to sea-water containing a definite concentration of hydroxyl ions, that fertilization membranes appear; and that the physiological properties of the fertilized eggs are acquired and are carried on at a speed equal to that produced by normal fertilization. It is now that the eggs become more permeable to water (R. S. Lillie, **22**) and to ions (McClendon, **25**; Gray, **18**), and that there is a marked increase in oxygen consumption. If the vitelline membrane be regarded as a continuous lipoid film, then an interesting parallel experiment can be carried out by the emulsification of olive oil.

Olive oil + no fatty acid forms no emulsion with distilled water.

Olive oil + no fatty acid forms no emulsion with alkaline water.

Olive oil + fatty acid forms no emulsion with distilled water.

Olive oil + fatty acid forms a complete emulsion with alkaline water.

The process of artificial membrane formation and that of oil emulsification are dependent on the same factors: (*a*) the existence of a fatty acid in the oil, (*b*) the existence of a minimum concentration of hydroxyl ions in the neighbouring aqueous phase. In each case the resultant product shows a marked increase in permeability to water and to ions.

There seems, therefore, reasonable grounds for believing that the unfertilized egg is surrounded by a continuous lipoid film, and that membrane formation occurs when this film is emulsified. When this occurs, however, we have seen that an electro-negative colloid is liberated; we must suppose, therefore, that either (*a*) this colloid exists in the lipoid layer as a dispersed phase or (*b*) lies immediately between the lipoid layer and the protoplasmic surface.

We have still to determine the origin of the fertilization membrane itself. Each unfertilized egg is surrounded by a wide gelatinous zona pellucida: this substance appears to be of a proteid nature—it is readily soluble in dilute acids, and so we may infer that it is electro-positive. If the zona pellucida is not removed before fertilization then the electro-negative colloid set free when the lipoid layer of the vitelline membrane is emulsified will come into contact with a colloid of opposite electrical charge. Mutual precipitation must occur—and this, it is here suggested, is the origin of the fertilization membrane. If, however, the zona pellucida be removed prior to fertilization, then no fertilization membrane is formed (McClendon, **25**; Gray, **8**). Nevertheless the egg develops normally.[1]

The complete mechanism of 'membrane formation' may therefore be as follows:

The unfertilized egg is surrounded by two membranes—the hyaline vitelline membrane, and the gelatinous zona pellucida. The vitelline membrane consists of a continuous lipoid structure, in which an electro-negative protein (*d*) [see Text-fig. 1] exists in solution as a dispersed phase (or below the lipoid structure is a layer of electro-negative protein). The zona pellucida consists of an electro-positive protein. When the continuous lipoid phase of the vitelline membrane is destroyed by emulsification, the enclosed protein (*d*) comes into contact

[1] Since writing the above I have found that a similar conclusion had been reached by McClendon (see 'Internat. Zeit. für Phys. Chem. Biologie', vol. i, p. 163, 1914); although the eggs of Toxopneustes examined by this author appear to have the reverse charges on the respective colloids when compared with those of Echinus, which were used by the present writer.

with the zona pellucida, i. e. with a colloid of opposite sign. Mutual precipitation must occur, giving rise to the fertilization membrane. This membrane is impermeable to the remainder of the protein (*d*), which draws in water through the fertilization membrane by osmosis. In this way the fertilization membrane is extruded from the protoplasmic surface of the egg.

The evidence for such an analysis of membrane formation is strong. (i) There is no doubt that the protein within the fertilization membrane has an opposite charge to that of the zona pellucida. (ii) If the zona pellucida be removed prior to fertilization no fertilization membrane is formed, but the egg is activated in a perfectly normal way. (iii) By micro-dissection it can be shown that the fertilization membrane is much tougher than any membrane possessed by the unfertilized egg.

It must again be emphasized that the essential act of activation is the emulsification of the vitelline membrane, and not the formation of the fertilization membrane or the extrusion of the latter by absorption of water.

If the above analysis of ' membrane formation ' be accepted, the question arises how can the spermatozoon act as an emulsifying agent? Loeb holds that the sperm introduces a specific ' cytolysin ' into the egg-surface. The evidence is, however, against this view: (i) in order that artificial membrane formation may occur as quickly as a normal fertilization membrane, fairly high concentrations of emulsifying agents are necessary: it is quite impossible for one spermatozoon to introduce sufficient quantities into the egg. (ii) Membrane-forming substances are not in any way specific, whereas spermatozoa are markedly so. The only alternative seems to be that the spermatozoon emulsifies the egg-surface by a different means to that effected by artificial agents. The action of the spermatozoon is at first local, and evidence has already been put forward in support of the view that its activating action on the egg is essentially a physical process. The suggestion made is that the destruction of the surface lipoid film is brought about by the spermatozoon electrolytically. Thin lipoid films are, according to Hardy, electrically charged, and are sensitive to the electric current.

It seems likely, therefore, that a disturbance of the electrical properties of the film will result in a loss in the stability of the film. Such reactions are well known in the case of non-lipoid films, e. g. in rhythmical catalysis (Bredig, **1**), and in the activation of passive iron [1] (Lillie, **23**).

Before passing on to the later stages of fertilization in the echinoderm or annelid egg, reference must be made to the activation of the eggs of Amphibia. There are two methods of artificial activation, (*a*) by mechanical puncture, (*b*) by electrical stimulation. These facts seem to indicate most clearly that the process is essentially physical in nature. In the first case the egg is subjected to an injury current with an inevitable wave of negativity sweeping over the egg-surface. In the second case the electrical disturbance is set up precisely as in the stimulation of a muscle or nerve. The only difference is that no recovery process ensues. It seems almost impossible to harmonize these facts with the theory of Loeb, or with that of F. Lillie.

The beautiful experiments of F. Lillie (**20**) enable us to be quite sure that the changes induced in the echinoderm or annelid egg by artificial 'membrane formation' are precisely those changes which are set up by contact of the egg with a spermatozoon. Lillie showed that the cortical changes in the egg of Nereis are completed on contact between the egg and spermatozoon: if the latter be now removed by means of the centrifuge, the eggs undergo a series of changes essentially similar to eggs which have been activated by means of artificial membrane-forming agents. Development of the egg depends in each case on events which take place subsequent to this phase. The whole process of normal fertilization is divisible into two well-marked phases, (*a*) activation, due to cortical changes produced by contact with the spermatozoon,

[1] The activation of an egg and a piece of passive iron appear parallel phenomena; and in this respect the activation of echinoderm eggs by means of metallic silver (Herbst, **13**) may warrant further investigation, although it is not clear whether the metal or one of its salts is the activating element.

(*b*) development, which usually depends on the exposure of the egg to hypertonic sea-water. Let us now consider the second or developmental phase of fertilization.

In the normally fertilized egg, the visible change which attends the second phase of fertilization, consists in the inclusion into the egg-substance of the head and middle piece of the spermatozoon; soon after this an aster appears in the vicinity of the sperm-nucleus. Eventually the male and female pronuclei, having approached each other, fuse together, and cell-division begins. We must suppose that as soon as the cell wall of the spermatozoon and that of the egg at the point of attachment are broken down[1] then the body of the spermatozoon will be very rapidly drawn into the egg by surface tension. On the mechanism of this process no experimental facts are available.

Once the sperm has been drawn into the egg, we can continue our experimental analysis. The experiment of Kupelwieser (**19**) shows that the sperm-nucleus plays no essential rôle, since although in certain cases it rapidly degenerates yet normal segmentation occurs: again in artificial parthenogenesis complete development takes place without any male pro-nucleus. Now, the only other visible structure which is associated with the male nucleus is the male aster. In Lillie's experiment with the centrifuged eggs of Nereis, it was found that eggs from which the sperm had been removed failed to develop a bipolar mitotic spindle; only one aster—the female aster—was present; otherwise the nuclear behaviour of the egg was normal. Again, in Kupelwieser's experiment, although the male nucleus degenerated the male aster remained functional. From a study of normal fertilization we therefore suspect that the developmental phase of fertilization is associated with the existence of two asters, one belonging to the female nucleus, and one introduced into the egg by the spermatozoon—or which comes into being when the male nucleus enters the egg.

[1] Until this occurs the two cells will remain essentially distinct from each other. Mere agglutination in a common matrix would not produce actual incorporation of the sperm and the egg.

Since the initial phases of fertilization and of artificial parthenogenesis are alike, and since the subsequent phase of development depends on the existence of a sperm-aster, is it possible that the process of artificial parthenogenesis can only be completed by treating the egg in such a way as to induce the formation of a second aster ?

As is well known, eggs which have been subjected to ' membrane formation ' will proceed to normal development if treated with hypertonic sea-water. The recent work of Herlant (**16**) shows clearly that such treatment does actually lead to the formation of a second aster. Without such treatment activated eggs behave in exactly the same way as eggs from which spermatozoa have been removed after normal initial activation.

The formation of accessory asters within fertilized, or within artificially activated eggs, when exposed to hypertonic sea-water, is now quite well established (Herlant, **16**; Vles and Dragoiu, **29**). Herlant has shown that one of these asters comes into communication with the female aster and forms a normal mitotic spindle in the case of artificially activated eggs ; further, he has shown that the optimum conditions for accessory aster formation and the optimum conditions for development are exactly equivalent. When it is remembered that Morgan (**26**) and Wilson (**30**) showed that similar treatment led to the formation of asters within unfertilized eggs, it will be realized that the work of Herlant has thrown much light on the whole process of artificial parthenogenesis. We are now able to give a reasonable explanation of the fact that membrane formation may either precede or follow treatment with hypertonic sea-water. There is no need to postulate the ' corrective substance ' of Loeb. Since the egg is more permeable to water after membrane formation than before, it is equally clear why treatment with hypertonic sea-water is more rapidly effective after membrane formation than before (Loeb).

We can, therefore, summarize the process of artificial parthenogenesis as follows. There are two phases. (i) An activation of the egg, by the destruction of a lipoid film at the surface.

This process raises all the physiological activities of the egg to the values reached in a normally active cell. (ii) A developmental phase, whereby the necessary machinery for development is introduced into the egg in the form of an artificially produced aster.

It has been stated, however, that the theory of fertilization advanced by F. R. Lillie is based on a very different series of facts to that of Loeb. Lillie's theory is based on the behaviour of the normal gametes. It is necessary for any alternative theory to cover the whole of the facts.

Lillie has shown that sea-water which has been in contact with the unfertilized eggs of the same species has a remarkable effect on the spermatozoa. Such sea-water contains a substance which (i) usually causes a marked increase in the activity of the spermatozoa, (ii) causes them to form macroscopic clusters—usually rounded in shape—of intensely active sperm, (iii) in some cases causes the spermatozoa to adhere to one another for a considerable time, in large immobile clumps. The essence of Lillie's theory is that fertilization is effected by the union of the egg and the sperm by this intermediate and specific substance (given out by the unfertilized egg) which Lillie calls 'fertilizin'. The sperm contains a substance which is agglutinated by fertilizin, and so the sperm becomes attached to the egg. Immediately fertilization has been effected the production of fertilizin ceases, and so no more spermatozoa can adhere to the egg.

I think it is a just comment to say that the above theory (with its marked analogy to the side-chain theory of Erhlich) does not purport to indicate the nature of the forces, physical or chemical, which underlie the various processes of normal fertilization. It does, however, stress the necessity of the existence of specific substances, 'fertilizin, &c.', without which union between egg and sperm is impossible; further, Lillie makes no attempt to extend his theory to the process of artificial parthenogenesis. Let us attempt to examine the properties of 'fertilizin' from a physico-chemical point of view.

The presence of fertilizin usually stimulates normal sperma-

tozoa to a high degree of activity: it is not, however, the sole means whereby an increased activity may be brought about. The same effect can frequently be obtained by exposing the spermatozoa to a slight increase of hydroxyl ions in the surrounding medium, in fact the activity of spermatozoa can readily be regulated by this means (Gray, **9**; Cohn, **3**); the gametes of Luidia have been frequently observed to show no activity when exposed to egg-secretions, whereas intense activity is aroused by hydroxyl ions (Gray, **11**). Again, the sperm of Sphaerechinus will not fertilize the eggs of Strongylocentrotus, unless hydroxyl ions are added to the medium. These facts indicate that either (i) the efficiency of fertilizin depends upon the concentration of hydroxyl ions present, or (ii) 'fertilizin' is itself a weak base which stimulates the spermatozoon by virtue of its basic properties. The second hypothesis covers all the facts, has the advantage of simplicity, and is supported by the fact that the essential constituent of the egg-secretion is readily destroyed by acids, but not by alkalies.

Since, however, there is unanimous agreement that a certain degree of activity on the part of the spermatozoon is necessary for fertilization, let us consider the more unique effects of egg-secretions on the sperm, viz. aggregation and agglutination. There can be no doubt that the aggregation of the sperm into active clusters must be due to some effect which the sperm have on each other: the observations of Lillie on the spontaneous aggregations of Nereis sperm provide strong evidence that aggregation is due to the production of CO_2 and that the sperm aggregate at regions of optimum CO_2 tension. There is nothing to indicate that such clusters are due to any other cause than the clusters of protozoa which have been described by Jennings (**12**). It is, therefore, reasonable to suppose that whenever the activity of the sperm is sufficient to produce the critical amount of CO_2 in the medium immediately surrounding each sperm, then aggregations will form; naturally, they will only be temporary owing to (i) a gradual abatement in CO_2 production in an increasingly acid solution, (ii) a gradual

abatement of movement owing to the supply of available energy being used up within the cells.

Again, Loeb's comment on the significance of sperm aggregations is important. There is no evidence that a spermatozoon must take part in an aggregation before it can effect fertilization. Aggregation need be nothing more than an interesting corollary to the activation of the sperm by the egg-secretion.

Subsequent to forming active aggregations in water containing egg-secretions, the sperm may adhere to each other in dense masses (e.g. Nereis); in certain species no such agglutination takes place. A consideration of the agglutination effect of egg-secretions would involve a discussion of the whole mechanism of cell agglutination; such a discussion is not possible, but the lucid summary given by Buchanan (**2**) may be recommended to the notice of any who feel disposed to follow Lillie's argument of specific agglutinations. The fundamental fact is that agglutination depends primarily on the presence of free ions. This can readily be demonstrated in the case of spermatozoa or of eggs. The addition of a trivalent cation such as cerium causes a very marked agglutination (Gray, **9**), which Lillie regards as comparable to the effect of heterofertilizin (i.e. the fertilizin of one species on the spermatozoa of another). Again, the addition of a small amount of sodium phosphate to normal sea-water causes a marked agglutination of the sperm of Echinus miliaris. These phenomena depend upon the deposition of an insoluble precipitate on the surface of the cells; in the case of cerium agglutination, insoluble cerium hydroxide (probably in the colloidal form) is deposited on the cell-surface and the cells adhere to each other by virtue of this common matrix—just as particles of oil are agglutinated by ferric hydroxide (Ellis, **6**). Any substance which breaks up or dissolves this matrix reverses agglutination; thus acid dissolves $Ce(OH)_3$ forming cerous chloride, while alkali or sodium citrate pectises or breaks up the film into non-coherent parts.[1]

[1] It may be noted that this explanation differs from that offered in a previous paper (Gray, **9**). The conditions for the deposition of such

Agglutination by sodium phosphate is somewhat different: in this case agglutination is due to the formation at the cell-surface of insoluble calcium phosphate. This agglutination only occurs in the presence of calcium ions, and is reversed by any substance which dissolves calcium phosphate, e. g. acids.

The parallel drawn by Lillie between the phenomena of agglutination of germ cells and those of bacterial cells is no proof that the agglutinative properties of the germ cells is an essential condition for fertilization. Both bacteria and germ cells exhibit the phenomena of spontaneous agglutination. The latter phenomenon has been described by the writer (**8**) for the eggs of Strongylocentrotus lividus, and the physiological properties of the cells indicates clearly that the same factors are involved as in experimental agglutination.

Apart from such considerations it does not follow that because the eggs give off a substance which causes spermatozoa to adhere to one another, the same substance will cause a spermatozoon to adhere to the egg. As pointed out elsewhere, a mere agglutination of the egg and sperm is an inadequate picture of the events which ultimately lead to the inclusion of the sperm into the cytoplasm of the egg; it is only when the cell-membranes break down at the point of contact that an actual fusion can occur much as a small drop of orthotoluidine is drawn into a large drop of di-methyl-aniline (Darling, **4**).

According to the present view, therefore, the only essential effect of egg-secretions upon the spermatozoa lies in the capacity of these substances to increase the activity of the sperm. In certain cases egg-secretions appear to have no effect on spermatozoa and yet fertilization readily occurs; this fact is obviously explicable on the physical analysis outlined elsewhere in this paper.

Summary.

1. The theory of artificial parthenogenesis put forward by Loeb meets with considerable difficulties when applied to the

precipitates depends on the charge on the cell-surface, but the mechanical effect of flocculation seems certainly due to these precipitates acting as a common matrix for the cells.

facts of normal and hybrid fertilization. The theory of fertilization put forward by F. R. Lillie does not appear to be applicable to the facts of artificial parthenogenesis.

2. The facts of normal fertilization appear to indicate that the action of the spermatozoon on the egg is essentially of a physical nature.

3. Evidence is advanced in favour of the view that the activation of an unfertilized egg by a spermatozoon is due to the electro-motive force set up when the two gametes come into contact. The inert egg is activated by the spermatozoon in the same way as any other resting cell is activated when in intimate contact with an active neighbour.

4. After activation normal development only occurs if two asters are present in the egg. Under normal circumstances the second aster arises in the egg in conjunction with the male pro-nucleus; in artificially activated eggs the second aster arises when the egg is treated with hypertonic solutions.

5. In the case of the echinoderm egg the formation of the fertilization membrane is discussed. One essential step in the activation of these eggs is the removal of a continuous lipoid film from the surface of the unfertilized egg.

6. The view is expressed that the only essential effect of egg-secretions on spermatozoa is the capacity of these substances, in certain cases, of increasing the activity of the male gametes.

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