Diese Tatsache, die zunächst (was Hohlmuskeln [1]) betrifft), nur für Aplysia festgestellt ist, bedarf weiterer Untersuchungen.

Wie in so vielen anderen Muskeln, finden wir also beim Fusse von Aplysia, neben dem gewöhnlichen schnellen Kontraktionssystem, ein langsames, und der visköse Tonus ist nichts anders als der sehr lang gedehnte Gipfel der Kontraktion des langsamen Systems. Die Gesamterscheinung des viskösen Tonus besteht daher bei Aplysia aus einem dynamischen und einem statischen Teile. Offenbar leidet die statische Komponente auch unter normalen Umständen dauernd Verlust, den die dynamische Komponente dauernd ersetzt. Man kann die dynamische Komponente ausschalten, dann verschwindet der statische visköse Tonus zuletzt vollkommen. Bei Aplysia ist dazu etwa ½ Stunde nötig. Bei Helix (unter anderen Bedingungen, Versuche im Eisschrank hatten bislange keine entsprechenden Resultate) behauptet sich die statische Komponente, also der Gipfel der langsamen Verkürzung etwa 4 Tage lang [2]); die Kontraktilität des schnellen Systems bleibt dabei erhalten!

Ich habe in früheren Arbeiten gefunden, dass das Pedalganglion dauernd den Tonus reguliert; die dynamische Komponente erzeugt ihn dauernd in übertriebenem Masse, dauernd wird er durch jenes spezifische Zentrum des langsamen Systems vermindert. Das ganglienlose Tier schrumpft im Laufe der Zeit mehr und mehr und wird hart. Auch die tonische Kontraktion nach Vorkühlung eines ganglienlosen, im Uebrigen aber intakten Tieres in warmem Aquariumwasser ist ausserordentlich übertrieben. Die Dualität von statischer und dynamischer Komponente des Tonus scheint uns auf befriedigende Weise das paradoxale Verhalten des Muskels bei verschiedenem Vorkühlungsgrad zu erklären.

[1]) Beim Schliessmuskel der Muscheln ist ein solches Verhalten bekannt, doch darf man diesen Muskel nicht mit Hohlmuskeln vergleichen.

[2]) Nach Ch. S. KOSCHTOJANZ und A. MUSZEEFF (Russisch). Biologizeckii Journal T. 2, 1933, S. 503.

Embryology. — *Experimental Analysis of some Phenomena of Fertilization and Cleavage.* By M. W. WOERDEMAN.

(Communicated at the meeting of March 30, 1935.)

By means of the Zeiss-micromanipulator experiments were made on the eggs of Paracentrotus lividus (Stazione zoologica, Naples) and of Echinus miliaris (Biologische Anstalt, Heligoland) in order to study some phenomena of fertilization and cleavage.

TECHNIQUE.

The eggs were artificially inseminated after removal of the viscous jelly that surrounds them. Then a drop of the egg-suspension was brought on

a coverslip, which was inverted and placed on a microdissection moist chamber. Three holders of microdissection instruments were used, one provided with a mouthpipette, the two others with the micrurgical instruments, either glassneedle, electric micropipette or micropincette.

The eggs were divested of their fertilization membranes with the dissection instruments for some of the experiments (pipette investigations, cutting). The membranes could remain intact for other experiments (constrictions of cells). After the eventual removal of the membranes the hanging drop of seawater was sucked away with the mouthpipette so far that the cells got flattened against the coverglass in the shallow drop. By this the cell-structures became well visible and the cells were more or less immobilized. Then the operation was performed, after which by means of the mouthpipette seawater was applied again to the eggs. They were now regularly pursued in their further development and the resulting phenomena laid down in pencil sketches. Transmitted artificial light was used during the work, precautions have been taken against heating and drying up of the preparations. At the end of the observations, after staining by means of acetic carminic acid, they were checked in several instances with the stained preparations. Of the numerous experiments only a few are communicated.

EXPERIMENTAL RESULTS.

1. *Removal of penetrated spermium.*

As soon as the situation of the penetrated spermium could be determined by the appearing spermaster, the spermium was removed from the egg either by means of the micropipette or by cutting off part of the egg containing the spermium.

The egg will now exhibit a series of reactions, which must be ascribed to an activation issuing from the spermium. There appear namely reactions of the cytoplasm, changes in viscosity, changes in refraction; the cytoplasm becomes streaked (it seems as if radiations become visible in it, but these rays remain indistinct), sometimes little vacuoles are seen, which disappear again, till suddenly the nucleus, which had gradually swollen, becomes invisible, after the nuclear membrane has first shown folds. On the spot where the nucleus has disappeared, we now see a hyaline area come out (mixoplasm, WASSERMANN), which extends slowly and from which radiate mostly indistinct rays. This radiation, however, can sometimes be very strong, but never shows two centers (dicentric figure) as with the amphiasterstage.

Then the cytoplasm becomes more fluid, the surface of the egg displays bulges, slight constrictions, which disappear as fast as they have come. Jerking motions of the egg can even be observed as if it were contracting. But after some time the egg quiets down, the radiation disappears, the egg rounds up and soon afterwards the nucleus becomes visible again. It may occur that the nucleus turns out to have divided into

two, this, however, is not the rule. The egg now looks again exactly as at the beginning of the observation. Later the same sequence of processes can repeat itself, occasionally as many as four successive cycles have been observed, but then the cell dies, mostly in the phase in which the egg displays the forming of bulges at its surface. The latter can break off, the exoplasm is often broken through, effusions of the endoplasm through the ruptured cortical layer form, the cytoplasm shows vacuolization and finally the egg breaks up to drops of cytoplasm. In the instances observed the whole process lasted 2½ to 3 hours from after the operation.

In the literature it is known as monaster-cycle and appears also e.g. after artificial activation of the egg with fatty acids, as with the artificial parthenogenesis according to the method of LOEB.

The regulation of the cytological reactions started by the entrance of a spermium evidently fails, when the sperm has soon afterwards been removed from the egg. With the activation by means of fatty acids according to LOEB the processes of development had also afterwards to be regulated by a second treatment of the egg (a.o. with hypertonic seawater).

When we compare the reactions of the eggs from which the penetrated spermia have been removed with those of normal eggs after insemination, then they prove to elapse considerably slowlier. The female pronucleus disappears for instance at a moment when the control-cells have passed already through their first cleavage.

If we cut off part of the egg in which the penetrated spermium finds itself, then in this egg-fragment a normal cleavage-figure (amphiaster with spindle, disappearance of nucleus etc.) forms (see Fig. $1._2$ and Fig. $2._2$) and the egg-fragment shows cleavage simultaneous with, or sometimes even rather sooner than the control-cells. This process is already sufficiently known (merogony-experiments).

We can make use of the above mentioned difference between the reactions in egg-fragments with a sperm-nucleus and with an egg-nucleus for a second series of experiments.

2. *Disturbance of the sequence of the fertilization phenomena.*

If an egg immediately after the penetration of the spermium is constricted between egg-nucleus and spermium the meeting of the two pronuclei can be prevented. Then the male pronucleus prepares for an independent division; in the egg-part in which the female pronucleus finds itself processes of development find also place, but in a slower tempo. When after some time the constriction is discontinued, then we see the two swollen pronuclei still move towards each other and in very characteristic instances (see Fig. 1) I could observe that the egg-nucleus landed into the spindle which had formed between the two asters of the sperm-amphiaster. Shortly afterwards division of the sperm-nucleus takes

place. The egg-nucleus, however, is found back undivided beside the two sperm-daughter-nuclei. The insufficiently prepared female pronucleus

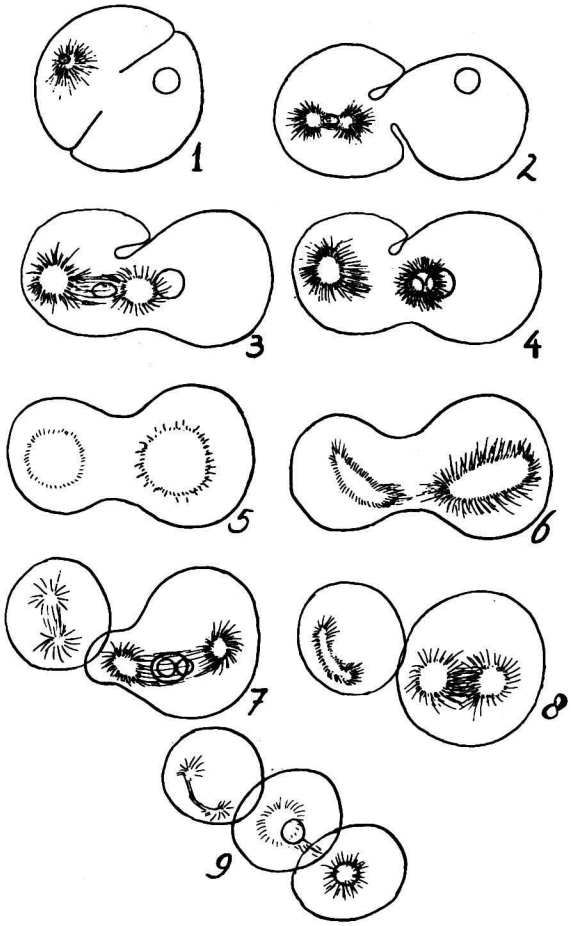

Fig. 1. *Egg of Paracentrotus liv.*

1. Constricted in two places. Spermaster at the left, egg-nucleus at the right.
2. Formation of an amphiaster in connection with sperm-nucleus.
3. Approach of the sperm-nucleus and egg-nucleus.
4. Egg-nucleus comes to lie with sperm-nucleus near one of the asters.
5. and 6. Nuclei have disappeared, irregular asters in cytoplasm.
7. Egg divided. In the right "blastomere" three nuclei visible (two sperm-daughter-nuclei and the undivided egg-nucleus).
8. The three nuclei disappeared, amphiaster.
9. The right "blastomere" divided. In each cell one nucleus. So the egg-nucleus has now also divided.

could consequently not be divided simultaneously with the sperm-nucleus. With the then following cycle of nuclear division also the egg-nucleus is now divided. A similar observation was made by HERBST (1912) when he allowed spermia to penetrate into eggs, which had already started their

development in consequence of artificial activation. In these instances the egg-nucleus divides, the sperm-nucleus not so, however. Consequently the nucleus cannot divide independently, but is dependent on the condition of the environing cytoplasm. The changes in the cytoplasm are the cause of nuclear modifications, which have to take place synchronously with the cytoplasmic changes, if the nucleus is to proceed to a co-ordinate division.

3. *Removal of spermium and egg-nucleus.*

Described was that after the dissolution of the egg-nucleus the cell displays a number of reactions, which point to an altered viscosity of the cytoplasm and to irregular changes of surface-tension (formation of bulges, irregular constrictions of the surface etc.). As experiments of PÉTERFI and of CHAMBERS (puncturing of nuclei) have shown that mixing of karyoplasm and cytoplasm leads to decrease in viscosity and even to cytolysis, the question rises if not the breaking down of the nucleus before the cell-division is responsible for the above described cytoplasm changes, which were seen in the monaster-cycle. In a number of experiments after the spermium had entered, one fragment of the egg containing the spermium was cut off, and one part in which the egg-nucleus found itself. Or in another instance first the spermium and a little later the female pronucleus were sucked away by means of the micropipette. The remaining non-nucleated egg-fragments have never shown the above described cytoplasm-changes, which we can undoubtedly consider as incomplete endeavours to cell-cleavage.

Summarizing we can say: the penetrated spermium introduces cytoplasm changes, which can lead to a series of changes of the nuclei, accompanied with swelling, by which the nuclei finally lose their nuclear membrane and break down. The mixing of nuclear contents and cytoplasm causes again the appearance of further cytoplasmic changes, which are necessary as preparation for the division of the cell-body.

There is considerable evidence for assuming that for this last process the astrospheres (asters) are indispensible.

4. *Isolation, complete and partial removal of an aster.*

When, after a fusion-nucleus and a distinct amphiaster have formed, one of the asters is separated from the amphiaster, the development is seriously disturbed. In most cases we see that an already dissolved nucleus is reconstructed; we find then a considerably swollen nucleus beside a large irregular hyaline mass, emanating indistinct rays (see Fig. 2). This reminds us very strongly of the phenomena of a monaster-cycle. But after some time the hyaline mass divides, two asters arise, the nucleus disappears again and now cleavage is effected after all. In other instances the egg passes through an actual monaster-cycle, because a division of the hyaline mass fails to come. Of course there is then no question of cleavage.

When not an entire aster, but part of it is removed, nothing is to be

seen immediately after the operation (asters soon become invisible on agitating the cytoplasm), but after a few minutes a little aster reappears, which, if the aster has not been diminished too much, is able, together

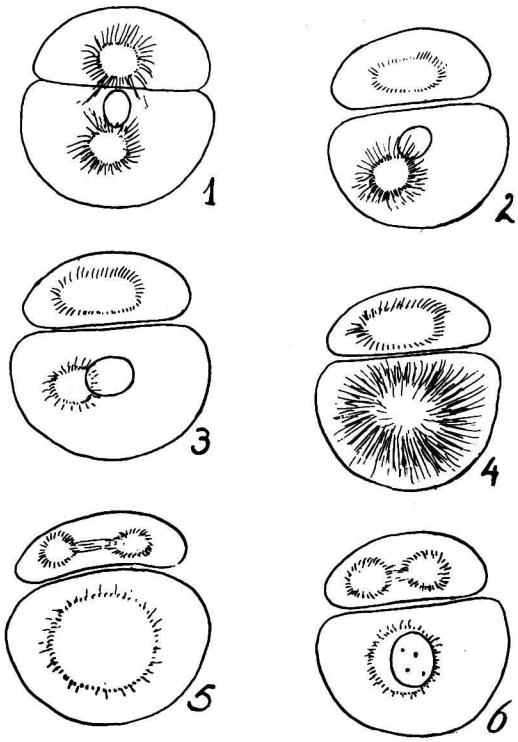

Fig. 2. *Egg of Paracentrotus liv.*

1. Section, by which an aster is isolated.
2. and 3. Swelling of the nucleus, indistinct radiation round hyaline area.
4. Dissolution of nucleus.
5. Isolated aster divides (pseudo-spindle-formation). In the other egg-fragment monaster.
6. Reconstruction of nucleus, no cleavage.

with the normal, intact aster to induce cleavage of the egg. If an aster has been mutilated too much, regeneration has first to take place, before cell-division is effected. In most instances a cleavage is then passed over.

Asters have a strong regenerative power.

If one of the asters of an amphiastral stage is isolated, then that aster divides at regular intervals, while between the two daughter-asters for some time something is formed, which looks like a spindle of an amphiaster (see Fig. $1._7$ and Fig. $2._5$). In reality it is something quite different (other viscosity, etc.). So it appears that the asters of an amphiaster are capable of independent division. For this it is not essential that they are in contact with a nucleus, for they also proceed to division in non-nucleated egg-fragments. At the same time above mentioned experiments teach us

that the nucleus only divides when it comes to lie between two asters and that it is necessary that asters have a certain dimension (degree of activity?) to cooperate successfully to the cleavage of the cytosome.

5. *Cleavage between two asters of different origin.*

As is well known, asters can be produced in the cytoplasm under the influence of various agents, which are called „cytasters". They play an important part in artificial parthenogenesis and have therefore been subject of numerous investigations.

FRY considers them to be less vigorous in constricting the cytosome then asters arisen from division of the spermaster. Moreover it was asserted that they could not divide and therefore had every time to be formed de novo. But for the rest it seems that there is no essential difference between cytasters and what are called „nuclear" asters.

Again and again I have been able to observe cytasters in cytoplasm activated by the penetration of a spermium, after the spermium had been removed from the egg. When a dissolution of a nucleus takes place

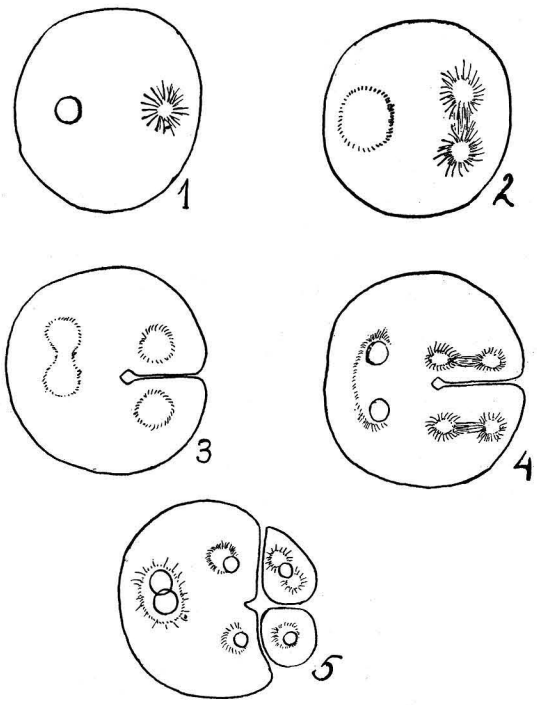

Fig. 3. *Egg of Paracentrotus liv. flattened against cover-slip.*

1. Sperm-radiation at the right, egg-nucleus at the left.
2. Division of sperm-nucleus; monaster near egg-nucleus.
3. Constriction of cytosome between spermasters.
4. Egg-nucleus divided, no constriction between the two daughter-nuclei. The sperm-nuclei in second division.
5. Again constriction of cytosome between sperm-daughter-nuclei, monaster-cycle of egg-nucleus begins.

between two cytasters, the nucleus can divide, though this does not always occur, but I never observed that cleavage of the cytosome followed. Fig. 3 gives a convincing evidence that cleavage is possible between two asters arisen from the spermaster and not between two asters formed beside the female pronucleus (which I shall call "cytasters" for the sake of convenience, though in theory it is conceivable that they are asters of the same nature as spermasters, but which are latent beside the egg-nucleus). By strong flattening of eggs just fertilized, against the coverslip of the moist chamber, fusion of the pronuclei has been prevented on the example represented in Fig. 3. Afterwards an amphiaster develops round the sperm-nucleus and also an aster is seen in connection with the egg-nucleus. Only a constriction of the cell-body perpendicular on the axis of the sperm-

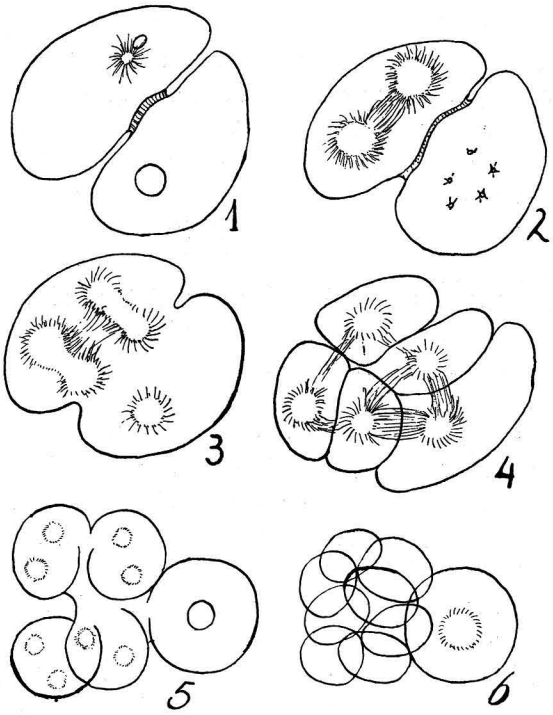

Fig. 4. *Egg of Paracentrotus liv.*

1. Constriction between penetrated spermium at the left and egg-nucleus at the right.
2. Division of sperm-nucleus (distinct amphiaster), egg-nucleus dissolved.
3. Constriction vanished by liquefaction of cytoplasm. Irregular division of sperm-nucleus. Its second division in state of preparation. Monaster near egg-nucleus.
4. Sperm-nucleus divides into four daughter-nuclei. Between "nuclear asters" derived from sperm-aster and the "cytaster", near the egg-nucleus, cleavage is seen.
5. Spermasters divide again. Egg-nucleus reconstructed.
6. Egg-nucleus dissolved again. Monaster. "Blastomeres" with sperm-asters have again divided. Egg-fragment with egg-nucleus shows no cleavage.

amphiaster is formed, but not between the two daughter-nuclei arisen from the female pronucleus.

Figure 4 proves that cleavage may take place between a nuclear aster (arisen by the division of a spermaster) and a cytaster. The blastomere, which after cleavage only contains the cytaster, shows after this no further division, but a monaster-cycle, ending in the death of the blastomere. So it is plain that there are indeed asters of different "activity" and that the spermasters ("nuclear asters") are strongest. They can divide independently of the nucleus and are able to induce cell-division either together with a similar aster or in combination with a cytaster. As said before I never saw cleavage of the cytosome between two cytasters, but division of a nucleus I sometimes saw.

6. *Isolation of the spindle.*

In some experiments I have succeeded in isolating the spindle from the two asters in an early metaphase-stage. As a strongly refractive gelatinous body it then presents itself. In a short time a light radiation issues from the spindle in the surrounding cytoplasm (currents of liquid towards the spindle?). I have also observed division of the spindle into two round bodies, though no distinct amphiaster had formed. Division of the cytoplasm did not follow. Evidently the cells had been too much injured, for they have developed no further. The possibility of a division of the nuclear spindle without the presence of asters has, however, been rendered probable by these observations.

7. *Cleavage between asters with and without nucleus.*

In his study about cytasters FRY has also intimated his opinion that a normal division is only possible when nuclear substance is near the asters and DALCQ also has pointed out the necessity of the nucleus for cell-division.

In connection herewith it may be mentioned that in my experiments I have never seen cell-division between two asters without a nucleus, at best irregular constrictions of the cell-body were seen. The indispensability of nuclear substance for cleavage appears from figure 5. If namely in an amphiasterstage an aster is isolated, then this aster will divide. The remaining aster forms after some time again an amphiaster, which envelops the nucleus. Only this last amphiaster induces constriction of the cytosome. Between the daughter-asters, arisen from the aster, which was isolated from the nucleus, cell-division does not take place.

Finally a few observations about conjugation of the pronuclei may be mentioned.

8. *Prevention of karyogamy.*

On the penetration of the spermium the sperm-nucleus swells and moves in the direction of the female pronucleus (penetration-path). When the

male pronucleus has arrived at a certain distance of the female then the latter begins also to move towards the male pronucleus (copulation-

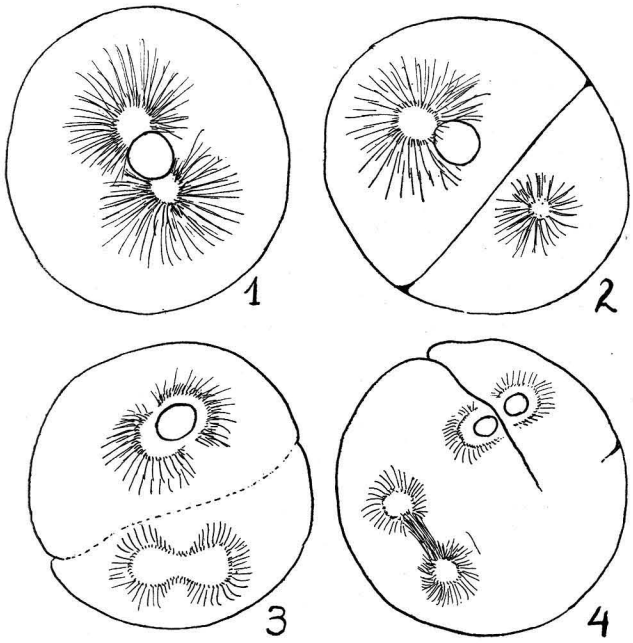

Fig. 5. *Egg of Echinus miliaris.*

1. Amphiasterstage.
2. Artificial constriction, by which an aster is isolated.
3. Constriction vanishes by liquefaction of cytoplasm. Isolated aster divides. Small deformed amphiaster round nucleus.
4. Nucleus has divided and between daughter-nuclei constriction of cytoplasm has formed. Isolated aster in state of division (pseudo-spindle), but this division does not lead to constriction of cytoplasm.

path). At some distance from each other the pronuclei come to a pause and finally union of the nuclei is seen.

If the male pronucleus is prevented to move then the female pronucleus can cover the distance to the male pronucleus itself. If the meeting has been retarded and if the male pronucleus is already lying in an amphiaster, then on approach of the female pronucleus the male is sometimes seen to move to one pole of the amphiaster to meet the female pronucleus (see Fig. $1_{.3}$ and $1_{.4}$).

If one succeeds in moving the two pronuclei just before karyogamy out of the amphiaster enveloping them and when they lie then at some distance from each other in the cytoplasm, one can observe them come together and fuse outside the amphiaster (see Figure 6). The two asters now seem to be attracted by the fusion-nucleus, the distance covered can be discerned by a hyaline streak left by the asters during their displacement in the cytoplasm and one gets a strong impression that the fusion-nucleus imbibes itself with liquid from the cytoplasm and causes currents, which take the asters along to the cleavage-nucleus. The phenomenon that

the two asters lie pressed against the cleavage-nucleus can perhaps be explained by this.

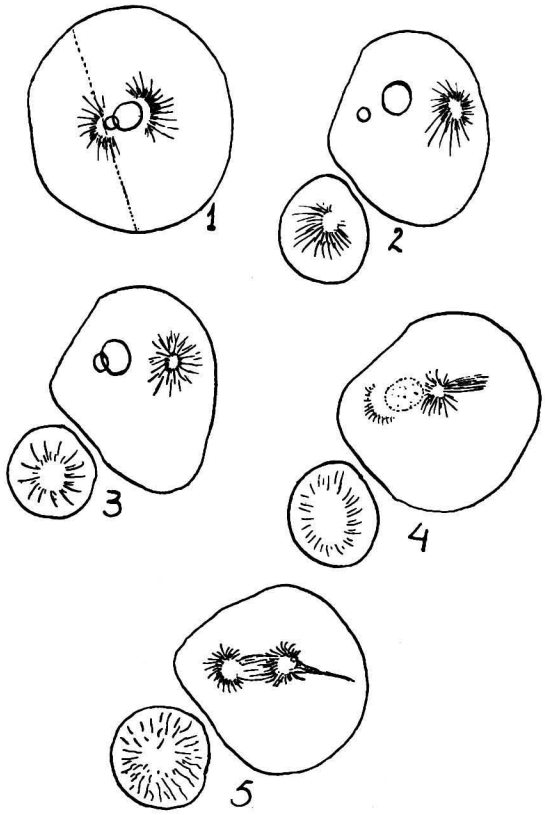

Fig. 6. *Egg of Paracentrotus liv.*

1. Section before karyogamy.
2. Fragments rounded up. Nuclei lie free from asters in cytoplasm. Asters small (immediately after operation).
3. Nuclei approach each other. Irregular radiation in the egg-fragment without nuclei.
4. Nuclei fused and dissolving. Aster moves to place where nuclei have vanished.
5. Forming of amphiaster. Path of right aster visible.

Summary.

The penetrating spermium activates the cytoplasm of the egg. So a series of physico-chemical changes is effected in the cytoplasm, by which in their turn the two pronuclei begin to change and swell. In the meantime they may exercise an attraction on each other. After fusion the cleavage-nucleus goes on to imbibe, an amphiaster arises (by division of the sperm-aster) and the two asters are pressed against the imbibing fusion-nucleus. After some time the nucleus dissolves, the mixoplasm (WASSERMANN) forms and this mixing calls forth a further sequence of cytoplasmic changes

by which cleavage is made possible. Cleavage can only be effected between two asters of a certain intensity of efficacy in the presence of nuclear substance. For a normal cleavage the spermaster is therefore essential and the removal of the spermium from the egg leads to a monaster-cycle because the spermaster is wanting. The spermaster can divide independently of the nucleus, the nuclear spindle can probably divide independently of the asters.

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Physics. — *An X-Ray Goniometer for the Investigation of Crystal structures of solidified Gases.* By W. H. KEESOM and K. W. TACONIS. (Abstract of Communication No. 235*b* from the KAMERLINGH ONNES Laboratory at Leiden.)

(Communicated at the meeting of March 30, 1935.)

A method was developed for determining the crystal structures of solidified gases in those cases in which the DEBIJE-SCHERRER method does not yield sufficient data.

We produced single crystals by very slow crystallization of the liquid that had been condensed in a small thin-walled tube dipping in a cup filled with mercury, cooling the mercury, and regulating its temperature.

Such a crystal in arbitrary orientation was rotated in the axis of the DEBIJE-SCHERRER camera. We made two exposures of the substance on the same film. In the second exposure the film was continuously moved in the direction of the axis of the camera, just as in WEISENBERG's goniometer.

The data from such a film make it possible to determine the crystal structure unequivocally.

We treated the diagram by making a gnomonic projection on a plane perpendicular to the axis of rotation of the crystal.

For ethylene we deduced from this gnomonic projection a rhombic cell with axial ratios 1.327 and 0.850. The absolute dimensions were found by measuring the spacings. The result is a rhombic cell with two molecules:

$$a = 6.46, \quad b = 4.87, \quad c = 4.14 \text{ Å} \quad \text{at} -175^\circ \text{ C.}$$

Density 0.717.
