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A CYTOLOGICAL STUDY OF ARTIFICIAL PARTHENOGENESIS IN STRONGYLOCENTROTUS PURPURATUS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF CALIFORNIA

BY

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A Cytological Study of Artificial Parthenogenesis in Strongylocentrotus purpuratus

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Edward Hindle

With one plate

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A Cytological Study of Artificial Parthenogenesis in Strongylocentrotus purpuratus[1].

By

Edward Hindle.

With Plate V.

Eingegangen am 14. Mai 1910.

Introduction.

The following investigation was undertaken mainly with the object of tracing the cytological changes that follow the chemical fertilization[2] of sea-urchin eggs by treatment with a monobasic fatty acid, followed by treatment with a hypertonic salt solution (LOEB, '05, '09).

With this method LOEB has shown that it is possible to obtain from the unfertilized eggs of echinoids practically 100 per cent of larvae, a large percentage of which are normal. In external features the process of development is almost identical with that occurring in normally fertilized eggs and, therefore, it is of interest to determine whether the cytological changes are also similar to those that ordinarily follow the entrance of a sperm into the ovum.

The cytological effects of various solutions, mainly on the eggs of echinoderms, have been investigated by a number of authors.

R. HERTWIG ('96) studied the effect of dilute solutions of strychnine on the unfertilized eggs of *Echinus* and *Sphaerechinus*, and found that the nucleus, after this treatment, may give rise to a typical mitotic spindle in which division of the chromosomes takes place. The

[1] A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of California. Berkeley, May, 1910.

[2] For the sake of brevity the term »Chemical fertilization« is employed to denote the starting of development in unfertilized eggs by chemical means.

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cleavage centrosomes were supposed to arise from the achromatic part of the nucleus and the chromosomes from the nucleolus.

MORGAN ('96, '99, 1900) found that the cytoplasm of eggs of *Arbacia*, when treated with hypertonic solutions of NaCl and $MgCl_2$, or strychnine, became filled with »artificial astrospheres« each containing a centrosome. The centrosomes were therefore considered to arise de novo and MORGAN expressed agreement with HERTWIG's view that they may develop out of the achromatic part of the nucleus.

In 1901 appeared WILSON's important paper on the cytological changes following the chemical fertilization of the eggs of *Toxopneustes variegatus* by means of LOEB's older method, in which magnesium chloride was the agent employed. He found that, in accordance with LOEB's observations, the eggs vary considerably in their response to the hypertonic solution, even when derived from the same female and lying side by side in the same solution. He goes on to say (p. 531): »First, even those eggs that segment and give rise to swimming larvae show wide differences in both the rate and character of the internal changes and in the ensuing form of cleavage. Second, a great variety of asymmetrical mitoses and other pathological phenomena, leading to the production of monstrous forms always occur in a considerable number of the eggs. Third, numerous gradations of the mitotic processes occur, the incomplete mitoses showing innumerable modifications ranging from a hardly perceptible change up to a nearly complete division.« As a result of his examination, WILSON showed that both cleavage-asters and cytasters together with their centrosomes may arise de novo. Further, the cleavage aster arises from the nucleus and its centrosome is more distinct than that of the cytasters. The chromosomes were found to arise in two different ways; in one case, they arise in the ordinary way direct from the reticulum, whereas in the other case they are built up by the nucleolus. Moreover only eighteen chromosomes were found instead of the normal number of thirty six.

In the same year DELAGE (1901) published the results of his cytological study of artificial parthenogenesis in the eggs of *Strongylocentrotus lividus*. His results differed markedly from those of WILSON, for the full number of chromosomes was stated to be restored in the artificial embryos, DELAGE having found nine chromosomes in the unfertilized ovum of this species and eighteen in the cells of the parthenogenetic larvae.

Boveri ('02) and Petrunkewitsch ('04) have both shown that the number of chromosomes normally occurring in the cells of this species is 36, and that the number occurring in the unfertilized egg is 18. The latter is the number of chromosomes found by Delage in the parthenogenetic larvae, and therefore his own observations support the idea that after chemical fertilization the full number of chromosomes is not restored during subsequent development. In spite of this, however, in a recent paper Delage ('08) still adheres to his former view that the number of chromosomes constant for the species is restored in the cells of the parthenogenetic larvae. This view seems to be based mainly on theoretical considerations for no observations are brought forward in support of it[1]).

Wassilieff ('02) and Petrunkewitsch ('04) have studied the cytological effects of artificial fertilization in echinoids, but in both cases their methods and results were imperfect, as they used the older method of treatment with a hypertonic salt solution in order to cause development.

The only complete account of the cytological details of artificial parthenogenesis in echinoids is Wilson's ('01), for the later studies of Herbst ('07, '09) and other authors, do not concern the finer cytology of development. As Godlewski ('09) has given a summary of this subject it will be unnecessary to discuss in detail the various studies of artificial parthenogenesis in other groups that have appeared during the last few years. In all cases where the number of chromosomes in the parthenogenetically developing eggs was determined the reduced number was found to occur, but these observations have been confined to the first few divisions. On these grounds, therefore, Delage considers the question undecided, as the restoration to the full number is supposed to take place gradually.

In the following account it will be seen that the reduced number of chromosomes persists in the cells of parthenogenetic embryos of *Strongylocentrotus purpuratus* as far as the free swimming blastula, and beyond this stage it is impossible to count them owing to the small size of the cells. These observations, therefore, agree with those of Kostanecki ('04, '08) on *Mactra*, Lefevre ('07) on *Tha-*

[1]) »On voit que, si les partisans de la non-régulation du nombre des chromosomes avaient raison, leur nombre devait se reduire de moitié à chaque génération, jusqu'à ce que la reduction devienne impossible en présence d'un nombre impair ou, finalement, de l'unité« (Delage, p. 497). The logic of this remark is not very forcible.

lassema, and other investigators, but are opposed to the theory of DELAGE.

LOEB ('09) has shown that in sea-urchins, and many other classes of animals, the developmental effects of fertilization consist essentially of two processes; first, in an alteration of the cortical layer of the egg, presumably a cytolysis, resulting in the formation of a membrane; and, secondly, in a modification of the processes of oxidation, probably by the destruction of some substance within the egg that is injurious to the synthetic processes started by the membrane formation.

The first process may be effected by any cytolytic agent and if cytolysis is not carried too far the egg begins to develop. Among the agents that are able to cause membrane formation in the egg the following may be mentioned: In the first place we have those substances having a specific cytolic action on cells in general, such as saponin, solanin, digitalin, bile-salts and soap; then we have the simple fat solvents such as benzol, toluol, amylen, and chloroform, which cause membrane formation very rapidly and, in addition, completely cytolize the eggs in a short time; alcohol, ether, and even distilled water are also effective; but the most convenient agents to employ are solutions of alkalies, or of acids, especially monobasic fatty acids.

In the presence of free oxygen this membrane formation starts the oxidations underlying the synthesis of nucleins and other processes of development, and in some forms (e. g. *Thalassema* and *Asterina*) the eggs continue to segment normally and develop into free swimming larvae. But the eggs of sea-urchins seem to contain some substance that has an injurious effect upon the syntheses underlying development. By the time that the first spindle is formed the eggs begin to disintegrate and at ordinary temperatures (15° C.) the majority do not complete the first division. It is true that by keeping them at a low temperature (2—5° C.) they may segment normally for some time, but only a very small percentage develop into blastulae. Therefore a second process is necessary in order to eliminate this disturbing factor of development. After membrane formation, the subsequent treatment with hypertonic sea water, containing oxygen, for from thirty to fifty minutes is sufficient to destroy this injurious substance, and if the eggs are then transferred back into ordinary sea water development proceeds regularly and a large percentage develop into normal larvae. Instead of employing a hyper-

tonic solution, the same result may be obtained by preventing the oxidations in the eggs for about three hours, either by putting them in sea water containing a little potassium cyanide, or simply by removing the oxygen from the sea water by means of a stream of purified hydrogen.

Technique.

The eggs of *Strongylocentrotus purpuratus* were the only kind employed, and in all cases were obtained by taking out the gonads from a female and allowing them to remain in a dish of sterilized sea water for a few hours. The ripe eggs gradually drop out of the ovaries and fall to the bottom of the dish where they may be collected by means of a pipette.

Membrane formation was effected by allowing the eggs to remain in a mixture of 50 cc.s. sea water + 2.9 cc.s. of N/10 butyric acid from 90 to 150 seconds, according to the temperature. The eggs were then transferred back into normal sea water and if they had been in the butyric acid solution for the correct length of time all of them formed membranes. As it was desired to study the cytological effects of butyric acid alone, some of these eggs were allowed to develop without further treatment but at 15° C. very few of them completed even the first division.

When the eggs from the butyric acid solution had remained in normal sea water for about 20 minutes, they were then placed in a mixture of 50 cc.s. sea water + 8 cc.s. of $2^1/_2$ N-NaCl and exposed to the action of this solution for times varying from 30 to 60 minutes. The length of exposure necessary depends mainly upon the temperature, but also on the length of time the eggs have remained in the sea water after removal from the butyric acid solution. Finally, the eggs were again transferred into normal seawater and, if the time of exposure to the hypertonic solution had been correctly chosen, practically all them developed and gave rise to free swimming larvae.

The various stages in the formation of spindles and segmentation were followed under the low power of the microscope and embryos were obtained at all stages of development.

The material was fixed in FLEMMING's strong solution, and imbedded in paraffin in the usual way. Sections were generally cut 3 μ in thickness and stained on the slide by one of the three following methods:

a) ARNOLD's Method: Mordant the sections for ten minutes in a dark brown solution of Iodine and Kl in 70% alcohol. Rinse in 70% alcohol and stain for two hours in a saturated solution of Basic Fuchsin in 75% alcohol. Wash in water and then stain for ten minutes in a 1% aqueous solution of Polychromatic Methylene Blue. Rinse with water and pass up rapidly through the alcohols into a saturated solution of Orange G in clove oil. Allow the sections to differentiate in this solution until they appear more pink than blue, then wash in clove oil, followed by xylol, and mount in Canada Balsam.

b) HEIDENHAIN's Haematoxylin Method (slightly modified). Mordant the sections for ten minutes in an alcoholic solution of Iodine in Kl. Then mordant in a 3.5% aqueous solution of iron alum for about twelve hours. Stain for an equal length of time in a 0.5% aqueous solution of haematoxylin, artificially ripened by the addition of a few drops of lithium carbonate solution; then differentiate and mount in the usual way.

c) MALLORY's Phospho-Tungstic Haematoxylin Method. Mordant the sections for ten minutes in a 0.25% aqueous solution of potassium permanganate. Rinse in water and clear in a 5% aqueous solution of oxalic acid for twenty minutes. Wash thoroughly and then stain for twelve to twenty four hours in a solution of phospho-tungstic haematoxylin. Rinse in water, pass up rapidly through the alcohols into xylol, and mount in canada balsam.

ARNOLD's stain was found to give the most reliable results but for the sake of comparison sections were stained by all three methods.

The experimental part of the work was performed at the Herzstein Research Laboratory, Pacific Grove, under the direction of Professor LOEB, without whose assistance and kindly criticism it would have been impossible to complete this study. The cytological examination of the eggs was completed in the Zoological Department of the University of California, with Professor KOFOID, to whom it gives me great pleasure to express my indebtedness for much help received in this part of the work.

External Changes after Artificial Fertilization.

Under a low power of the microscope the unfertilized egg of *S. purpuratus* appears as an opaque spherical body about 60 μ in diameter. During treatment with butyric acid it presents no morpho-

logical changes, but as soon as the egg is transferred back into normal sea water the process of membrane formation commences. Small vesicles appear over the whole surface increasing in size until they run together and form a clear transparent layer completely surrounding the egg and bounded on the outside by the surface film that has been raised up by this cytolytic process. The thickness of this transparent outer layer is usually about 7 μ but it may vary considerably according to the amount of cytolysis that takes place after the action of the butyric acid.

The membrane formation is complete a few minutes after transference to normal sea water. It is accompanied by the more definite appearance of a light space in the cytoplasm, marking the position of the nucleus. The greater distinctness of this clear space at this stage is due to the dissolution of the cytoplasmic granules immediately surrounding the nucleus to form a clear perinuclear zone.

About 30 minutes after the completion of the subsequent treatment with hypertonic solution, the cleavage aster appears. As it develops the nucleus becomes still more distinct and the various stages in the formation of the spindle and subsequent division of the nucleus can be observed. In those eggs that segment normally the first three cleavages give rise to eight cells of approximately equal size. The fourth cleavage results in the formation of four smaller cells at one of the poles. After this stage the divisions become less regular but eventually a blastula is produced which develops cilia on its outer surface and becomes free swimming. These blastulae swim about at the surface of the water and behave in exactly the same way as normal larvae[1]). The blastula undergoes the process of invagination giving rise to a gastrula and from this is developed the pluteus with its typical skeleton.

The Unfertilized Ovum.

In sections the unfertilized egg of *S. purpuratus* is rather opaque owing to the large number of yolk granules scattered through the cytoplasm. These granules are more abundant at the periphery, thus forming a kind of ectoplasmic layer. The nucleus appears as an oval, lightly-staining area, bounded by a definite membrane, and

[1]) Those that develop from eggs treated simply with a hypertonic solution (LOEB's first method) are unable to rise to the surface of the water but can only swim about the bottom.

contains a fine meshwork of chromatin together with a deeply staining nucleolus. The latter usually contains numerous vacuoles, and is not a mere aggregation of chromatin.

Internal Changes after Treatment with Butyric Acid.

After the membrane formation is complete the cytoplasmic granules become aggregated together in clumps and therefore the protoplasm loses its regular appearance.

A few minutes later the nucleolus shows unmistakable signs of dissolution, as it stains much more lightly, and may even break down into two or more smaller ones (Fig. 2, *nl*). Meanwhile the granules in the vicinity of the nucleus disappear, and thus it becomes surrounded by a clear zone, from which faint radiations extend into the cytoplasm (Fig. 2).

Certain of the granules immediately surrounding this region stain much more deeply than others (Fig. 2, *ch.gr.*). Probably this appearance is due to the formation of acids, as a result of oxidation processes starting from the nucleus and extending out into the cytoplasm, and Loeb ('06) has shown that acids are formed in developing eggs.

This is succeeded by a rapid growth of the nucleus which in some cases increases to as much as three times its original diameter. The chromatin then aggregates together and assumes the form of a spireme which subsequently breaks up into 18 chromosomes.

In eggs that are developing at ordinary temperatures, about two hours after treatment with butyric acid very distinct astral rays appear, extending radially from the now indistinct nuclear membrane into the cytoplasm and apparently centring in the nucleus. This radiation does not usually resolve itself into a bipolar figure but persists as a monaster. Frequently the chromosomes divide and become drawn out along the rays so as to appear scattered throughout the cytoplasm (Fig. 14). This may be succeeded by a reduction of the rays and a reconstruction of the nucleus with an increased number of chromosomes. A redevelopment of the monaster may now follow and the whole series of processes be repeated two or three times. The changes observed agree, therefore, with Wilson's description of the behaviour of monasters in eggs that have been treated with magnesium chloride (Wilson, '01). Within 2 or 3 hours these eggs begin to degenerate by a process of cytolysis (Fig. 13), by means of

which the protoplasm becomes broken up into a mass of small particles lying within the fertilization membrane.

If, after treatment with butyric acid, the eggs are kept at a low temperature (2—5° C.) the first few divisions may be accomplished. In this case the period of nuclear growth is succeeded by the almost complete disappearance of the perinuclear zone, and the subsequent development of a typical amphiaster in the region of the nucleus. The succeeding changes are identical with those described below, that take place in eggs that have been subsequently treated with hypertonic solution.

We have never observed any cytasters to be developed in eggs treated with butyric acid alone; usually a monaster appears which may divide to form a typical cleavage-aster or, more often, remains undivided. In all cases, therefore, the rays that develop in these eggs originate from the nucleus and do not have an independent cytoplasmic origin as in the case of cytasters.

Internal Changes after Treatment with Butyric Acid followed by a Hypertonic Solution.

(Loeb's improved method of artificial fertilization.)

The interval (about 20 minutes) between the transference of the eggs from butyric acid to normal sea water and their subsequent treatment with hypertonic salt solution is characterized by the alterations in the appearance of the cytoplasm and nucleolus, and the subsequent development of a perinuclear zone, as described above. The nucleus then commences to grow and faint radiation can some times be seen extending from the perinuclear zone into the surrounding cytoplasm (Fig. 2).

During immersion in the hypertonic solution there are no apparent changes beyond a slight reduction of the clear zone of hyaloplasm surrounding the nucleus (Fig. 3).

After the eggs are put back into normal sea water the internal changes resulting in the first cleavage follow each other in quick succession. The first change noticed is an increase in the development of the perinuclear zone, followed by further growth of the nucleus. Meanwhile, the meshwork of chromatin becomes coarser and more aggregated together and the nucleolus gradually disappears. This stage is succeeded by a reduction of the perinuclear zone together with its radiations.

About half an hour after transference to normal sea water, from one pole of the nucleus a definite aster begins to develop, its rays focussing in a more or less indistinct centrosome situated on the nuclear membrane (Fig. 4). By division of the centrosome a typical amphiaster is formed in the nuclear area and as it develops the nuclear membrane disappears (Fig. 15). At the same time the chromatin assumes the form of a spireme, which subsequently breaks up into about 18 long and slender chromosomes. At this stage it is impossible to clearly distinguish their number, but, as the chromosomes are gradually drawn into the equator of the cleavage amphiaster (Fig. 6), they shorten considerably and become quite distinct by the time that the equatorial plate is formed (Fig. 5).

At this stage we have made numerous counts of the chromosomes and invariably found it in the neighbourhood of 18, which is half the number that is normally present in this species.

During the metaphase each chromosome splits longitudinally, and in the succeeding anaphase the halves move away from each other along the spindle fibres, 18 going to each pole (Fig. 7). As they approach the centrosomes the chromosomes swell up and become indistinct, and finally fuse together to form two daughter nuclei. These changes are accompanied by a division of the cytoplasm into two equal parts, followed by complete disappearance of the spindle fibres, and the first cleavage is complete (Fig. 8).

Each daughter nucleus remains partially surrounded by a clear region that seems to represent the centrosome together with a slight accumulation of cytoplasm, and here the cleavage aster of the next division first makes its appearance. After the first cleavage is complete the succeeding divisions follow each other in regular succession and present all the features of ordinary mitosis (Fig. 9).

In each case the nucleus becomes completely surrounded by a clear zone of hyaloplasm. This is followed by a period of nuclear growth during which this perinuclear zone almost disappears. From the centrosome, that has persisted at one pole of the nucleus, rays now appear extending out into the cytoplasm (Fig. 10) and by the subsequent division of the centre of attraction a typical amphiaster is produced (Figs. 9—10). As it develops the nuclear membrane disappears and the chromatin breaks up into 18 chromosomes which arrange themselves about the equator of the amphiaster (Fig. 9). In this way a typical mitotic spindle is formed in the nuclear area

and the succeeding stages in the division of the cell are identical with those described above.

The cytoplasmic granules are gradually used up as development proceeds and disappear before the gastrula stage is reached.

As the cells become smaller at each division, it becomes increasingly more difficult to count the number of chromosomes with any degree of accuracy, owing to their smaller size and also to the smallness of the cells, which prevents the chromosomes separating distinctly. But it is possible to obtain a close approximation of their number as late as the free swimming blastula stage, in which there are at least 512 cells, and we have invariably found it to be in the neighbourhood of 18 (Fig. 11). The reduced number of chromosomes, therefore, may reasonably be supposed to persist throughout the further stages of development, for a multiplication of their number has never yet been observed in any dividing cells.

In addition to the normal processes of development described above, which occur in the majority of the eggs, abnormal phenomena are always found in some of them. The reason for this is evident, for it is obviously impossible to secure for all the eggs exactly the same supply of oxygen and hence the effect of the hypertonic solution cannot be the same for all the eggs taken out at one time.

The most commonly occurring abnormality is the development of a varying number of asters in the cytoplasm quite independently of the cleavage aster, which arises from the nuclear region. These cytasters develop in eggs that have been over-exposed to the action of the hypertonic solution and have not been observed after treatment with butyric acid alone.

Each cytaster consists of a number of fibres radiating from a more or less indistinct central granule into the surrounding cytoplasm (Fig. 15). As a rule they do not attain much development but remain scattered around the periphery of the cell and disappear before the completion of the first division. In certain cases, however, they may become so well developed as to interfere with the normal division of the egg, the rays of cytasters in the region of the nucleus becoming attached to the chromosomes and thus producing multipolar mitoses. In these cases the chromosomes are divided irregularly between the two poles of the cleavage amphiaster and the centres of as many cytasters as form a connection with the nucleus.

A cell wall may develop between any two centres of attraction

connected by spindle fibres, and therefore, in the case of multipolar, mitoses, the egg may divide into as many cells as there are centres of attraction Cytoplasmic cleavage does not necessarily follow a multipolar mitosis, but the resulting nuclei may remain scattered through the undivided cytoplasm of the egg and redivide, thus producing a multinucleate cell.

In addition to the formation of multipolar spindles the centres of the cytasters may divide and thus produce a typical amphiaster which may operate as a centre of cytoplasmic division independently of the cleavage amphiaster.

Occasionally a cell wall is developed between the two centres of one of these cyt-amphiasters and thus an enucleated portion of cytoplasm may become divided off from the main mass.

In every case the formation of cytasters results in the production of more or less irregular segmentation, or may completely stop development.

These cytasters resemble those described by WILSON in the eggs of *Arbacia* after treatment with hypertonic salt solutions. The phenomena observed by us in the eggs of *S. purpuratus* entirely support his conclusions that »there is no possibility of drawing any other than purely an arbitarary distinction between cytasters and nuclear asters in their relation to the nucleus« and »that the central bodies of the cytasters are true centrosomes that are formed de nove in the cytoplasm«.

Comparison of the Cytological Effects of Natural and Chemical Fertilization.

In comparing these two processes it is necessary to avoid consideration of those pathological phenomena that are always present in a certain percentage of the chemically fertilized eggs. When the sperm enters the ovum it introduces the exact amount of substances (lysins, etc.) necessary to start normal development and there is little chance of error. But when one attempts to imitate this process chemically the possibilities of error are much greater. In the first place the cytolytic substance may enter in excess; and similar difficulties arise in the subsequent exposure of the eggs to the action of the hypertonic solution, through the impossibility of ensuring exactly the same exygen supply for all of the eggs.

Therefore, the embryos that present pathological features should

be ignored in comparing the processes of natural and chemical fertilization.

In both cases the first effect of fertilization is to start a cytolytic process resulting in the formation of a membrane. In naturally fertilized eggs the sperm nucleus in its passage through the cytoplasm is accompanied by a radiation which comes in contact with the egg nucleus and there spreads out on one pole of it. The fusion of the two nuclei which now takes place is followed by a period of nuclear growth during which the radiations almost disappear. Meanwhile the nucleolus exhibits unmistakeable signs of dissolution, as it changes from being a dense spherical body to a faintly staining and often indistinct mass.

In the chemically fertilized eggs the changes are very similar. The egg nucleus becomes surrounded by a perinuclear zone with faint radiations extending into the cytoplasm. This stage probably corresponds with the radiation that appears near the sperm nucleus and forms a clear zone at one end of the cleavage-nucleus after the two germ nuclei have fused. Moreover, just as in the normal eggs, it is succeeded by a period of nuclear growth during which the perinuclear zone with its radiations practically disappear and also the nucleolus shows signs of dissolution. The appearance of this radiation is probably the result of oxidation processes starting from the nucleus, which result in the solution of the yolk granules and is followed by the flowing of these solutions towards the nucleus. They are used up in the synthesis of chromatin, and hence this process is followed by an increase in the size of the nucleus. With the cessation of nuclear activities preparatory to division cytoplasmic solutions no longer flow towards the nucleus and hence the radiations caused by these centripetal currents disappear. From the alteration in its appearance it is probable that the nucleolus also takes some part in the nuclear syntheses.

In both kinds of eggs the period of nuclear growth is succeeded by an apparent cessation of nuclear activities preparatory to division.

In naturally fertilized eggs a distinct aster (cleavage aster) appears at one pole of the nucleus, its rays centering in a clear area which represents a diffuse centrosome. This area divides and the two halves move apart until they come to lie at opposite sides of the nucleus and form the poles of a typical amphiaster which is developed in the nuclear region. Meanwhile the chromatin assumes the form of a spireme, which breaks up into 36 chromosomes that

arrange themselves about the equator of this amphiaster to form a nuclear spindle. In the chemically fertilized eggs a nuclear spindle arises in a similar way and the chromatin assumes the form of a spireme preparatory to breaking up into chromosomes, but, instead of 36, only 18 of these latter bodies appear. The subsequent changes are identical in both kinds of eggs. The chromosomes split longitudinally and each half moves along the spindle fibres towards its respective pole. As they approach the poles the chromosomes swell up and eventually fuse together to form a single nucleus in the region occupied by each of the diffuse centrosomes. Meanwhile a cell wall develops between the two nuclei dividing the cytoplasm into two, and finally the spindle fibres disappear. The succeeding processes of development, both internal and external, are similar in both naturally and chemically fertilized eggs, with the exception that at each succeeding division only 18 chromosomes appear in the latter instead of the normal number, 36.

Summary of Results.

A cytological study of the changes occurring in the eggs of *Strongylocentrotus purpuratus* after chemical fertilization by means of Loeb's improved method.

(A.) Effect of treatment with butyric acid.

1) The first change is the starting of a process of cytolysis resulting in the formation of a fertilization membrane.

2) This is accompanied by an alteration in the appearance of the nucleolus, which, from being a dense mass of chromatic substance, changes to a lightly-staining body of somewhat indefinite shape.

3) A dissolution of the cytoplasmic granules in the immediate neighbourhood of the nucleus results in the appearance of a clear perinuclear zone. Probably as a result of currents flowing centripetally from the cytoplasm towards the nucleus, radiations appear in and around this zone.

4) The appearance of these radiations is succeeded by a period of growth during which there is an increase in the size of the nucleus.

5) In eggs that are developing at ordinary temperatures a large monaster is now developed, it's rays centering in the nucleus. The nuclear membrane disappears and the chromatin breaks up into 18 chromosomes. These may undergo division and be drawn out of the

nuclear area along the rays, in which case, they appear scattered through the cytoplasm. Such eggs never divide but simply disintegrate by a process of cytolysis.

6) In eggs that are developing at a low temperature (2—5° C.) the first few divisions may be accomplished, in which case the period of nuclear growth is succeeded by exactly the same changes as those occurring in eggs that have been subsequently treated with a hypertonic solution, described below.

7) Cytasters are not developed.

(B.) Effect of treatment with butyric acid followed by treatment with hypertonic solution.

8) The interval (15—20 mins.) between treatment with butyric acid and with hypertonic salt solution is characterized by the membrane formation and an alteration in the staining properties of the nucleolus. These changes are accompanied by the appearance of a clear perinuclear zone as described above.

9) During the treatment with hypertonic salt solution there is a slight increase in the size of the nucleus and the clear zone almost disappears.

10) After transference of the eggs back into normal sea water the perinuclear zone reappears, and is followed by further growth of the nucleus.

11) A typical cleavage aster develops in the nuclear region, its rays being focussed in two areas, one at each pole, that probably represent diffuse centrosomes.

12) A varying number of asters, quite independent of the cleavage aster sometimes appear in the cytoplasm. If excessively developed they interfere with normal division of the cell and multipolar spindles are often formed. In eggs that have not been exposed too long to the action of the hypertonic solution, the cytasters disappear before the completion of the first division.

13) The chromatin assumes the form of a spireme and then breaks up into 18 chromosomes, which is exactly half the number occurring in normally fertilized eggs. They arrange themselves in the cleavage aster, and divide in the usual way forming two daughter nuclei each with 18 chromosomes. This process is accompanied by division of the cytoplasm.

14) The reduced number of chromosomes, 18, persists in the cells of the parthenogenetic larvae at least as far as the blastula,

and beyond this stage it is impossible to count them owing to the small size of the cells.

Zusammenfassung der Ergebnisse.

Die cytologischen Änderungen, welche nach der Behandlung des Eies von *Strongylocentrotus purpuratus* mit Loebs verbesserter Methode der künstlichen Parthenogenese eintreten, wurden untersucht mit folgendem Resultat:

A. Behandlung der Eier mit Buttersäure allein.

1) Die erste Änderung im Ei nach der Behandlung mit Buttersäure ist ein cytolytischer Prozeß an der Oberfläche des Eies, der zur Bildung einer Befruchtungsmembran führt.

2) Gleichzeitig findet eine Änderung im Aussehen des Nucleolus statt, der vorher eine dichte Masse chromatischer Substanz war und nun sich in einen Körper umwandelt, der sich nur leicht färbt und etwas unbestimmte Form hat.

3) Später erfolgt eine Auflösung der cytoplasmischen Körner in der unmittelbaren Umgebung des Kerns, so daß eine helle perinucleare Zone sichtbar wird. Strahlungen erscheinen in und um diese Zone.

4) Auf die Bildung dieser Strahlung folgt eine Periode der Volumzunahme des Kerns.

5) In Eiern, die sich bei Zimmertemperatur entwickeln, bildet sich jetzt ein Monaster, dessen Strahlen ihren Mittelpunkt im Kern haben. Die Kernmembran verschwindet, und das Chromatin sammelt sich in 18 Chromosomen. Solche Eier gehen an einem cytolytischen Prozeß zugrunde.

6) Bei niederer Temperatur (2—5° C.) kann es zu einer Reihe von Zellteilungen kommen. In diesem Falle folgen auf die Periode des Kernwachstums dieselben Änderungen, welche sich bei Eiern finden, welche nach der Buttersäurebehandlung für kurze Zeit in eine hypertonische Lösung gebracht werden und die sub B. erwähnt werden.

7) Durch die Buttersäurebehandlung werden keine Cytaster hervorgerufen.

B. Veränderungen, welche bei Eiern eintreten, die erst mit Buttersäure und dann mit hypertonischem Seewasser behandelt werden.

8) In dem Zeitraum zwischen Membranbildung (durch Buttersäurebehandlung) und der Anwendung der hypertonischen Lösung (15—20 Minuten) findet eine Änderung in der Färbbarkeit des Nucleolus statt, und die Bildung einer hellen perinuclearen Zone.

9) Während dem Verweilen der Eier in der hypertonischen Salzlösung findet eine kleine Volumzunahme des Kerns statt, und die helle Zone verschwindet fast gänzlich.

10) Nachdem die Eier aus der hypertonischen Lösung in Seewasser zurückgebracht sind, erscheint die perinucleare Zone wieder, und der Kern entwickelt sich.

11) Eine typische Spindelfigur mit zwei Strahlensystemen bildet sich in der Kernregion; in jedem der beiden Centren ist vermutlich ein diffuses Centrosom.

12) In manchen Eiern bildet sich unabhängig von der Kernspindel eine variierende Zahl von Astrosphären im Protoplasma. Wenn sie ungewöhnlich stark entwickelt sind, stören sie die normale Zellteilung, indem es zur Bildung

multipolarer Spindeln kommt. In Eiern, die nicht zu lange dem hypertonischen Seewasser ausgesetzt gewesen sind, verschwinden aber diese Cytaster, ehe die erste Furchung vollendet ist.

13) Das Chromatin nimmt die Form eines Spirems an und trennt sich dann in 18 Chromosomen, also in halb soviel, wie in normal befruchteten Eiern vorhanden sind. Diese ordnen und teilen sich in der Spindel in der gewöhnlichen Weise, indem sie zwei Tochterkerne mit je 18 Chromosomen bilden. Dieser Prozeß wird von einer Teilung des Cytoplasmas begleitet.

14) Die reduzierte Chromosomenzahl, nämlich 18, bleibt dauernd in den Zellen bestehen, zum mindesten bis zum Blastulastadium; in späteren Stadien gelang es nicht, dieselben zu zählen, wegen der geringen Zellgröße.

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Explanation of the Figures.

The figures are drawn to a magnification of 750 diameters with the exception of Fig. 11, which is magnified 1500 diameters. In all cases the drawings were made from sections stained by one of the three methods described above.

Plate V.

Figs. 1—11. Various stages in the development of eggs of *S. purpuratus* chemically fertilized by means of butyric acid, followed by treatment with a hypertonic salt solution.

Fig. 1. Unfertilized ovum. Std. ARNOLD.

Fig. 2. Ovum, 15 minutes after treatment, for 120 secs., with a solution of butyric acid, showing the appearance of a clear perinuclear zone (*p.z.*), surrounded by chromatic granules (*ch.gr.*). Std. HEID.

Fig. 3. Ovum 5 minutes after transference from hypertonic salt solution to normal sea water (treatment undergone: butyric acid 2 mins., water 20 mins., hypertonic salt solution 45 mins., water 5 mins.), showing the appearance of the perinuclear zone. Std. MALL.

Fig. 4. Appearance of cleavage aster 30 mins. after transference from hypertonic solution. (But. 2 mins., water 20 mins., hyper. 45 mins., water 30 mins.) Std. HEID.

Fig. 5. Polar view of equatorial plate showing 18 chromosomes. (But. 2 mins., water 20 mins., hyper. 45 mins., water 50 mins.) Std. ARNOLD.

Fig. 6. Lateral view of nuclear spindle, drawn from same slide as Fig. 5.

Fig. 7. Anaphase of first division of egg. (But. 2 mins., water 20 mins., hyper. 45 mins, water 80 mins.) Std. MALL.

Fig. 8. Completion of first division. (But. 2 mins., water 20 mins., hyper. 45 mins., water 100 mins.) Std. HEID.

Fig. 9. Anaphase of second division. Std. ARNOLD.

Fig. 10. 8-cell stage of chemically fertilized egg showing stages in aggregation of the chromatin and development of the cleavage aster. Std. ARNOLD.

Fig. 11. Parthenogenetic blastula, 24 hrs. old, one cell showing equatorial plate containing 18 chromosomes. Std. HEID.

Fig. 12. Normally fertilized embryo, 4- to 8-cell stage; showing equatorial plate containing 36 chromosomes. Std. HEID.

Fig. 13. Ovum 3 hours after treatment for 2 mins. with butyric acid (Temp. 16° C.), showing the cytolytic nature of degeneration. Std. MALL.

Fig. 14. Large monaster developed $2^1/_2$ hours after treatment with butyric acid alone. Std. HEID.

Fig. 15. Abnormal embryo showing the development of cytasters (*cyt.*) 60 mins. after chemical fertilization. (But. 2 mins., water 20 mins., hyper. 50 mins., water 60 mins.) Std. HEID.

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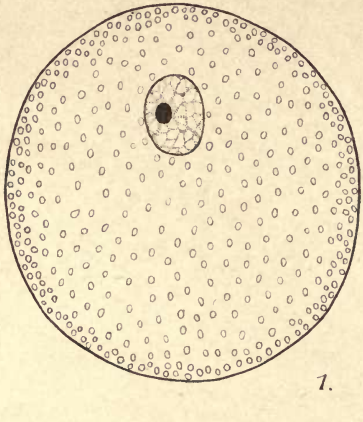

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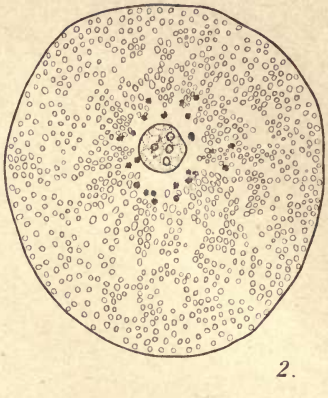

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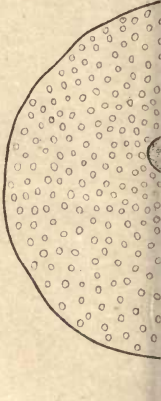

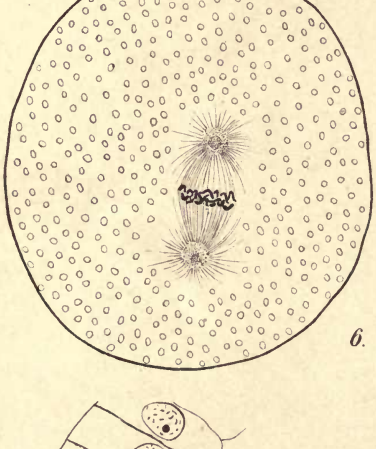

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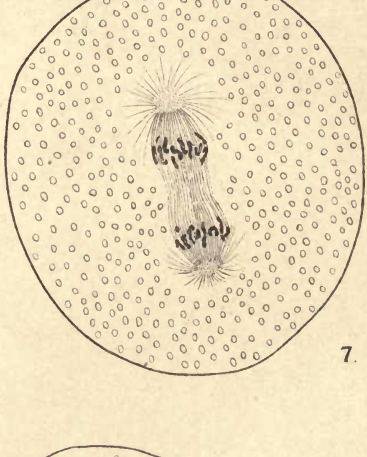

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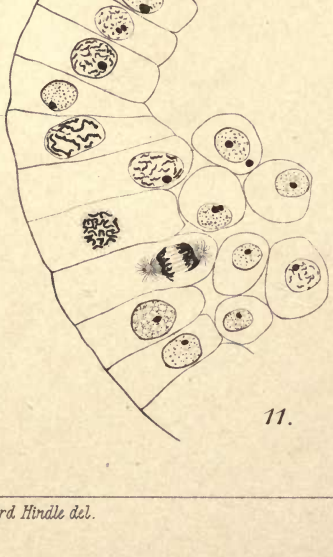

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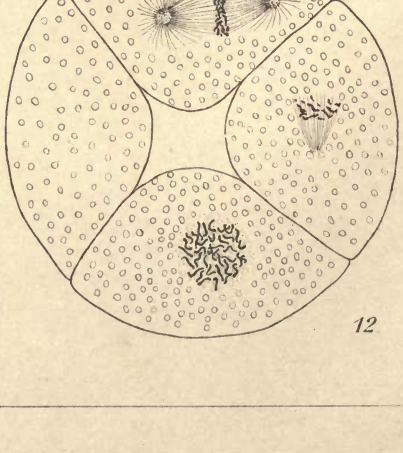

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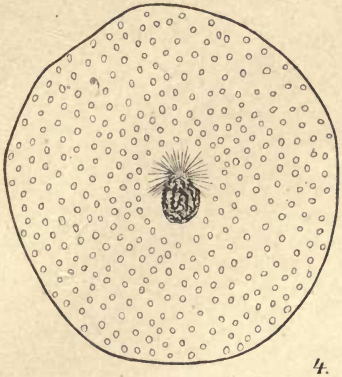

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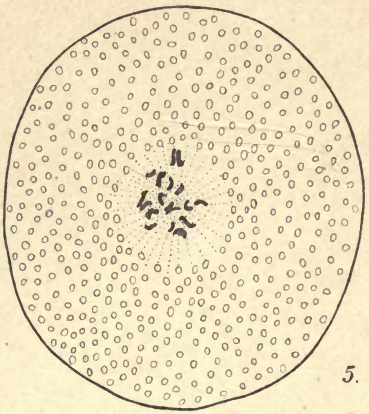

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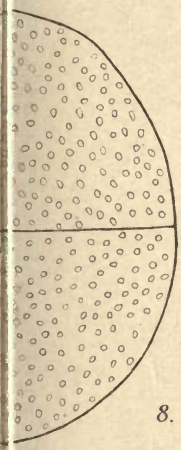

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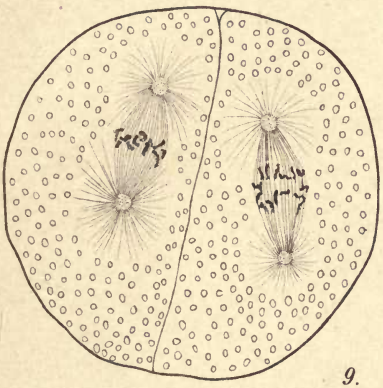

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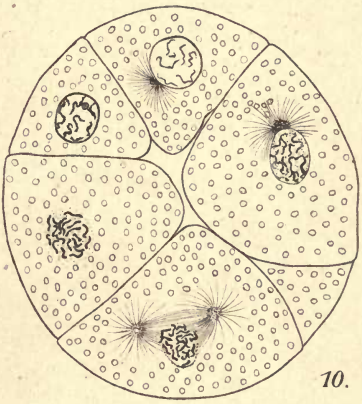

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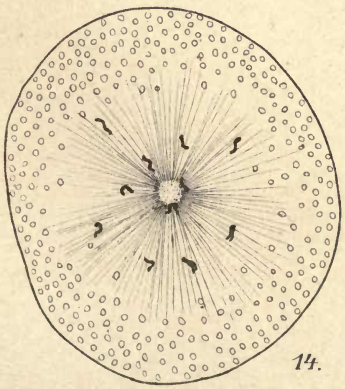

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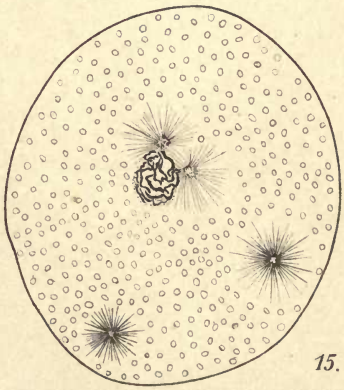

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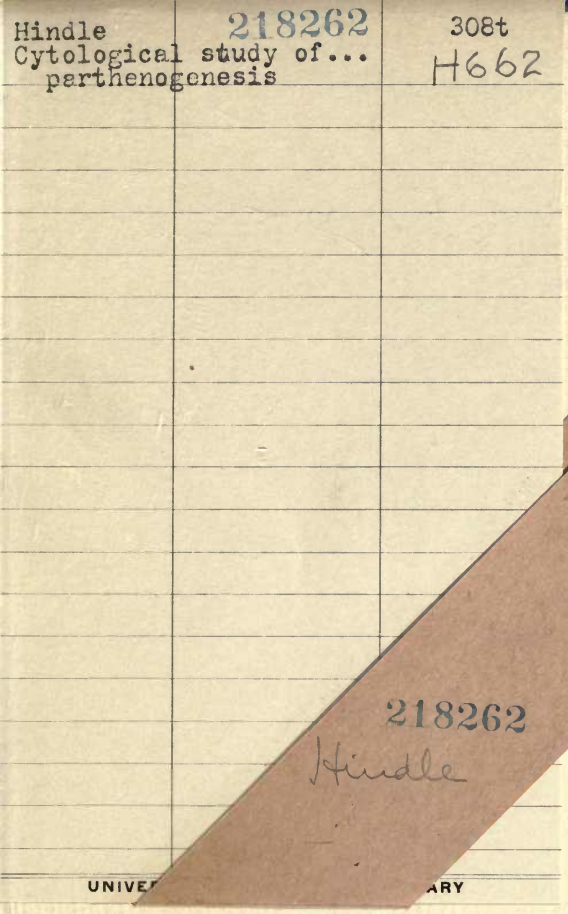

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