Artificial Parthenogenesis and Fertilisation: A Review.

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THIS article is an effort to gather together, in so far as they relate to the phenomena of fertilisation in the sea-urchin egg, the results obtained by experiments. It does not pretend to consider the problem of fertilisation as a whole, nor the phenomena save in Echinoderms, and no attempt will be made to establish comparisons with other forms in which the details may to some extent differ. The limitation to one form is in so far appropriate, that practically all the experiments have been made on Echinoderm eggs.

I have personally studied fertilisation in the egg of Echinus esculentus—specially in sections,—and though I have nothing fresh to add to the description of the facts, this article may in a measure be considered a sequel to a paper on maturation in the same form. In that paper my attention was chiefly directed to the chromosomes, and I did not follow out the results of observers in the experimental field, but as some of the phenomena described are of interest in connection with these results, I shall take the opportunity of returning to them.

The two mitotic divisions characteristic of the maturation phases, differ markedly from those which take place in the segmentation phases. In many respects there is a close resemblance to phenomena observed in the eggs of Toxopneustes, which develop parthenogenetically under the influence of magnesium chloride solution (Wilson, 1901).

On the dissolution of the nuclear membrane the site of the germinal vesicle is occupied by a "kinoplasmic" mass, derived either entirely from the nuclear network, or also partly from protoplasm differentiated on the distribution of the nuclear substance into it. In fixed material this area has a fibrillar appearance. This may be the result of the fixing reagents used, but in any case it indicates the accumulation at this part of protoplasm which has undergone some change in constitution physical or chemical. In the nuclear area, out of this material, asters are formed, and ultimately the first polar amphiaster. Besides the asters concerned in the formation of the bipolar figure, there are secondary asters, which seem to have only a temporary existence. In some few cases multipolar figures were observed. No structure recognisable as a centrosome or centiole was found before the germinal vesicle broke down, and therefore the centrosome was either derived from the nucleus, or formed de novo in the nuclear area. The astral radiations are confined to a small and superficial part of the egg, and a very unequal division results in the formation of the polar bodies. When the two divisions are over all the radiations and the remains of the kinoplasmic area disappear, the cytoplasm assumes its alveolar structure throughout, the nucleus retires from the surface, and no centrosome can be recognised in relation to it.

On the breaking down of the germinal vesicle the greater part of the nuclear material disappears as such, and not only is a change in the constitution and distribution of the protoplasm to be recognised, but experiment proves that the egg has undergone a physiological change of state. Whereas a spermatozoon can neither fertilise an egg with the germinal vesicle intact, nor a fragment without the nucleus (Delage, 1901), after the polar bodies are formed the egg becomes capable of fertilisation.[1] This cytoplasmic maturation, dependent, probably (Delage, 1901), on the influence of the

[1] For evidence and theories regarding the influence of the stage of maturation in Amphibian eggs see Bataillon (1901).

nuclear sap set free from the germinal vesicle, is accompanied by the conversion of the large vesicular nucleus related to the metabolic changes underlying the growth of the ovum, into a small morphologically equivalent nucleus, possessing the same number of chromosomes as the sperm nucleus. This has been proved by their enumeration when the nuclei undergo independent transformation, and the number is one half that found in the segmentation divisions.

The eggs thus matured remain, in the case of the sea-urchin, for a considerable time quiescent within the ovary before they are discharged—for the process of ripening in the ovary is a gradual one. When discharged into sea water it seems that, like the eggs of some other forms (O. Hertwig, 1893, p. 239), after lying for many hours unchanged, the sea-urchin eggs show spontaneously, karyokinetic transformation; for instance, R. Hertwig (1896) observed in eggs which had been deposited prematurely during transport, analogous changes to those produced by treatment with strychnine. This phenomenon is one apparently of wide range. In an interesting review entitled "Giebt es bei Wirbeltieren Parthenogenesis" (1900), Bonnet, after examination of all the literature up to that date, comes to the conclusion that, according to our present knowledge, the phenomena in vertebrates are due to degenerative divisions, and in meroblastic eggs to fragmentations, and the alleged parthenogenetically divided tubal, uterine or laid eggs, are either over-ripe, and therefore badly fertilised, or are eggs normally fertilised with defective spermatozoa. In the light of the facts of artificial parthenogenesis, it may be that this segmentation in unfertilised eggs, at least in certain invertebrates, is an effort in the direction of true parthenogenesis which is abortive, the egg dying before the tardy process is accomplished.

In 1876 Greeff described parthenogenetic development in Asterocanthion. The eggs were obtained from animals early in the season, before the spermatozoa were mobile, and the blastulæ formed differed from those produced in normal fertilisation. O. Hertwig (1890) recorded some observations on spontaneous parthenogenesis. In confirmation of Fol, he found that eggs from fully-matured animals did not segment spontaneously, but only after a considerable time underwent changes considered pathological. The nucleus enlarged more and more, and after ten to fifteen hours the eggs died and fragmented. Only among hundreds of eggs here and there one had divided into two. At Trieste, however, in a season when the animals were late in maturing, and at a time when males were rarely got, he observed in a limited number of cases (in Asterias glacialis, and Astero pecten) that after the polar mitosis had occurred, the nucleus did not come to rest, but continued to divide. There resulted an irregular division, but here and there a blastula was found which had no vitelline membrane. Into the interesting observations and suggestions regarding the failure of the second polar body extrusion, and the union of two vesicular nuclei in the egg, we cannot here enter. The main point established was, that fully-matured eggs did not develop parthenogenetically, but that in some few cases immature eggs did divide irregularly, and in a small number of cases blastulæ were formed. A number of observers have described the occurrence of natural parthenogenesis in Echinoderms, and it is an open question; but apart from its possible relation to immaturity of the ovum, the sources of error in the matter of infection by spermatozoa are so many, and the causes which artificially start parthenogenetic development in certain cases are so slight, that all cases of so-called "natural parthenogenesis" are open to suspicion, but even granting that it may occur, it is a matter of no great moment in the question of "artificial parthenogenesis." It would be only additional evidence of the fact, that there is in these forms a tendency to parthenogenetic development, which, however, does not normally occur.

Mitotic division may be excited in unfertilised eggs in a variety of ways.

First, by increasing the degree of concentration of the sea water (Morgan, Hunter), or by increasing the osmotic pressure in various other ways (Loeb). This may be done by adding various inorganic salts to the sea water, especially the salts of magnesium, potassium, sodium, and calcium, in definite proportions (Loeb, Morgan, and others), or by adding sugar or urea (Loeb). Other salts have also given results, e.g. chloride of manganese (Delage). The effect is produced when the eggs, after being left from half an hour to two hours in the solution, are transferred to pure sea water, and is due to the disturbance of the osmotic pressure leading to loss of water by the egg, followed by rehydration (Bataillon, Loeb, Giard, etc.), not to specific chemical stimulation.

The nuclear activity may also be roused by other chemical bodies, as strychnine (Hertwigs, Morgan), chloroform, ether, alcohol, by lack of oxygen (Mathews), by very dilute hydrochloric acid (Loeb, Delage).

Further, purely physical agents may have the same effect—heat (Mathews, Bataillon, Delage, Viguier), cold (Morgan, Greeley), and, most important, agitation (Mathews). Mathews had previously proved for Asterias, and Morgan for Arbacia also, that shaking of unripe eggs caused them to form the polar bodies—the shaking presumably causing dissolution of the nuclear membrane. Ripe eggs of Asterias, but not of sea-urchin, act in the same way, but only after they have lain some time in water; after two hours larvæ begin to appear on shaking; after four hours, hard shaking produces a large proportion of larvæ, and the mere transference of the eggs by a pipette from one vessel to another is sufficient to form a few larvæ. A few hours later the slight amount of shock experienced in the transference of the eggs, causes a large number to begin to develop, though they do not go beyond the late segmentation stages. At this time shaking causes all to develop, but none reach the blastula stage. Loeb and Fischer have extended this observation to the Annelids, Chætopterus and Amphitrite.

The mitotic phenomena produced artificially are apt to be irregular, and the division of the cell body is often unequal when it occurs. Thus the nucleus may divide repeatedly without division of the cytoplasm, and then the egg may break into as many segments as there are nuclei (Wilson and others). It is only in a relatively small proportion of eggs that division is regular enough to permit of development to the larval stage. Further, the eggs of the same species behave capriciously to the same agents under different conditions (temperature, etc.), and the eggs of closely allied species seem to react differently to the same agent.

There is no reasonable doubt, however, that true artificial parthenogenetic development has been demonstrated for the Echinoderms—sea-urchins and star-fish—and for at least two Annelids, though the same amount of independent testimony is not available for the latter.

Actual development to a larval stage has been obtained only by certain of the agents enumerated above.

1. Increase of Osmotic Pressure.—The most successful results (Loeb, 1902) are obtained at a temperature about 20° C., by the addition of the chlorides of potassium or sodium to sea water, the optimum degree of concentration being determined by experiment for each set of observations.[1] After the eggs have remained in this for half an hour to two hours, the optimum being again tested by experiment, they are restored to normal sea water.

Sea-urchins (Loeb, Wilson, Giard, Prowazek, Delage, Viguier, Hunter). Annelids : Chætopterus, Amphitrite, Nereis (Loeb, Fischer).

2. Agitation. — Asterias (Mathews), Chætopterus and Amphitrite (Loeb, Fischer), but not sea-urchins (Mathews, Viguier).

3. Elevation of Temperature.—Asterias during maturation (Delage) ; not for ripe eggs (Greeley, Viguier).

4. Depression of Temperature.—Asterias (Greeley) ; not sea-urchins (Viguier).

[1] Loeb (1902) uses a stock solution of $2\frac{1}{2}$ n. HCl, and adds this in different proportions, 8, 10, 12, 14, 16, 18 c.cm., to 100 c.c. of sea water in six vessels to determine the best grade of concentration.

5. Exposure to weak HCl in sea water, and subsequent restoration to pure sea water. Asterias (Loeb, Delage).

6. Continuous exposure to a solution of a specific chemical substance at the same osmotic pressure as normal sea water.

Potassium Chloride. Chætopterus (Loeb).

Calcium Chloride. Amphitrite (Fischer).

The Ions of potassium and calcium are said to be specific for these forms respectively.[1]

With regard to the influence of the state of maturation, Delage gives results to show that in Asterias glacialis, when the eggs are placed in sea water to which is added an equal quantity of a solution of HCl, raising the molecular concentrations of the mixture to 0·660, different results are got according to the stage of maturation. Among the eggs placed in the liquid before maturation, 20 per cent. of blastulæ were got, at the appearance of the first polar body 95 per cent., and after the appearance of the second polar body 5 per cent., while none of the controls showed any normal segmentation.

From all this it seems that changes in the osmotic pressure between the egg and its surrounding medium, and mechanical agitation, are the chief agents so far as yet

[1] Delage ('Compt. Rendus de l'Acad. des Sciences,' October 13th and 20th, 1902) announces that he has found an agent which is as certain and effective as the spermatozoon, in producing development to advanced larval stages, in Asterias. It is sea water aërated by carbonic acid gas, and at the same osmotic pressure as ordinary sea water (or lower?). When the eggs, at what he calls the "critical stage"—i.e. when the nuclear membrane of the germinal vesicle is dissolved, up to the expulsion of the first polar body—are placed in this, and after one hour transferred to pure sea water, practically all the eggs develop. His view is, that the maturation is arrested temporarily, and on restoration to pure sea water, the carbonic acid gas is quickly eliminated and division proceeds; but it is not partial, as in the polar mitoses, but complete, and goes on to the formation of the normal larval forms. The result is not obtained at a stage after the polar bodies are extruded and the ovum has again come to rest, nor is it applicable in sea-urchin, in which the maturation is over before the ova are shed. His theory as to the action of the gas is, that it is a temporary poison which arrests maturation completely, and is quickly removed afterwards without altering the characters of the protoplasm.

known, which tend to the production of artificial parthenogenesis, but that in the case of the Annelids there is evidence to show that certain Ions may have a specific effect.

According to Loeb (1902) the solutions must act, first, by favouring the solution or dissolution of the nuclear membrane; and second, by changing, in some sense, the physical properties of the protoplasm (viscidity, etc.).

Mathews (1900), as a conclusion from his experiments on Arbacia eggs, pointed out that the known methods of causing liquefaction in protoplasm will induce karyokinesis in these eggs, and also shows that loss of water has a liquefying action.

Before considering further the bearing of the physiological and physico-chemical conceptions regarding fertilisation, I shall proceed to the morphological changes which have been described in unfertilised eggs which undergo parthenogenetic development.

R. Hertwig (1896) studied the changes in the egg after treatment with strychnine. On the breaking down of the nucleus, half spindles, and in a few cases whole spindles, supposed to arise from the fan spindles, were formed. The fan spindle fibres he regarded as derived from the achromatic network of the nucleus. The chromosomes derived from the nucleoli became attached to the primary rays. Later, protoplasmic rays also appeared, centering on the focal point of the half spindle. At this central point, and derived from the central parts of the rays, there appeared a rounded body resembling in every way a centrosome, though none such was to be found before the nucleus broke down. The body was an ovocentrum, formed from the achromatic portion of the nucleus, and, according to Hertwig, the individualised centrosome is ultimately a derivative of the nucleus—is, in fact, an achromatic nucleus.

Doflein (1897), contrariwise, examined the phenomena of karyokinesis of the sperm nucleus in eggs which, after fertilisation, had been treated by chloral solution after the manner of the experiment of O. and R. Hertwig. The nuclei did not unite, but underwent independent transformation. Doflein, like R. Hertwig, considered the middle piece of the spermatozoon as equivalent to the centrosome, and from the experiments concluded, that from the centrosome a complete spindle could form, and out of this, again, the achromatic nuclear network. Thus, compared with Hertwig's results, the ripe sperm nucleus contains all the parts, even as the ripe egg nucleus, which are necessary for a further development.

In Hertwig's results we have evidence of a centrosome arising from the nucleus de novo. Morgan, in 1896, described the formation of artificial astrospheres in the cytoplasm of the eggs of Arbacia treated by salt solutions, and from his further observations published in 1899 and 1900 he decided, that in spite of certain differences these artificial astrospheres corresponded to the normal spheres which occur at the apices of the spindles in the segmentation stages; further (1900), that both artificial and normal spheres are due to accumulation of a specific substance, and that the yolk spheres are excluded from the substance of the astrospheres.

His view of the astral radiations is that they serve to transport the chromosomes, but are not concerned in the division of the cytoplasm.

Evidence of free formation of the centrosomes is found also in the appearance of asters in the cytoplasm in various forms, Echinus among them, on the breaking down of the germinal vesicle in maturation.

Boveri (1901) in essence accepted Hertwig's definition of the structure described by him as an ovocentrum, and its origin apparently de novo. He argued that phylogenetically the centrosome is an individualised cytocentrum, derived from a centro-nucleus in which the centrosome or its equivalent is not differentiated from the chromatin nucleus. To the nucleus of the sea-urchin egg must necessarily be attributed the properties of a centro-nucleus, with the capacity of producing out of itself, under the action of certain stimuli, individualised centrosomes, when such fail to be supplied in the normal way in fertilisation. If even under similar conditions, the sperm centrosome be present, the cytocentrum remains latent. The centrosome looked at in this way, is not a specific cell organ in the sense that it must consist of a specific chemical substance, but that parts of a substance contained in the nucleus, undergoing certain changes, and grouping themselves together, are organised into a centrosome.

Thus the ovocentrum of the sea-urchin egg is not to be considered an individualised centrosome, but an intranuclear latent cytocentrum, and the nucleus is a centronucleus. Thus the centrosome in such a case is not something strictly new, but arises by the transformation in a definite manner of a cytocentrum already present. It is a case not of new formation, but of "reparation." "Gervisse Centronuclei sind im stande unter bestimmten Bedingungen Centrosomen zu reparieren."

Morgan's artificial astrospheres he did not admit to have true centrosomes—the essential character of capacity for division was not proved for them.

This brings me to Wilson's very interesting and important paper on the morphological phenomena in parthenogenetic eggs.

The main results are that under the influence of the magnesium chloride solution, not only are asters produced *de novo* in connection with the nucleus, but also in the cytoplasm. "Not only the asters connected with chromosomes (nuclear asters), but also the supernumerary asters unconnected with nuclear matter (cytasters), may multiply by division; the cytasters contain deeply staining central granules indistinguishable from centrosomes, that divide to form the centres of the daughter asters. These asters operate with greater or less energy as centres of cytoplasmic division. Typical cytasters, often containing deeply staining central granules resembling centrosomes, are formed in the magnesium solution in *enucleated egg fragments* produced by shaking the unfertilised eggs to pieces, and these asters likewise may *multiply by division*, though no cytoplasmic cleavage takes place. The cleavage centrosomes first make their appearance outside the nucleus, but directly on the nuclear membrane, and the evidence renders it nearly certain that they arise by the division of a single primary egg centrosome that is formed *de novo*. All the evidence goes to show that the cleavage centrosomes are of the same general nature as the central bodies of the cytasters."

Among many interesting details I will refer here only to the changes described for eggs which underwent segmentation, and were capable of developing into swimming embryos, because in certain particulars they are reminiscent of what takes place in the formation of the first polar amphiaster.

I may summarise as follows :—(1) The first change that occurs is a coarsening in the appearance of the protoplasm, better marked in eggs treated by stronger solutions. (2) A primary radiation appears centering on the nucleus, better marked in eggs treated with weaker solutions. (3) A varying number of secondary radiations appear in eggs especially treated with stronger solutions. The extent of the primary radiations is inversely in proportion to the number of the secondary radiations. These latter appear as vague clear spots in the cytoplasm, which gradually become surrounded with radiations, and finally assume the form of asters. They always appear *in situ*, and do not change their position till a later period. (4) Coincident with the appearance of the radiations there is a gradual growth of the nucleus. (5) Round the nucleus appears a clear perinuclear zone of hyaloplasm. (6) The nuclear membrane fades out, and a vague irregular clear space is left, to which the hyaline zone contributes. (7) The rays then diminish, and, indeed, almost disappear.

The eggs at this point were restored to pure sea water, and after a pause the radiations reappear and advance centrifugally towards the periphery. In eggs capable of development the principal rays are now focussed on two centres at opposite poles of the nuclear area, which now forms a spindle connecting the two asters. If the amphiaster is typical, division proceeds as in normal fertilisation. If more than two asters are formed from the nuclear area, multipolar figures form, and irregular cleavage results. If there is only a single radiation which does not resolve itself into a bipolar figure, the egg never properly segments, but there are regularly alternating phases of nuclear transformation.

Analysing the meaning of the phenomena, Wilson says, " We may therefore state that the first general effect of the stimulus, whether the magnesium solution or the spermatozoon, is to arouse an activity of the cytoplasm, one result of which is the establishment of a centripetal movement of the hyaloplasm towards one or more points at which the hyaloplasm accumulates." The rays in this view are the expression, in part at any rate, of centripetal currents, and the substance flowing in, is the hyaloplasm or interalveolar substance. The hyaloplasm spheres at the centres of the asters are local accumulations of this hyaloplasm. In fixed material, studied in sections, the radiations are fibrillar in appearance, and as they stain much more deeply than the general network the hyaloplasm in the rays must probably have undergone some physical or chemical change. The centrosome is a well-defined body of considerable size and of spongy consistence, composed of intensely staining granules, which often give the centrosome the appearance of a minute nucleus containing a chromatin reticulum. The hyaloplasm spheres in the living egg correspond to the centrosome, the clear area round it, and the innermost darkly staining radiated zone of the aster taken together.

Thus Wilson has proved that structures which cannot be distinguished morphologically from "true centrosomes" appear in the cytoplasm *de novo*; and further, that they divide to form the apices of bipolar figures, even in enucleated fragments.

In a recent paper Meves (1902), using Boveri's nomenclature, expresses the view that the centrosome is only the mantle of the centriole, and is only present in rapidly-dividing cells like the blastomeres. The " Doppelkörchen " of the tissue-cells are to be considered as centrioles, and "nur von den Centriolen nicht aber von den Centrosomen, kann daher gelten, dass sie allgemeine und dauernde Zellorgane sind." The results of Morgan and Wilson can only then be held to prove that centrioles under certain conditions may, by the action of salt solutions, be excited to form centrosomes and radiations round them, for their results might be explained by a multiplication of the two centrioles which the egg has derived from the last division of the division period, and the distribution of these centrioles through the cell. Even in enucleated fragments there is no proof that the fragment did not contain the centriole of the cell.

Such a supposition admits of neither proof nor disproof, and the presence of a free "centriole" in the unfertilised sea-urchin egg has not been demonstrated. I have seen in young oocytes minute bodies, stained black with iron hæmatoxylin—sometimes double bodies,—but I have not been able to convince myself that they are more than accidents of staining and fixing.

Turning now to the phenomena of fertilisation in the sea-urchin, there is to be recognised (1) a local stimulation at the place of contact of the chosen spermatozoon,[1] manifested by the streaming out of the protoplasm to form the entrance cone. (2) A general stimulation, manifested by the throwing off of the vitelline membrane, and by a change in the constitution of the protoplasm. It becomes more viscid for a time (Morgan); a funnel-shaped area of darkly staining substance follows the path of the sperm head (Wilson). (3) A protoplasmic movement focussed on the situation of the middle piece giving rise to the sperm aster. This appears soon after the entrance of the spermatozoon, when the head has begun a movement of rotation. The rotation goes on

[1] Buller (1902) has studied the question of the bearing of chemotaxis on fertilisation in Echinoderms. His conclusion is that chemotaxis plays no rôle in bringing the sexual elements together. The meeting is a matter of chance. The passage through the gelatinous coat is radial in direction, and probably purely mechanical, though possibly due to stereotaxis.

through 180° till the base of the conical sperm head is directed inwards. The rays of the aster now extend widely, and at their centre is a clear area. Meantime the sperm head becomes converted into a small round nucleus. The movement of the sperm head is, at first, radial; then there is a change, and it assumes a new direction towards a point not quite in the centre of the egg; when this change of path is taken up the egg nucleus begins to move towards the point where the nuclei ultimately meet (Wilson and Giardina). The aster now comes in contact with the egg nucleus, and as the nuclei approach, the clear area at its centre spreads out over its side. The aster then divides and the nuclei conjugate. The radiations now die down during a pause in which the nucleus grows in size (Wilson), to redevelop again focussed at the poles of the nucleus.

According to Hertwig, Doflein, Erlanger, and Wilson's earlier account, the centrosome corresponds to the whole middle piece, but later Wilson described the middle piece as cast aside, and in the centre of the aster is a small darkly-staining granule. Boveri (1901) represents the sperm centrosome as a spherical body smaller than the middle piece, and containing within it two centrioles shortly after its entrance into the egg.

Various other observers have represented a dark-staining granule at the centre of the aster. My own observations are inconclusive, and do not warrant me in expressing an opinion.[1]

[1] The character of the fully-formed centrosome in the sea-urchin egg is still subject to difference of opinion. The form in which I see it in osmic acid material is that of a largish sphere of very finely alveolar structure. In Wilson's papers on magnesium and etherised eggs, "it appears as a well-defined body of considerable size, consisting of intensely stained granules, which often give the centrosome exactly the appearance of a minute nucleus containing a chromatin network." This becomes in the anaphases more homogeneous, and flattens down into a plate-form, which in the telophases often lies directly on the membrane of the newly-formed nucleus precisely as Boveri (1901) has described for Echinus. Boveri (1901) represents it in several forms. In one set of preparations it is a largish sphere of very finely alveolar

The essential difference between the processes seen in magnesium eggs and normal fertilisation is that whereas in fertilisation there is only one, and that a definitely localised point of astral activity, in the magnesium eggs there are a number of foci, and development in large measure depends on the accident of their number in the nuclear area.

There is the same want of unity of purpose that is seen in polyspermic eggs, in which the number of points of astral activity depends on the number of spermatozoa which gain an entrance.

It has long been recognised that the union of the nuclei and the initiation of division are co-ordinated, but in a measure independent factors in fertilisation. Parthenogenetic development under artificial agents is the latest proof of this. The possibility of the development of enucleated egg fragments when entered by a spermatozoon, as described by Boveri, and afterwards named merogony by Delage, is another. Either nucleus is sufficient in itself.

With the problems underlying the nuclear conjugation this article is not concerned. It starts from the assumption that the union of equivalent nuclei is the end of fertilisation, but not the means (Boveri).

The cause of the nuclear conjugation is not as yet understood. The first possibility is that the aster is concerned in bringing them together. Giardina (October, 1902) brings the latest suggestion on this line. Starting from the basis of the alveolar structure of protoplasm, he suggests that the aster is the expression of both centripetal and centrifugal currents. The centrosome is concerned in the diffusion of chemotrophic substances into the egg, while at the same time

structure. In another set, in which the centrosome had reacted differently, there is a centriole within the centrosome, which divides before the centrosome, so that it is double in the metaphase. In Wilson's earlier account there was no central body, but in later descriptions there was a mass of granules in a well-defined sphere, which succeeded a single granule of earlier stages. In my previous paper, I regret that I misrepresented Professor Wilson's nomenclature by referring to this as his centrosome. The sphere, as a whole, is named the centrosome. See note to page 314, "The cell, etc.," 1900.

the hyaloplasm flows in towards the centre. He points out that the germ nucleus does not move till the rays of the aster have reached it, and the aster has assumed a position of equilibrium towards the centre of the egg. The union is thus the result of the chemotactic forces of which the aster is the expression.

Wilson (1901 B) shows, however, that the nuclei may unite in the entire absence of an aster. When eggs, immediately after fertilisation, are placed in a weak solution of chloral (O. and R. Hertwig), or ether (Wilson), no aster is developed, but when replaced in sea water the rays reappear and the nuclei unite. In a certain proportion of cases, which will be referred to later, the nuclei remain apart and undergo independent transformation; but in some instances, also while the eggs are still in ether, the nuclei enlarge, and later conjugate in the entire absence of an aster. This happens, however, only when the spermatozoon has entered at a point not too far from the egg nucleus. Giardina holds that this fact, and the other—that the nuclei quickly unite whenever the eggs are put in pure sea water, and the aster develops,—makes Wilson's observation insufficient to exclude his hypothesis. Other explanations, such as mass attraction and direct chemical attraction, both observers reject. Wilson thinks the latter improbable. Again, the idea of protoplasmic currents such as suggested by Butschli, Erlanger, and Conklin, is not proved by actual evidence in normal conditions in the sea-urchin egg (Wilson). The changes of shape of the germ nucleus might suggest amœboid movement on its part; but, again, this does not apply to the sperm nucleus, which travels through a longer path (Wilson). The changes in form might be due to the exercise of chemotactic forces on the nucleus (Giardina).

The phenomenon described by Boveri (1888) under the name of "Partial Fertilisation," has recently been worked out in detail in Boveri's fixed preparations by Teichmann (1902). The method by which the results were obtained was that eggs which had lain fourteen hours in unrenewed sea water were fertilised with spermatozoa, which were treated with a ·05 per cent. solution of potassium hydrate until only a few were mobile. While polyspermy occurred in more than half the eggs, the remainder were fertilised by a single spermatozoon. In these cases, however, the sperm nucleus did not unite with the germ nucleus, but the aster became detached from it, and advanced alone to the germ nucleus, a bipolar figure was formed and division proceeded. The sperm nucleus took no share in the process, but passed unaltered into one of the blastomeres. Later, however, either in the two- or the four-cell space, it broke up into its chromosomes, which entered into the equatorial plate of the cell in which it was included, and which now divided like its neighbours. Such eggs were capable of developing to the blastula stage.

The question presented itself: Was this aster and the amphiaster the result of the activity of an ovocentrum, or were they the product of the sperm aster?

In monospermic eggs Teichmann found the early stages very scarce, and, though very suggestive, too few for absolute proof, and the phenomena seen in dyspermic eggs are described to fill the gap. It may be admitted that in these eggs, in spite of the apparent inactivity of the sperm nucleus, the sperm aster with its centrum is the operative factor in starting the developmental process. The appearances are very similar to those in the etherised eggs described by Wilson (1901). In that form, as in Echinus, the nuclei conjugate when they are very unequal in size, and before the division of the aster. In Asterias and other forms an amphiaster is developed before conjugation, and the nuclei are nearly equal in size. In the experiments the union was delayed, as in "partial fertilisation," and the amphiaster was formed before the conjugation.

Among Teichmann's observations I shall refer only to those of monospermic eggs. The main feature is the detachment of the sperm aster from its nucleus, its application to the egg nucleus, and its normal division, followed by normal segmentation. The fate of the sperm nucleus depends on the position it assumes in the egg, relative to the cleavage plane. If it lies outside the equatorial plate of the spindle it passes unchanged into one of the blastomeres; if it lies within the field of the first spindle, it does not actually unite with the chromatin of the female nucleus, but its chromatin undergoes a marked relaxation. Though it shows a marked resistance to the tractive forces, it is drawn out and torn into several shreds. It thus passes undivided into one of the blastomeres, and no chromatin elements derived from it are found at the poles of the spindle. In several cases where the nucleus lay exactly at the equator, and the traction of the poles was nearly equal, it was observed that the chromatin mass was much broken up, and was torn into two parts. The cleavage of the cell body may have helped to complete the division. The loosening of the sperm chromatin mass in the first spindle seems to have broken its power of resistance, for when the next division is initiated, the two nuclei lying side by side in one of the blastomeres unite in the equatorial plate stage, and the chromatin of both is equally distributed in the next division. The number of chromosomes is now different in the blastomeres, sometimes double, perhaps quadruple in some cases (though an accurate count was not possible), as if the chromosomes of the sperm nucleus emerged in double number, though the first division was suppressed.

The sperm nucleus in the case where it has not lain within the power of the spindle in the first division, may now, in analogous fashion, be caught in the second division, to unite later with the chromatin of one of the blastomeres of the four-cell stage, or it may even pass over into the eight-cell stage, as seen in living eggs by Boveri.

Teichmann concludes that the radiations are derived from the sperm centrosome as their starting point, and on the supposition that the centrosome is introduced by the spermatozoon into the egg, that it has suffered less from the chemical reagent than the nucleus. The centrosome behaves as in ordinary fertilisation, the sperm nucleus is passive, and seems to form no hindrance to the normal processes in the egg, and it seems to be of no significance, whether it enters earlier or later into union with one of the descendants of the egg nucleus. The difference between the phenomena of "partial fertilisation" and the normal process is the non-union of the nuclei. In certain cases Boveri (1890) described an independent transformation of the nuclei under normal conditions, but the elements from both entered into the equatorial plate of the first cleavage spindle, and normal division took place. The mere want of union is not of moment, if the sperm nucleus lies near enough the germ nucleus to be influenced by the nuclear fluid of the egg nucleus. The absence of this hastening factor may explain the fact that the karyokinesis of the sperm nucleus in enucleated fragments is much slower than in the cleavage spindle. But such an explanation alone will not hold for cases of dyspermy in these experiments, where the sperm nuclear descendants remain far behind the derivates of the egg nucleus, and it must be assumed that a change has taken place in the sperm nucleus itself, a kind of paresis, produced by the potassium hydrate. This holds for the monospermic eggs also, and explains why, even in spite of its position, the nucleus does not enter into union.

Another factor is a change in the egg, defined as an over-ripeness. In many cases the germ nucleus is a stage ahead, compared with the normal process, of the centrosome. When the centrosome met the egg nucleus, the latter must already have been in a way prepared for division, and this great readiness to enter into division may be part explanation of the lagging behind of the sperm nucleus. There has not been time for the sperm nucleus to undergo transformation before the egg nucleus has submitted to division. Teichmann does not explain in what the over-ripeness consists. It may perhaps be that, since the eggs had lain fourteen hours in unrenewed sea water, the early preparatory stages of the natural transformation had supervened, which takes place in eggs after lying long in sea water.

Regarding the main point, it may be admitted that the aster and its centrosome here concerned is that belonging to the sperm nucleus, and that in its behaviour we have a beautiful demonstration of the independence of the two factors in fertilisation, or, in other words, of the two functions of the spermatozoon, and that the two functions have been disturbed in unequal degree. At the same time the egg protoplasm, after the fourteen hours' sojourn in unrenewed sea water, was approaching to that stage in which it acquires spontaneously the tendency to develop astral activities, and it might be held that the conditions are the same as in magnesium eggs in which, as a result of a general stimulation, asters and centrosomes which are unconnected with the nucleus appear de novo in the cytoplasm. While the results are of interest in connection with the apparent independence of the factors in fertilisation, they also show how they are co-ordinated together. The sperm nucleus becomes dissociated from the aster, and fails of union, because it has not undergone the transformation which properly corresponds to the phase reached in the cycle of the centrosomal changes. Further, while the nuclei may be resolved into chromosomes before union, and yet unite in the equatorial plate stage, a certain stage in the transformation of the dense mass of chromatin of the sperm head into a nucleus with distinct chromatin network, must be reached before union can take place. This seems to show that several co-ordinated factors are at work in the nuclear conjugation.

Further insight into the behaviour of the factors in fertilisation is given by an experiment described by Ziegler (1898). This consisted in carrying newly-fertilised eggs by a gentle current of water in his compressorium against threads of cotton wool. The egg was caught on a thread and nearly cut through, leaving only a slender bridge of protoplasm between the two portions of the egg. The one contained the sperm nucleus, the other the germ nucleus. While the sperm nucleus regularly divided, followed by division of the cytoplasm, the egg nucleus merely underwent alternate changes of disintegration and reconstruction without division of the cytoplasm. Radiations appeared and disappeared, and after three cycles, owing to the segmentation of the portions containing the sperm nucleus, the egg-nuclear portion became detached and disintegrated. In another experiment the egg-nuclear portion underwent changes of form suggesting abortive attempts at cleavage. The mitotic transformation of the egg nucleus was not synchronous with that of the sperm nucleus, but always a little behind.

These observations show that under the conditions of the experiments the egg nucleus is excited to division without direct contact with the sperm nucleus or aster, but that the mitotic phenomena are ineffective to produce cytoplasmic cleavage. Ziegler refers this to the general stimulation of the egg by the spermatozoon, manifested also by the throwing off of the vitelline membrane. Boveri has shown that the same phenomena occur in egg fragments produced by shaking some minutes after fertilisation, and he (1902) refers to cases of this kind in which he has observed divisions of the nucleus followed by cell cleavage. The division was repeated a second time, and thus the four-cell stage was reached, but development then ceased. Another example of the effect of this general stimulation is to be seen (Boveri, 1902) in the cases in which the egg is incited to throw off the polar bodies by the entrance of the spermatozoon.

This brings me to a further reference to Wilson's observations on etherised eggs (1901 B). As has already been said, under this treatment the sperm and germ nuclei remain apart and undergo independently karyokinetic transformation. "The most striking fact is that, while the sperm aster often gives rise to a perfect and symmetrical bipolar figure, the egg nucleus in a great number of cases produces a monaster, which seems at first incapable of resolving itself into a bipolar figure." In typical cases the egg nucleus gives rise to a monaster such as described by Hertwig ('96), and such as occurs in magnesium eggs. While the egg monaster does not at first give rise to a dicentric figure, it does so later, as may be gathered from the description of an egg continuously observed in the living state. At the height of its development the egg monaster lay at one side, the sperm amphiaster at the other, and no spindle was formed between them. The egg divided into three cells, two larger and somewhat irregular containing two daughter sperm nuclei, and a small one in which the single egg nucleus re-formed. At the second division each of the sperm nuclei gave rise to a perfect amphiaster, and divided into two, the accompanying cytoplasmic division resulting in the formation of two complete cells and one binucleate cell. The single egg nucleus gave rise to a tetraster, and divided into three cells, one binucleate, the nuclei of the latter quickly fusing together. The embryo now consisted of six cells—three containing maternal, three paternal nuclei. At the ensuing division fifteen cells were formed, of which eight larger ones contained paternal nuclei, while seven much smaller ones containing maternal nuclei lay in a definite group at one side. The egg observed afterwards died. Wilson has not seen an egg monaster become dicentric at the first division, but the above observations prove that it may operate as an effective division centre, without establishing a spindle connection with either of the sperm asters, and that it may divide later. A centrosome was demonstrated in the monaster, in the same form as in the sperm aster, and as in magnesium eggs. The possible action of the chemical as the exciting agent of the karyokinetic transformation was excluded by control experiments, and it was therefore concluded, that it was due to a stimulus effected by the spermatozoon, as in Ziegler's experiment. These observations, added to the results obtained in the magnesium eggs, "demonstrate that under appropriate stimulus the egg may give rise to a centrosome capable of progressive division, but the etherised eggs show in the clearest manner that this centrosome is less effective than the sperm centrosome."

I shall not venture on the general problem of the asters and centrosomes. It will suffice for the present purpose if it be admitted that, without prejudice to the question either of the individuality, or the persistence of the centrosome, the body and its aster represent a kinetic phase of protoplasm, which reveals itself in cycles of activity, and that the centrosomes and asters constitute together, in some sense, a divisional apparatus, though that term is not used in any definite mechanical sense.

The egg both before and after maturation lacks the power, for and by itself, to produce in normal circumstances such a divisional apparatus as will regularly and equally divide the cell.

In Ziegler's and Boveri's experiments on separated portions of the egg containing only the egg nucleus, a divisional apparatus is called up under the general stimulation of the spermatozoon; but it is ineffective, or only very partially effective. In the etherised eggs it is slow in appearing, and less effective than that associated with the sperm nucleus. In magnesium eggs the effect of the disturbance of equilibrium is to cause a change of state in the protoplasm which results in the differentiation at many foci of kinetic centres, and it is only in the cases where a single such centre, which divides into two, appears in the nuclear area, or at most two centres, that normal division proceeds.

In fertilisation there is only one kinetic centre, and this is localised on the middle piece of the spermatozoon. Its activities are rapidly unfolded, and dominate all the other latent astral activities of the egg. "The latent capacity of both nucleus and cytoplasm to give rise to centrosomes is in this case wholly inhibited."[1] By union of the nuclei its activity is transferred to the cleavage nucleus, and "becomes a part of an activity on the part of the egg nucleus that would have ensued even had the germ nuclei not united."[1]

Thus it may be said that the spermatozoon supplies the lack in the egg, by providing a powerful and effective "divisional apparatus." How is this effected? Does the spermatozoon act by giving a general or diffused stimulus

[1] Wilson, 1901 A, pp. 581, 582.

to the egg, or by disturbing the general equilibrium in some such way as the loss of water does, when the normal osmotic relations are disturbed? Or does the spermatozoon carry into the egg some specific chemical substance which produces a local differentiation, of which the centrosome and the aster are the expression? Or does it import "a highly active centrosome or centroplasm about which the cytoplasmic energy is brought to a focus?"

Boveri (1902) holds that a general stimulation of the egg, with the sperm head as the point of predilection for the formation of the aster, as in magnesium eggs the egg nucleus is the point of predilection, is insufficient as an explanation. There is much rather something special present in the spermatozoon, which determines that the aster shall appear at that point, and that point only; and thus he thinks that still the appearances may best be described as being due to the introduction of a centrosome. Even admitting—which, as has just been indicated, he does not—that the spermatozoon acts like Loeb's agents, and in view of the demonstration by Morgan and Wilson that their effect is to cause the egg to produce centrosomes de novo, only a modification of secondary importance would be required in his theory of fertilisation, viz., that instead of saying that the spermatozoon brings a centrosome into the egg, it would be necessary to say that it causes the formation of a centrosome in the egg, from the division of which the rest follows.

Taking the sperm aster as the manifestation of activities produced by the spermatozoon, and looking to its sharp localisation on the site of the middle piece, it seems reasonable to suppose that the localised excitement is the effect of an agent operative in fertilisation, and that it is probably related to the middle piece; but the actual continuity between the centrosome of the spermatozoon and that in the aster has not been absolutely demonstrated, and the new facts in regard to the centrosome put the matter in another light. Thus it remains for the future to decide which of the two latter alternatives stated above shall be adopted, and perhaps after all there is only a formal difference, for the fundamental problem is the same when the question is raised how the centrosome exercises its activities.

Loeb (1901), on the physico-chemical side, suggests that a catalytic substance is carried by the spermatozoon into the egg, that is one which accelerates physical or chemical processes which would occur without it. The K ions act as catalysers, and the loss of water acts also, though less directly, in the same way, and it may be that it gives rise to substances which act catalytically. Inasmuch as in Chætopterus the normal development does not show the characteristics of a treatment of the eggs by K, it is probable that normal fertilisation is not brought about by K ions.

Delage (1901) considers that the egg is in an unstable state of equilibrium, which is readily upset by various agencies—loss of water, heat, etc., and he lays some weight on the specific action of the salts. He finds that the chloride of manganese has, for Asterias, a specific action superior to that of the alkaline salts. Together with his son, he showed that in the case of the sea-urchin there was less magnesium chloride in the sperm than in the eggs, by about 1 per cent., so that this salt could not have a specific action.

Among other possible factors in the action of the spermatozoon, he gives prominence to its abstraction of water from the cytoplasm. During maturation the nuclear sap is shed into the cytoplasm; until this is effected by the solution of the nuclear membrane, fertilisation is not possible; it is just at this "critical stage" in Asterias that he finds artificial parthenogenesis most liable to occur. In the specialisation of the sexual elements, the egg thus becomes rich, while the spermatozoon has become poor, in water. After the sperm head has entered the ovum it increases in size by abstraction of fluid from the egg protoplasm, and this abstraction of water by the sperm nucleus has to be reckoned with as a possible factor in fertilisation.

Apart from the large assumptions involved in such an hypothesis, the facts of "partial fertilisation, and the localisation of the aster on the middle piece, are in opposition to it."

It has been suggested that the centrosome is the seat of formation of a ferment. Mathews (1901), from the results of his experiments on the eggs of Arbacia, believes that "whatever the details of the process may prove to be, the essential basis of karyokinetic cell division is the production of localised areas of liquefaction in the protoplasm." "The centrosome might be a liquefying enzyme."

Experiments on this line have been tried, but without definite result. Pieri's results (1899), from which he supposed he had obtained a ferment "ovulase," have not been confirmed. Dubois (1900) showed that there was no question of a ferment being obtained by Pieri's methods. He made various experiments on sperm and eggs, from which he concluded that there was evidence of the existence of a "zymase," which he provisionally named "Spermase," in the spermatozoa, and in the egg a substance, at least modifiable by "spermase," provisionally named "Ovulase." Spermase cannot enter the egg by diffusion or osmosis, but only by a mechanical means, which is the raison d'être of the spermatozoon. Winkler's experiments (1900) are also inconclusive. He used sperm shaken for half an hour in distilled water and filtered five or six times through threefold filter-paper. The filtrate was added to sea water, the precaution being taken of keeping the mixture at the same degree of concentration as the sea water. While the sperm in heated sea water produced no results, the liquid caused in the case of Sphærechinus and Arbacia eggs, though in a relatively small number, the beginnings of segmentation. These results may have been due to osmotic influences.

Loeb (1900) states that up to that date he had found no enzyme save papain which had an effect in causing the egg to segment, and it was uncertain whether this was not due to some accidental constituent of the enzyme preparation used. Gies (1901) made a complete study of the effects of extracts of sperm made by the ordinary methods for the preparation of enzyme solutions. His results were wholly negative, and he concluded that, used in certain proportions and under certain conditions at any rate, such extracts did not possess any power of causing proliferation of the ripe ovum. No evidence could be furnished of the existence of a zymogen in spermatozoa. Extracts of fertilised eggs in the earlier stages of development seemed likewise devoid of any segmental activity. The results, Gies adds, do not, however, certainly show that enzyme action is impossible, after, or at the time of union of the spermatozoon with the ovum, within the latter.

The same negative result was got this spring by R. T. Lieper at Millport Marine Biological Station, using an extract of sperm prepared by spreading fresh sperm on sheets of glass, then drying in air and sun, and afterwards triturating the dried extract in sterilised sea water. The filtrate from this fluid produced no segmentations, though control experiments with eggs from the same ovaries normally fertilised, nearly all developed.

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