A FINE STRUCTURAL ANALYSIS OF CLEAVAGE INDUCTION AND FURROWING IN THE EGGS OF *ARBACIA PUNCTULATA*

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ABSTRACT

A fine structural study has been carried out on the various formed elements present before, during, and after the first cleavage division, not only in normally developing *Arbacia* eggs, but also in eggs which have been induced to cleave prematurely by high-pressure centrifugation. The aim has been to ascertain whether or not any of the morphologically identifiable components may be involved in initiating the furrowing process. Also, attention has been given to the fine structure of the cytoplasmic cortex, particulary in the walls of the furrow, in the hope of reaching a better understanding of the mechanics of cleavage. The annulate lamellae and the membranous envelope of the nucleus are the only formed elements which disappear shortly before cleavage, not only in eggs undergoing normal division, but also in eggs which have been induced to cleave ahead of schedule by high-pressure, high-force centrifugation. Therefore, it is suggested as a tentative hypothesis that materials liberated upon disintegration of the nuclear membrane and the annulate lamellae play an essential role in initiating and effecting the furrowing reaction, especially since the stratification of these elements in experimentally induced eggs corresponds to the position of the developing furrow. Another of the membranous elements in the egg, the Golgi complex, shows considerable modification as a result of high-pressure centrifugation, but these structures do not undergo disintegration. Rather, they become curled into rounded bodies. The vacuole population is not greatly affected by inducing treatments. During cleavage, both naturally occurring and experimentally induced, a considerable number of 50 A filaments appear in the denser cytoplasmic cortex, but only in the walls of the furrow. These filaments are similar to those which have been demonstrated in a number of contractile cells. Accordingly, it is suggested that this fibrillar system may be actively involved in the development of the cleavage force.

INTRODUCTION

The mitotic apparatus is known to exert considerable control over the prospective position of the presumptive cleavage furrows in naturally dividing eggs. In some manner the spindle-aster complex strongly influences the localization of factors concerned with triggering the furrowing reaction. This conclusion stems from the work of many investigators. However, the centrifugal studies of Harvey

(1935, 1956) and the microsurgical experiments of Hiramoto (1956) deserve particular mention. The latter showed that when the mitotic apparatus is displaced before anaphase, the furrow is shifted to a new position determined by the changed position of the spindle-aster complex. However, when the displacement is executed later, the furrow retains the position determined by the original location of the mitotic apparatus.

Experimentally, however, eggs can be induced to cleave many minutes before the appearance of any mitotic apparatus. The furrow-inducing treatment involves high-force (40,000–50,000 *g*) centrifugation with simultaneous application of hydrostatic pressure (8,000–12,000 psi) (Marsland et al., 1960; Zimmerman and Marsland, 1956). Some 4–5 min after such treatment, a high percentage (up to 100%) of the eggs begin to furrow and in most cases cleavage progresses to completion. All successful inducing treatments involve the disappearance of the nucleus and of certain cytoplasmic granules, which can be identified by their metachromatic staining with toluidine blue. Accordingly, Marsland et al. (1960) postulated that at least two factors, one from the nucleus and the other from the cytoplasm, are involved in the process of triggering the furrowing reaction.

In the present study the greater resolution achieved by electron microscopy has been used to investigate the morphological events which take place before and during experimental induction, and to compare these changes with those which occur during normal cleavage. Thus it may be possible to select the common factors that are essential to furrow-inducing mechanisms. In addition, the fine structure of the cytoplasmic cortex of the egg, especially in the region bordering the cleavage furrow, has been carefully analyzed. The force utilized in the execution of cleavage must be generated within this strongly gelled layer (see Discussion), and a study of the fine structural characteristics of this layer may provide a clue as to the mechanism of the furrowing process.

MATERIALS AND METHODS

Living Material

The eggs of *Arbacia punctulata* were shed by injection of 0.5 ml of 0.53 M KCl solution into the female specimen. The eggs were then washed twice in filtered sea water which had been previously equilibrated to the experimental temperature (20 ± 0.1°C). Insemination involved the addition of two drops of dilute sperm suspension (one drop of "dry" sperm, exuded from the excised testes, placed in 25 ml of sea water) to 100 ml of egg suspension.

The Pressure Centrifuge

This apparatus has been described fully by Brown (1934). Eggs contained in the experimental compartment of the centrifuge head can be subjected to high pressure (up to 20,000 psi) which is then sealed in by means of the needle valve. Thus, after centrifugation, the pressurized eggs can be compared with controls contained in the atmospheric section of the head.

Temperature Control

All glassware and solutions, including those containing the developing eggs, were kept in a thermostatic housing which maintained the experimental temperature at 20 ± 0.1°C. This housing also contained the pressure-centrifugation equipment.

Electron Microscopic Procedures

Both the experimentally treated and the untreated eggs were fixed in 2% glutaraldehyde in sea water or in 10% acrolein, also in sea water. The acrolein was prepared by distillation in the dark and stored at −20°C. The glutaraldehyde was first treated with $BaCO_3$ and then centrifuged, after which the pH was adjusted to 7.6 with 1 N NaOH. The eggs were fixed for 1 hr, then washed in sea water, and postfixed for ¾ hr in 1% OsO_4 in sea water. They were then rapidly dehydrated in ethanol and embedded in Epon 812 or Araldite. Sections were cut with a diamond knife on a Porter-Blum MT2 or LKB ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Philips 200, Hitachi 11C, or Hitachi 7S electron microscope.

RESULTS

Normal (Untreated) Eggs

FERTILIZED EGGS, 30 MIN AFTER INSEMINATION: Although the existence of a gelled cortex has been demonstrated experimentally (Chambers, 1938; Marsland, 1956; Hiramoto, 1957; Wolpert, 1960), no unequivocal fine structural differentiation of this layer before the time when cleavage furrows appear has been described. In some, perhaps better fixed eggs, a fine spongy matrix appears between the echinochrome pigment bodies immediately below the plasma membrane, but the boundaries of this zone are poorly defined and the spongy matrix grades imperceptibly into the matrix of the deeper cytoplasm. Most of the pigment bodies lie in the cortex, al-

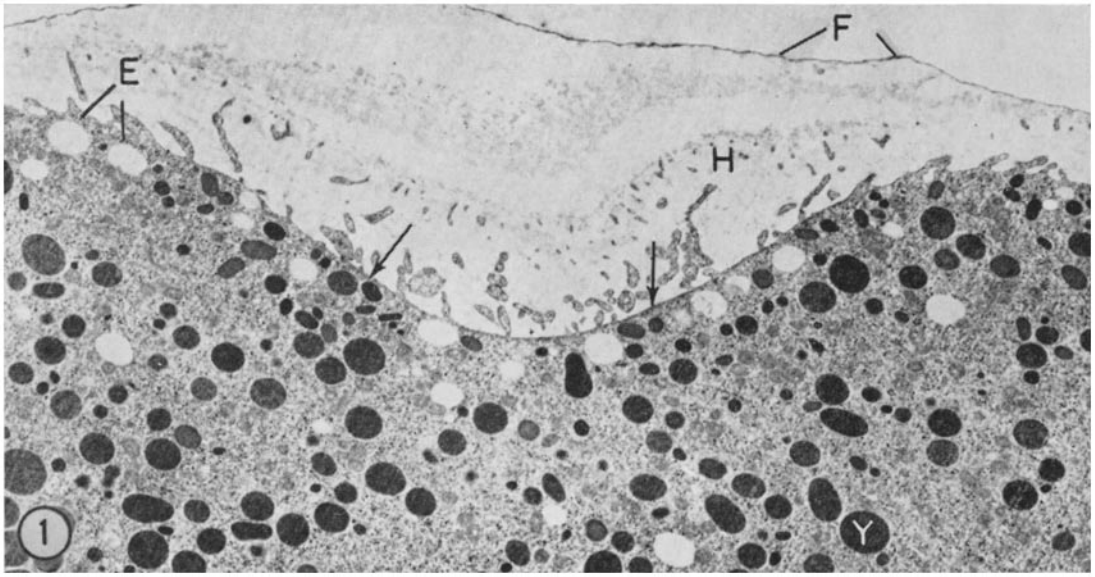


FIGURE 1 Low magnification electron micrograph of a section through the furrow region of a cleaving egg (60 min after insemination). Surrounding the egg is the fertilization membrane (*F*) and the hyaline layer (*H*) into which extend short microvilli. The most obvious constituents of the egg at this magnification are the yolk granules (*Y*) and the echinochrome pigment granules (*E*). Within the region of the furrow and only in this region is a thin dense layer of cytoplasm located on the inner side of the limiting membrane (see arrows). × 6000.

though a few may lie more deeply. The pigment bodies are membrane enclosed, but little, if any, internal structure is discernible.

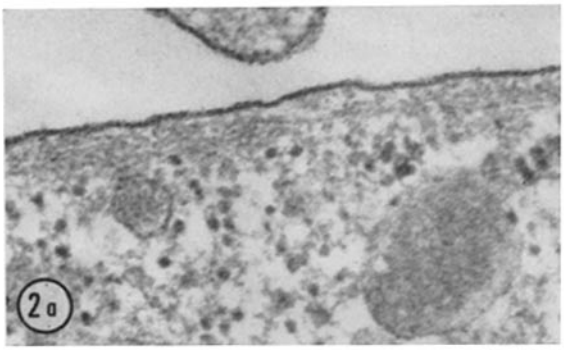
The organelles of the medullary cytoplasm have been described previously (see Anderson, 1968). They include both the rough- and smooth-surfaced endoplasmic reticulum, mitochondria, Golgi complexes, yolk granules, various vacuoles, and annulate lamellae. The annulate lamellae were followed carefully because of evidence indicating that they may be involved in the process of triggering the furrowing reaction. Typically, these lamellae appear in stacks of two or three (see Figs. 6 and 7). Vacuoles were also given special attention because some of them as well have been implicated as possible participants in the triggering process (Marsland et al., 1960; Kojima, 1959; Mulnard et al., 1959). The vacuole population is heterogeneous both in dimensions (0.1–1.0 μ in diameter) and in content. Some vacuoles appear to contain yolk which is in the process of breakdown, others appear as multivesicular bodies (Fig. 9), and still others contain short rods (Fig. 10), which are the "rod-like structures" described by Anderson (1968).

FURROWING EGGS, 60 MIN AFTER INSEM-

FIGURE 2 Micrographs taken of two parts of the cortex of an egg in an early stage in furrow formation. *a*. In the region of the furrow. Note the presence of fine filaments. These filaments make up the dense layer in the furrow of Fig. 1. *b*. At one of the poles. No filaments are present. × 70,000.

FIGURE 3 Portion of the cortex in the region of the furrow. The layer of fine filaments beneath the plasma membrane displaces the echinochrome pigment granules (*E*). A yolk granule (*Y*) is present as well. × 50,000.

FIGURE 4 A grazing section cut through the cortex of a cleaving egg in the furrow region. Note the meshwork of fine filaments. × 60,000.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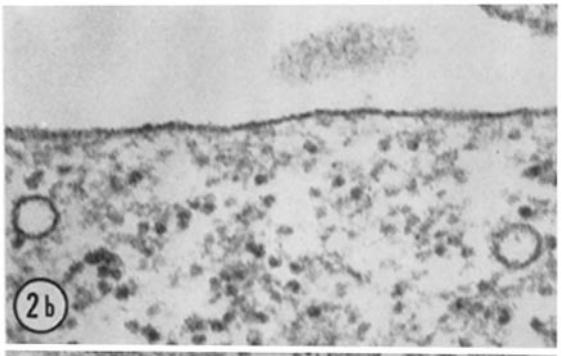
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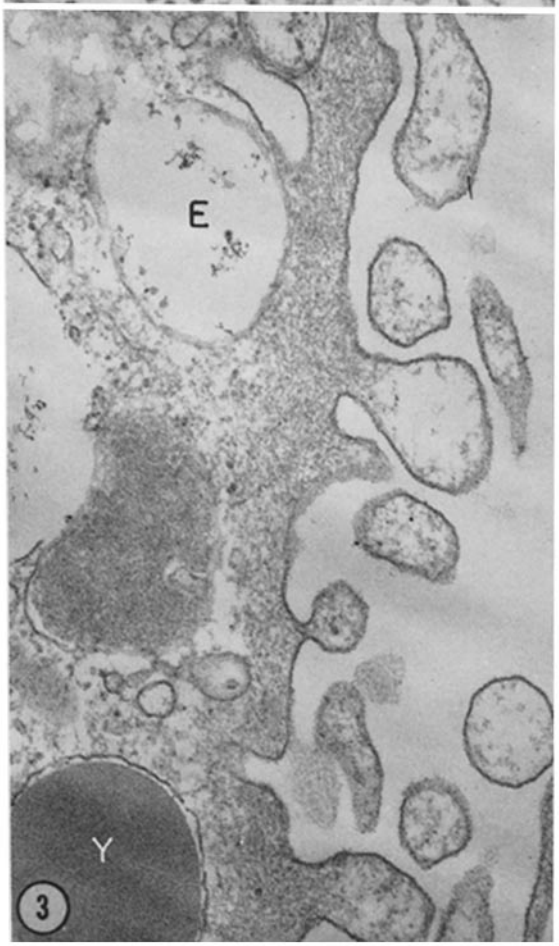
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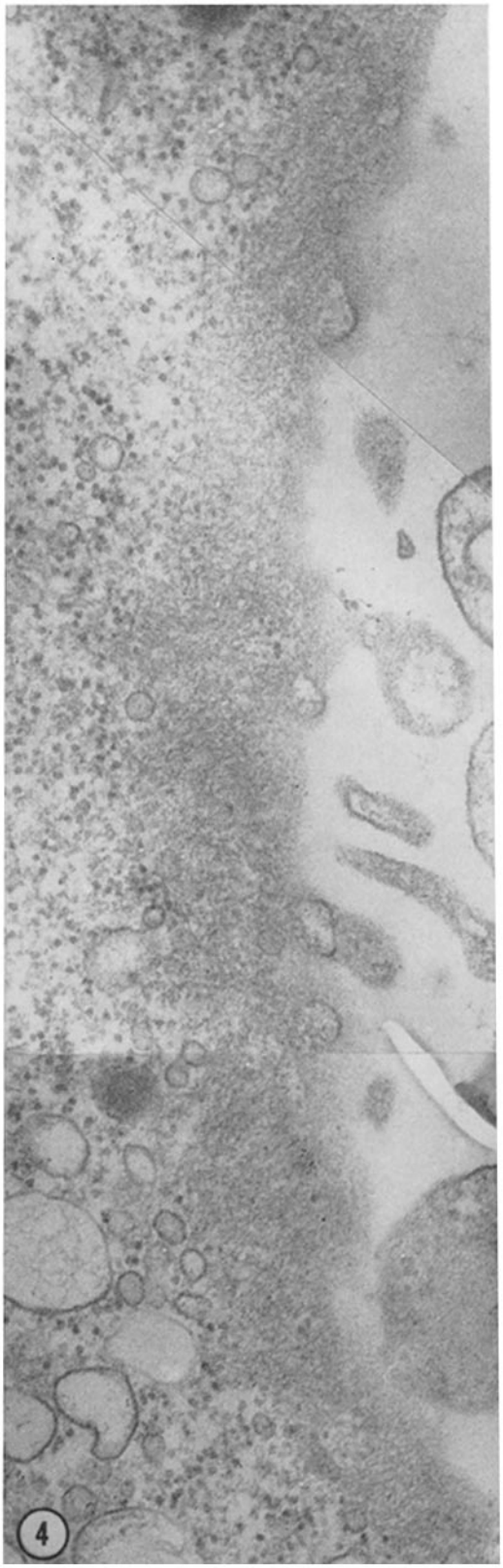
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INATION: At the time of cleavage a clearer differentiation of the cortex (as compared to the deeper cytoplasm) has been reported by Mercer and Wolpert (1958) in regard to the entire surface of the egg, and by Weinstein and Herbst (1964) and Robbins and Gonatas (1964) in regard to the surface of the furrow region only. The present observations tend to corroborate and amplify these earlier reports, particularly with reference to the cortex bordering the furrow proper. With our techniques, there is a dense layer of cytoplasm on the inner side of the plasma membrane in the furrow region (Fig. 1). This band is about 0.1 μ thick and appears to be constituted of an amorphous material in which are embedded numerous fine filaments, each about 50 A in diameter (Figs. 2 *a*, 3, and 4). Filaments are not found in the cortex away from the furrow region (Fig. 2 *b*). Bordering the furrow proper, however, the filaments always lie just beneath the limiting membrane, and thus displace the echinochrome granules inwards (Fig. 3). Occasionally, a few, but only a few, microtubules may be found among the filaments.

The chromosomes lie near the center of the egg and are surrounded by ribosomes and elements of the smooth-surfaced endoplasmic reticulum; the mitochondria and yolk granules appear to be excluded from this region. A nuclear envelope has begun to surround each chromosome.

The cytoplasmic organelles do not appear altered, the prominent exceptions being the annulate lamellae which could not be found, and the Golgi elements which appeared slightly less numerous than previously observed.

THE TWO-CELL STAGE, 15 MIN AFTER CLEAVAGE: The structure of the cortical and medullary cytoplasm at this time resembles that of the precleavage specimen. An important point to realize, however, is that there has been a reappearance of the annulate lamellae. At this stage the abundance of this organelle relative to the newly fertilized egg is difficult to assess, but it appears to be less.

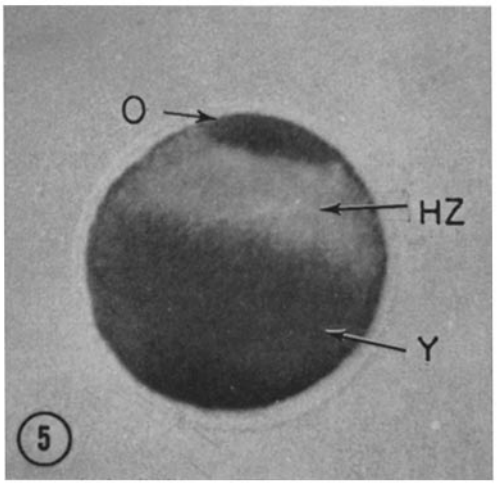


FIGURE 5 Light micrograph of an egg, centrifuged at 41,000 *g* for 3 min. It illustrates the following layers: the oil cap (*O*), the hyaline zone (*HZ*), an indistinct mitochondrial zone, and the yolk zone (*Y*). Most of the pigment remains embedded in the strongly gelated cortex. × 600.

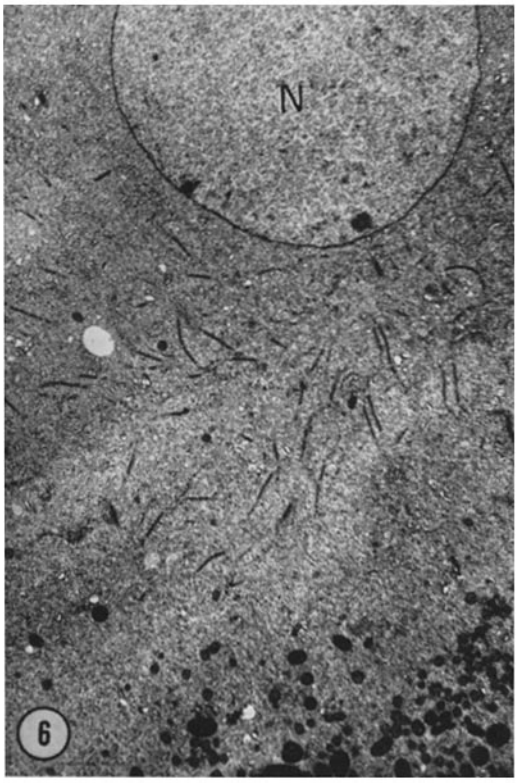


FIGURE 6 Low magnification electron micrograph through the hyaline zone of a non-pressurized fertilized egg centrifuged at 41,000 *g* for 3 min. A portion of the spherical nucleus (*N*) is included in this micrograph. Between the nucleus and the mitochondrial zone, present in the lower right-hand corner of this micrograph, are elements of the Golgi apparatus and the annulate lamellae. The latter are a particularly prominent constituent of this zone. × 3500.

Centrifuged Eggs

CENTRIFUGED, BUT NOT PRESSURIZED, 30 MIN AFTER INSEMINATION (41,000 *g* FOR 3 MIN): The stratification of these eggs has been described previously (Harvey, 1956). With light microscopy (Fig. 5), a distinct *oil cap* may be seen at the centripetal pole; in the specified order, one finds the following: (1) a clear *hyaline zone* in which the nucleus lies in close contact with the oil cap; (2) a narrow *mitochondrial zone*; and (3) a broad *yolk zone* which extends to the centrifugal extremity. However, without pressure there is little or no displacement of the pigment granules in fertilized eggs, except for the few granules that were found previously in the deeper cytoplasm (Marsland et al., 1960). Thin sections show a similar stratification of the cell components; with the added resolution of the electron microscope, it is possible to describe the position of other cytoplasmic elements. Both the annulate lamellae (Figs. 6 and 7) and the Golgi complexes (Fig. 8) appear in the hyaline zone on either side of the nucleus, although some of these elements may lie near the mitochondrial zone. Due to their plate-like form (Figs. 7 and 8), both the annulate lamellae and the Golgi complexes tend to become oriented in the field of centrifugal force, so that frequently their broad surfaces are presented to the sides of the egg and their ends point towards the poles (Fig. 6). Sometimes the cisternae of the annulate lamellae are in continuity with elements of the endoplasmic reticulum (Fig. 7). The vacuoles are usually present in the hyaline zone; however, some which appear to contain degenerating yolk material (Fig. 10), can also be found in the mitochondrial and yolk zones. As in uncentrifuged eggs, the vacuole population varies considerably from cell to cell. Multivesicular bodies (Fig. 9) and rod-containing vacuoles (Fig. 10) are not uncommon.

The hyaline zone is rich in ribosomes and in elements of the smooth surfaced endoplasmic reticulum (*SER*), the latter being very extensive in some cells. However, both ribosomes and SER may also be found in the other zones. The nucleus is generally spherical and displays numerous nuclear pores. By contrast, the surface of the egg is not evenly rounded. Often it shows fairly deep angulations, and many fine microvilli extend outward from the surface.

Stratification of the mitochondria is not complete, since a few of them may be found in the oil cap and in the upper part of the yolk zone. However, mitochondria are almost never seen in the hyaline zone. Some elements of the Golgi apparatus have been found in the mitochondrial and yolk zones; however, this is not the case for the annulate lamellae. The annulate lamellae, together with most of the Golgi elements, lie in a relatively narrow band in the hyaline zone on either side of the nucleus.

HIGH PRESSURE, HIGH FORCE CENTRIFUGATION, 30 MIN AFTER INSEMINATION: These eggs were centrifuged for 5 min at a force of 41,000 *g* while exposed to high pressure (10,000 psi). This constitutes an inducing treatment (Zimmerman and Marsland, 1956). In the present case, about 90% of the eggs began to display vigorous furrowing starting 4–5 min after deceleration and decompression. In most cases the furrows progressed and completed cleavage.

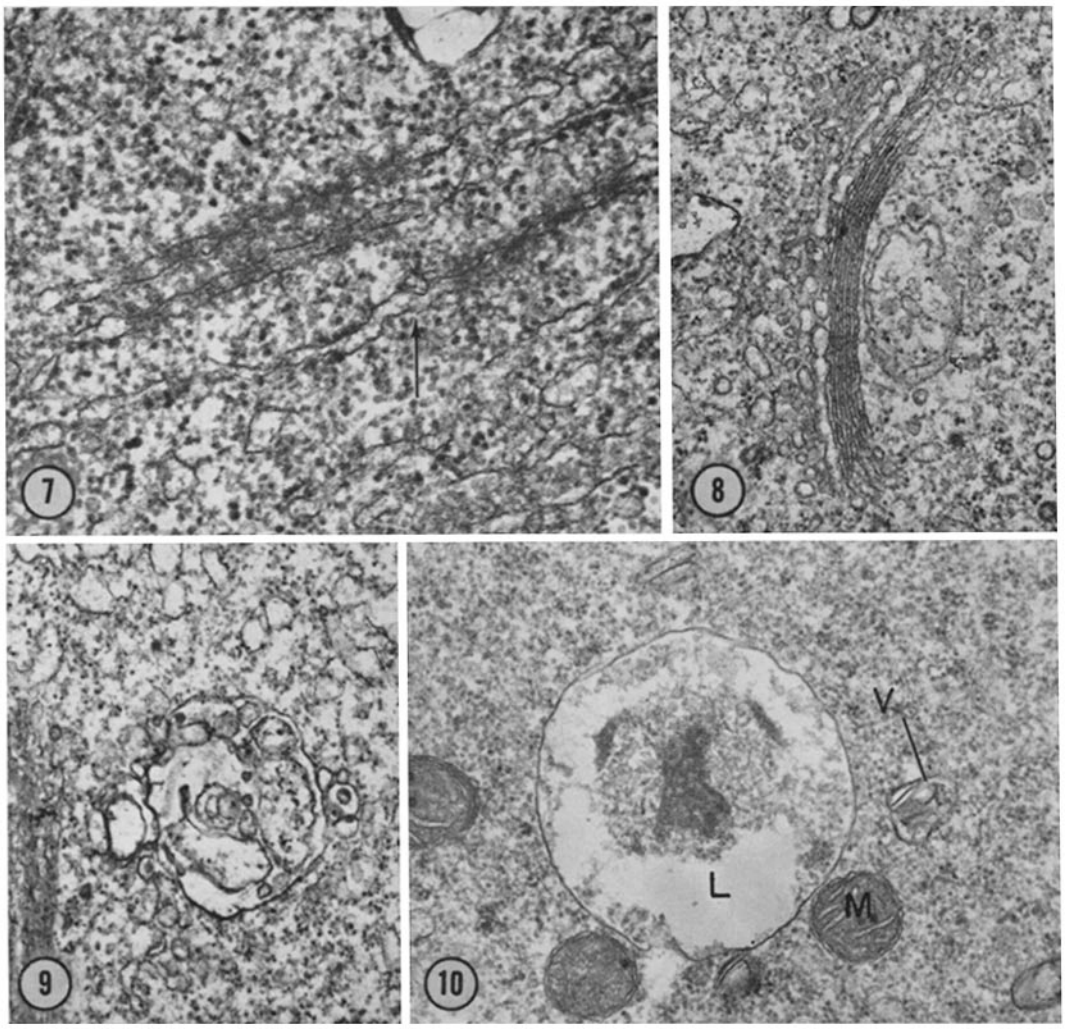
With light microscopy it could be seen that such pressure-centrifuged eggs display an additional stratification zone, namely a pigment zone, which occupies the extreme centrifugal pole and is about ⅓ as broad as the adjoining yolk zone (Fig. 11). Virtually all of the pigment granules had been displaced from the cortex of the hyaline zone, and most of them from the mitochondrial and yolk zones. Nuclei were never seen in any of the eggs that subsequently displayed experimentally induced furrowing.

In thin sections there were pronounced alterations in the elements of the hyaline zone. The annulate lamellae were totally absent in most of the sections and dramatically reduced in all others (Figs. 12 and 14). In the latter case a few small lamellar segments could be recognized, but organized stacks, such as those shown in Figs. 6 and 7, were definitely absent. The Golgi elements displayed a marked change of form, but were not reduced in number. Instead of appearing as flat plates, each consisting of five or more flattened cisternae, the Golgi elements now appeared as small spheres, due to a curling of the cisternae (Figs. 12, 13 *a* and 13 *b*). Coated pits attached to the ends of the cisternae remain unchanged. The SER was plentiful, but its form was irregular.

Although nuclei were absent from almost all of the pressure-centrifuged eggs, one cell did contain a nucleus. Instead of being smoothly rounded, the nuclear surface was deeply indented, with long finger-like strands of cytoplasm encroaching into the nuclear mass (Fig. 15). Moreover, the number of pores in the nuclear envelope appeared to be reduced.

The vacuole population of the hyaline zone showed little change as a result of the pressure-centrifugation. Approximately the same kinds and numbers of vacuoles were represented, including those containing "degenerating yolk material."

The cytoplasmic cortex in the hyaline zone, the region where cleavage furrows were developing in the nonfixed sample of the treated eggs, displayed a considerable number of 50 A filaments (Fig. 16) similar to those seen in the furrow region



FIGURES 7–10 Portions of the hyaline zone of nonpressurized centrifuged eggs (41,000 *g* for 3 min).
FIGURE 7 A stack of annulate lamellae. The arrow indicates continuity between the membrane of the annulate lamellae and the rough-surfaced ER. × 55,000.

FIGURE 8 A portion of the Golgi apparatus. This organelle appears as a series of isolated units, each of which is made up of stacks of flattened cisternae and associated vesicles. × 21,500.

FIGURE 9 Multivesicular body. × 18,000.,

FIGURE 10 In the center of this micrograph is a large vacuole (*L*) within which is material believed to be degenerating yolk. Morphologically this vacuole appears to be a lysosome. Mitochondria (*M*) and small vacuoles (*V*) containing short rods are also present. × 26,000.

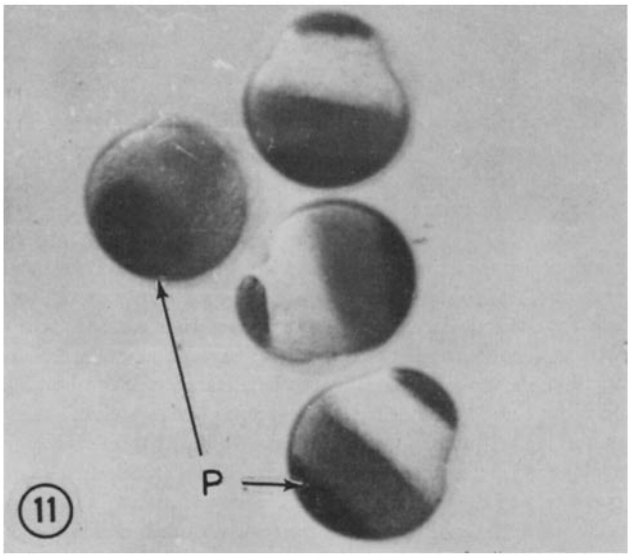


FIGURE 11 Light micrograph of an egg which has been subjected to high pressure (10,000 psi)) and centrifugation (41,000 *g*), an inducing treatment. This micrograph was taken 3 min after deceleration and decompression. Note that induced furrows are beginning to form. Also note the distinct pigment zone (*P*) and absence of pigment from cortex of the hyaline zone. × 300.

of the normally cleaving eggs (Figs. 3 and 4). These filaments were not found in the cortex of the other zones.

CENTRIFUGATION AT LOWER PRESSURE, 30 MIN AFTER INSEMINATION: This non-inducing treatment consisted of centrifugation for 5 min at a lower pressure (6000 psi). This sufficed to displace most of the pigment granules, although a few could still be found lodged in the cortex of the hyaline zone. The nuclei remained intact and could be seen clearly in the hyaline zone of all favorably oriented specimens. No sign of furrow induction was seen in any of the treated eggs.

Thin sections of such eggs revealed a state more or less intermediate between that of the centrifuged nonpressurized cells and that of cells which were centrifuged at higher pressure. In the hyaline zone the Golgi elements were either slightly curled or flat (Fig. 18). The annulate lamellae were somewhat reduced in number, but they retained their normal form and still were a conspicuous component of the zone (Figs. 17, 18, and 19). In many cases the nuclear surface was quite irregular (Fig. 19), sometimes with rather deep cytoplasmic intrusions. The pores in the nuclear envelope were not appreciably reduced in number. In one case we found 50 A filaments in the cortex of the hyaline zone.

DISCUSSION

Structural Elements Involved in Cleavage Induction

ANNULATE LAMELLAE AND NUCLEUS: Shortly before furrowing starts, both in naturally cleaving eggs (Verhey and Moyer, 1967; Harris, 1967) and in eggs that have been induced to cleave experimentally, the annulate lamellae and the nuclei disappear, i.e., they are no longer recognizable as morphological entities. Moreover, at the end of first cleavage or soon thereafter, both of these organelles reappear, only to disappear again shortly before the second cleavage division (Verhey and Moyer, 1967). These events, of course, may be purely coincidental. On the other hand, they may be related. Therefore it does not seem unreasonable to postulate that materials derived from the nucleus and the annulate lamellae are concerned with initiating and effecting the furrowing reaction. The fact that both the nucleus and the annulate lamellae become segregated during high-force centrifugation into the hyaline zone, in which the experimentally induced furrows develop, tends to strengthen the foregoing hypothesis. Essentially, this hypothesis is a more specific statement of the proposal made by Marsland (1958) that both nuclear and cytoplasmic factors are involved in cleavage induction.

Some evidence contradictory to the foregoing

proposal may be cited. For example, Harvey (1936) has shown that anucleate activated half-eggs may display a series of feeble, frequently abortive cleavages which are associated with the appearance of small, coarsely radiate asters; however, these cleavages are much delayed and could be regarded as a kind of fragmentation not related to the normal cleavage process. Furthermore, since the annulate lamellae are absent from the sea urchin blastulae or even absent after the second cleavage (Verhey and Moyer, 1967), as well as not being present in most dividing cells (see the recent review of Kessel [1968] for their distribution), we must conclude that the annulate lamellae play a minor role in inducing cleavage generally. On the other hand, it is known that the annulate lamellae are derivatives of the nuclear envelope (Kessel, 1963). Perhaps the nuclear envelope provides adequate amounts of inducing material in most cells, the annulate lamellae only being necessary for induction in very large cells such as eggs. It is also possible that the annulate lamellae may provide material needed for membrane production in the more extensive surface of dividing eggs. Here again egg cells, because of their size, would need a larger source of membrane precursor material.

THE GOLGI APPARATUS: Although the number of Golgi elements show a reduction at the time of cleavage, they remain numerous, though curled into little spheres, when experimentally induced cleavage occurs. Therefore, one cannot completely elimina thete possibility that Golgi elements play some small role in cleavage induction. It should be mentioned in this regard that in some dividing mammalian cells (Robbins and Gonatas, 1964) the Golgi apparatus breaks down concurrently with nuclear membrane dissolution.

CYTOPLASMIC GRANULES AND VACUOLES: No essential changes are found in the vacuole population in induced eggs as compared to eggs that were merely centrifuged. However, the vacuoles are very heterogeneous in size and contents, and it is difficult to say that there were no changes such as in the permeability of the boundary membranes. In some mammalian cells (Robbins and Gonatas, 1964) multivesicular bodies and membrane-enclosed dense bodies tend to aggregate near the cleavage furrows, as is also the case with the smooth-surfaced endoplasmic reticulum (Dougherty and Lee, 1967). Therefore, these other structures could conceivably be involved in cleavage.

Mechanics of Furrowing

CONTRACTILITY OF THE FURROW CORTEX: According to the hypothesis proposed by Marsland and co-workers (Marsland and Landau, 1954; Marsland, 1956) and by Wolpert (1960), furrowing results from the contraction of an equatorial band of strongly gelled cortical cytoplasm.

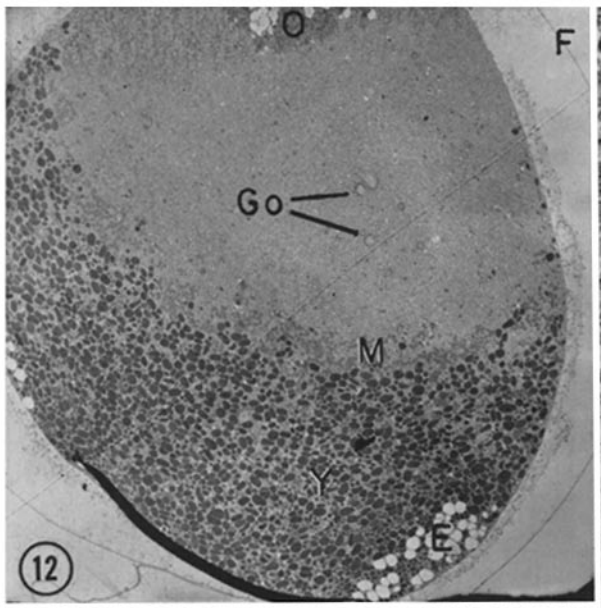
FIGURES 12–15 Eggs at 30 min after inseminations which were subjected to a pressure of 10,000 psi and a centrifugal force of 41,000 *g*. The eggs were fixed within 60 sec of release of compression and centrifugation.

FIGURE 12 Low magnification electron micrograph of a section through an egg. A portion of the oil cap (*O*), the hyaline layer containing nearly spherical Golgi elements (*Go*), the mitochondrial layer (*M*), the yolk layer (*Y*), and the echinochrome pigwent layer (*E*) are easily distinguished. Annulate lamellae are not present. The egg is enclosed in its fertilization membrane (*F*). × 1800.

FIGURES 13*a* and *b* These two micrographs illustrate typical examples of elements of the Golgi apparatus when subjected to pressure and centrifugation. The flattened cisternae curl, giving this complex the form of small spheres. × 49,000.

FIGURE 14 A portion of the cytoplasm in the hyaline zone. Even though annulate lamellae are no longer present, a few annuli or pores (*AL*) can be found on occasion, such as illustrated here. Many smooth-surfaced vesicles as well are present in this field. × 42,000.

FIGURE 15 Section through a portion of a nucleus of a pressurized, centrifuged egg. Nuclei are rarely encountered in such eggs. This nucleus (*N*) no longer presents a spherical profile, but is irregular, being indented by fingers of cytoplasm (*Cy*). Nuclear pores are sparse. × 20,000.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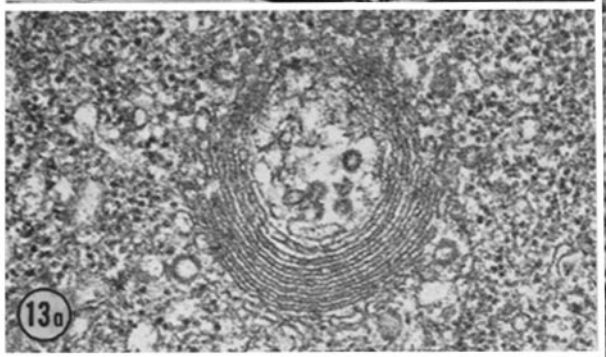
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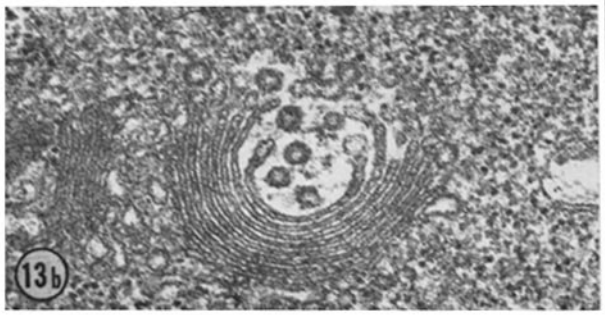
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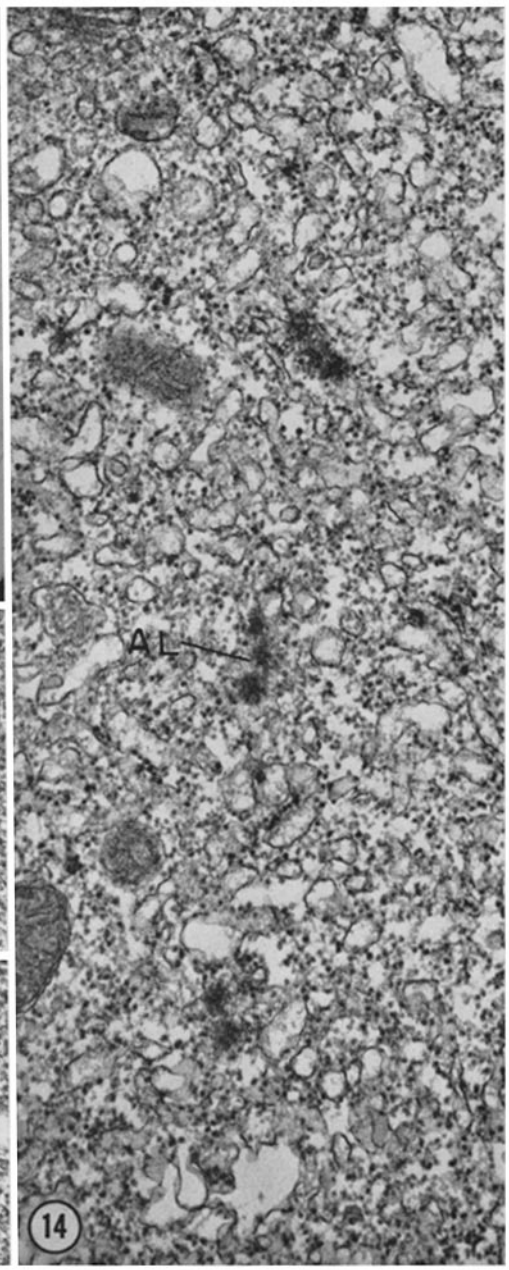
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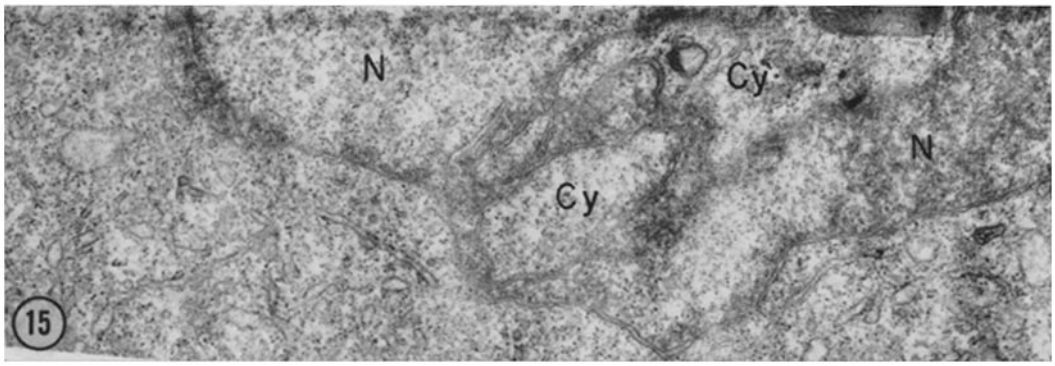
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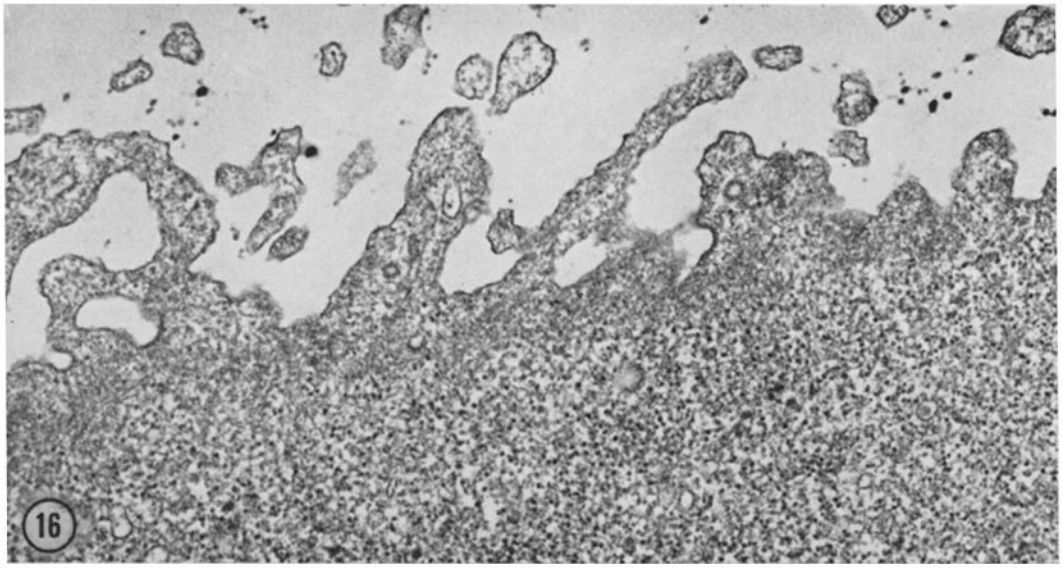


FIGURE 16 Cortex of an egg subjected to 10,000 psi compression and centrifugation at 41,000 *g*. This micrograph shows the region of the induced furrow. A dense layer of cytoplasm just beneath the limiting plasma membrane is composed of fine filaments. The cortex of the egg not in the furrow region appears like that in Fig. 2 *b*. × 37,000.

At first, during the elongation stage of cleavage, this band occupies a broad equatorial zone at the center of which the furrow will develop. Later, during active furrowing, the band is restricted to the walls of the furrow itself. This view has received support from a number of experimental studies (Hiramoto, 1956, 1965; Rappaport, 1966; Scott, 1960; Dan and Kojima, 1963).

Mercer and Wolpert (1958) have described in thin sections a dense layer immediately below the surface membrane. This layer is most strongly developed in the furrow region. Weinstein and Herbst (1964) report a similar layer restricted to the furrow walls in the cleaving blastomeres of sea urchin embryos, and Robbins and Gontas (1964) describe a dense layer in the furrow of dividing HeLa cells. The present demonstration[1] of a system of filaments embedded in the denser cortex of the furrow walls extends these earlier observations. Indeed, the presence of fine filaments in the furrow cortex during both natural and experimentally induced cleavage would seem to be of considerable significance with reference to the mechanics of the cleavage process.

Filaments of similar form and dimensions have been demonstrated in a number of cell types, especially cells characterized by a contractile capacity. For example, Cloney (1966) reports that filaments appear in the epithelial cells in the ascidian tadpole tail during metamorphosis at the time when these cells shorten rapidly, and that the orientation of the filaments suggests that they may play an active role in the contraction process. Similar filaments have been related to form changes in embryos, such as during blastopore formation (Baker, 1965), during the formation of the neural tube (Baker and Schroeder, 1967), and during the contraction of the filopodia of secondary mesenchyme cells.[2] Furthermore, in protozoa, filaments of similar dimensions appear to play a contractile role, such as in the retraction of the pseudopodia of *Difflugia* (Wohlman and Allen, 1968) and in the contractile stalks of *Stentor* (Randall and Jackson, 1958), *Vorticella* (Favard and Carasso, 1965), and *Spirostomum* (Yaqui and Shigenaka, 1963). Ac-

[1] While this paper was being reviewed, two abstracts appeared which reported the demonstration of filaments in the cleaving zone of coelenterate (Szollosi. 1968. *J. Cell Biol.* **39**:133A) and squid eggs (Arnold, J. M. 1968. *Biol. Bull.* **135**:408). Also, there has been a brief note describing filaments in coelenterate eggs (Schroeder, T. E.. 1968. *Exp. Cell Res.* **53**:272.).

[2] Tilney, L. G., and J. R. Gibbins. 1969. Microtubules and filaments in the filopodia of the secondary mesenchyme cells of *Arbacia punctulata* and *Echinarachnius parma*. *J. Cell Sci.* In press.

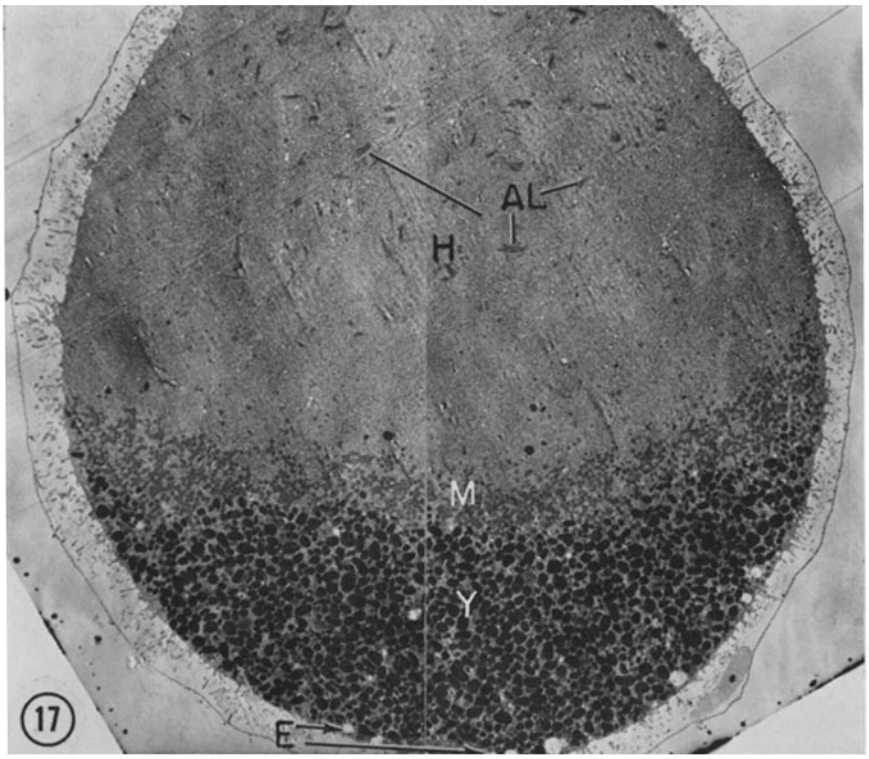


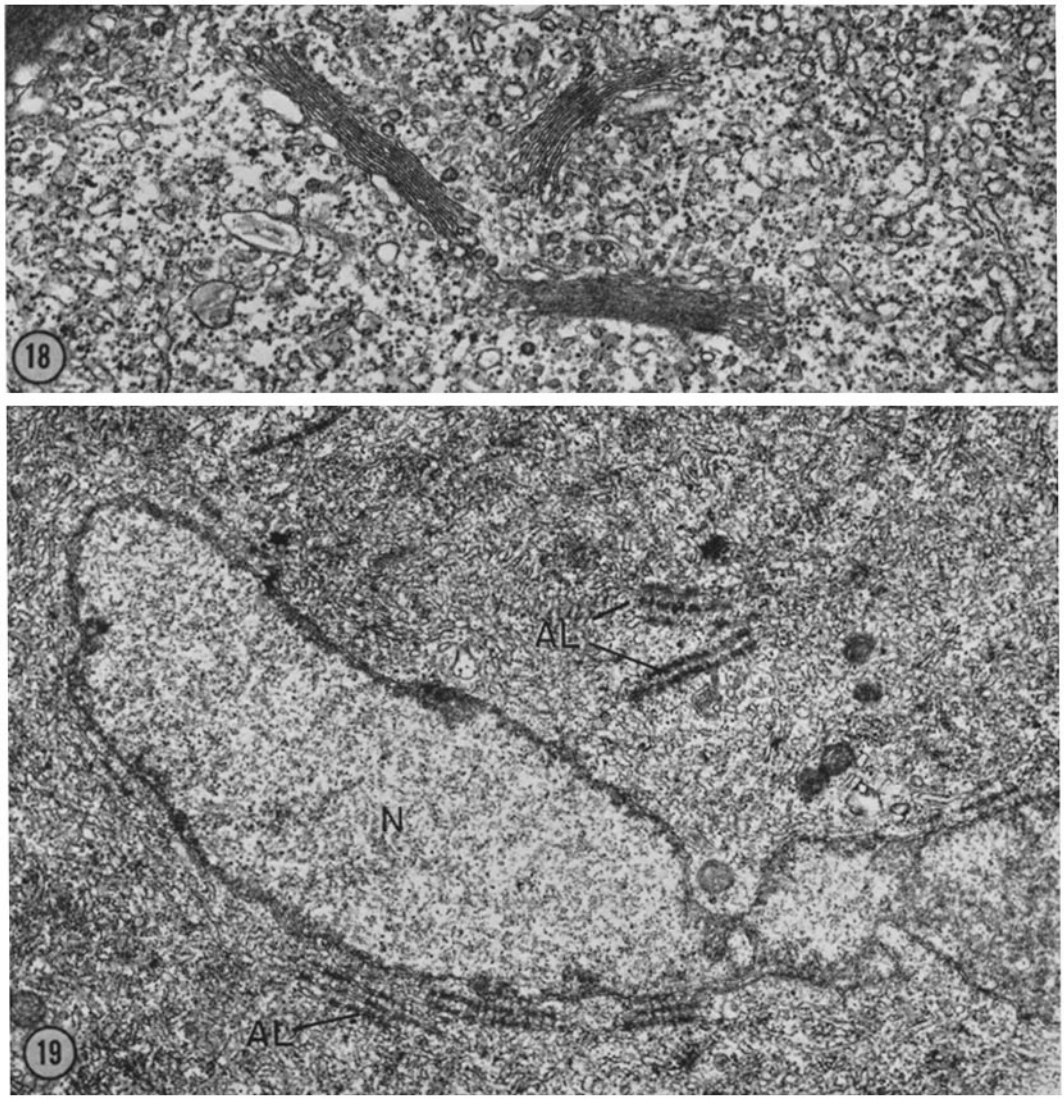
FIGURE 17 Low magnification electron micrograph of an egg 30 min after insemination which was subjected to a compression of 6000 psi and a centrifugation of 41,000 *g*. This is a "non-inducing" treatment. The hyaline zone (*H*), the mitochondrial zone (*M*), the yolk granule zone (*Y*), and a portion of the pigment zone (*E*) can be seen. Within the hyaline zone are stacks of annulate lamellae (*AL*) and Golgi elements. The fertilization membrane surrounds the egg. × 2300.

cordingly, it seems reasonable to postulate that the filaments of the furrow cortex play a contractile role in cleavage. On the other hand, an involvement of the microtubules does not seem likely. Their representation in the egg cortex is exceedingly sparse. Moreover, microtubules tend to disassemble in the presence of colchicine (Tilney and Gibbins, 1966), and successful cleavage can be induced in the presence of equivalent concentrations of colchicine (an observation made during the course of the present experiments; also see Zimmerman et al., 1968).

It seems possible that the deposition of contractile fibers in the furrow cortex may be initiated by materials, perhaps ionic calcium, liberated from the interior of the nucleus upon disintegration of the nuclear envelope. Such a view is in agreement with observations made in a number of other studies (e.g. Stephens and Kane, 1966; Sakai, 1968) and with the fact that at least some nuclei are particularly rich in calcium (Steffensen and Bergeron, 1959; Mirsky and Osawa, 1961).

RELAXATION OF THE POLAR CORTEX: In addition to a contraction of the cytoplasmic cortex in and near the equatorial zone of the dividing egg, a relaxation, or solation, of the gel structure in the polar regions would seem to be a necessary factor in the achievement of successful cleavage (see Marsland and Landau, 1954; Wolpert, 1960). Such polar relaxation has been demonstrated very clearly by the experiments of Just (1922), Chambers (1938), and Marsland (1939). Moreover, polar relaxation is of considerable importance in division, since it permits the egg to elongate just before the appearance of the definitive cleavage furrow (see Chambers and Chambers, 1961).

The recent work of Kinoshita and Yazaki (1967) seems to have an important bearing on the problem of polar relaxation. These workers were able to extract relaxing grana from sea urchin



FIGURES 18 and 19 Portions of eggs 30 min after insemination. These eggs were subjected to a non-inducing treatment (6000 psi compression, centrifugation of 41,000 *g* for 5 min).

FIGURE 18 Micrograph illustrating elements of the Golgi apparatus. The flattened cisternae present a linear profile, unlike the situation with an inducing treatment. × 24,000.

FIGURE 19 A portion of the nucleus (*N*) is illustrated in this micrograph. It is irregular in form and contains numerous pores in its envelope. Elements of the annulate lamellae (*AL*) are present nearby. × 15,000.

embryos and to demonstrate that such relaxing factors tend to be concentrated near the poles in glycerol-extracted, dividing eggs. It seems possible that these factors may be related to materials liberated by the disintegration of the nuclear membrane and its derivatives, i.e., the annulate lamellae.

Tentative Working Hypothesis

On the basis of this and other work, it is postulated that the deposition of contractile filaments in the furrow cortex of the dividing egg is triggered by the mobilization of material, perhaps ionic calcium, derived from the nucleus upon breakdown of

the nuclear envelope. Moreover, it is suggested that polar relaxation, which is an essential prerequisite of successful cleavage, is achieved by virtue of the calcium-sequestering activity of relaxing factors. In all probability the relaxing factors, whatever they may be, are distributed to the poles of the anaphase egg by virtue of the activity centered in and around the mitotic apparatus.

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BIBLIOGRAPHY

ANDERSON, E. 1968. Oocyte differentiation in the sea urchin, *Arbacia punctulata*, with particular reference to the origin of cortical granules and their participation in the cortical reaction. *J. Cell Biol.* **37**:514.

BAKER, P. C. 1965. Fine structure and morphogenic movements in the gastrula of the tree frog, *Hyla regilla*. *J. Cell Biol.* **24**:95.

BAKER, P. C. and T. E. SCHROEDER. 1967. Cytoplasmic filaments and morphogenic movements in the amphibian neural tube. *Develop. Biol.* **15**:432.

BROWN, D. E. S. 1934. The pressure coefficient of "viscosity" in the eggs of *Arbacia punctulata*. *J. Cell Comp. Physiol.* **5**:335.

CHAMBERS, R. 1938. The physical state of protoplasm with special reference to its surface. *Amer. Natur.* **72**:141.

CHAMBERS, R. and E. L. CHAMBERS. 1961. *Explorations into the Nature of the Living Cell.* Harvard University Press, Cambridge, Mass.

CLONEY, R. A. 1966. Cytoplasmic filaments and cell movements: epidermal cells during ascidian metamorphosis. *J. Ultrastruct. Res.* **14**:300.

DAN, K. and M. K. KOJIMA. 1963. A study on the mechanism of cleavage in the amphibian egg. *J. Exp. Biol.* **40**:7.

DOUGHERTY, W. J. and M. McN. LEE. 1967. Light and electron microscope studies of smooth endoplasmic reticulum in dividing rat hepatic cells. *J. Ultrastruct. Res.* **19**:200.

FAVARD, P. and N. CARASSO. 1965. Mise en évidence d'un reticulum endoplasmique dans le spasmonème de ciliés peritriches. *J. Microsc.* **4**:567.

HARRIS, P. 1967. Structural changes following fertilization in the sea urchin egg. *Exp. Cell Res.* **48**:569.

HARVEY, E. B. 1935. The mitotic figure and cleavage planes in the egg of *Parechinum microtuberculatus*, as influenced by centrifugal force. *Biol. Bull.* **69**:287.

HARVEY, E. B. 1936. Parthenogenetic merogony or cleavage without nuclei in *Arbacia punctulata*. *Biol. Bull.* **71**:101.

HARVEY, E. B. 1956. The American *Arbacia* and other sea urchins. Princeton University Press, Princeton, New Jersey.

HIRAMOTO, Y. 1956. Cell division without mitotic apparatus in sea urchin eggs. *Exp. Cell Res.* **11**:630.

HIRAMOTO, Y. 1957. The thickness of the cortex and the refractive index of the protoplasm in sea urchin eggs. *Embryologia.* **31**:361.

HIRAMOTO, Y. 1965. Further studies on the cell division without mitotic apparatus in the sea urchin eggs. *J. Cell Biol.* **25**:161.

JUST, E. E. 1922. Studies on cell division. I. The effects of dilute sea water on the fertilized eggs of *Echinarachnius parma*. *Amer. J. Physiol.* **61**:505.

KESSEL, R. G. 1963. Electron microscope studies on the origin of annulate lamellae in oocytes of *Necturus*. *J. Cell Biol.* **19**:391.

KESSEL, R. G. 1968. Annulate lamellae. *J. Ultrastruct. Res.* (*Suppl.*) **10**:1.

KINOSHITA, S. and I. YAZAKI. 1967. The behavior and localization of intracellular relaxing systems during cleavage in the sea urchin egg. *Exp. Cell Res.* **47**:449.

KOJIMA, M. K. 1959. Relation between the vitally stained granules and cleavage activity in the sea urchin. *Embryologia.* **4**:191.

MARSLAND, D. 1939. The mechanism of cell division: hydrostatic pressure effects on dividing egg cells. *J. Cell Comp. Physiol.* **13**:15.

MARSLAND, D. 1956. Protoplasmic contractility in relation of cytokinesis. *Pubbl. Sta. Zool.* (*Napoli*). **28**:182.

MARSLAND, D. 1958. Nuclear and cytoplasmic factors in cleavage induction. *Anat. Rec.* **132**:473.

MARSLAND, D. and J. LANDAU. 1954. The mechanisms of cytokinesis: Temperature pressure studies on the cortical gel system in various marine eggs. *J. Exp. Zool.* **125**:507.

MARSLAND, D., A. M. ZIMMERMAN, and W. AUCLAIR. 1960. Cell division: Experimental induction of cleavage furrows in the eggs of *Arbacia punctula*. *Exp. Cell Res.* **21**:179.

MERCER. E. H. and L. WOLPERT. 1958. Electron microscopy of cleaving sea urchin eggs. *Exp. Cell Res.* **14**:629.

MIRSKY, A. E. and S. OSAWA. 1961. The interphase nucleus, *In* The Cell. Brachet, J. and A. E. Mirsky, editors. Academic Press Inc., New York.

MULNARD, J., W. AUCLAIR, and D. MARSLAND. 1959. Metachromasia observed in the living egg of *Arbacia punctulata* and its cytochemical analysis. *J. Embryol. Exp. Morphol.* **7**:223.

RANDALL, J. T. and S. F. JACKSON. 1958. Fine structure and function in *Stentor polymorphus*. *J. Biophys. Biochem. Cytol.* **4**:807.

RAPPAPORT, R. 1966. Experiments concerning the cleavage furrow in invertebrate eggs. *J. Exp. Zool.* **161**:1.

ROBBINS, E. and N. K. GONATAS. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**:429.

SAKAI, H. 1968. Contractile properties of protein threads from sea urchin eggs in relation to cell division. *Intern. Rev. Cytol.* **23**:89.

SCOTT, A. C. 1960. Furrowing in flattened sea urchin eggs. *Biol. Bull.* **119**:246.

STEFFENSEN, D. and J. A. BERGERON. 1959. Autoradiographs of pollen tube nuclei with calcium-45. *J. Biophys. Biochem. Cytol.* **6**:339.

STEPHENS, R. E. and R. E. KANE. 1966. Studies on a major protein from isolated sea urchin egg cortex. *Biol. Bull.* **131**:382.

TILNEY, L. G. and J. R. GIBBINS. 1966. The relation of microtubules to form differentiation of primary mesenchyme cells in *Arbacia* embryos. *J. Cell Biol.* **31**:118A.

VERHEY, C. A. and F. H. MOYER. 1967. Fine structural changes during sea urchin oogenesis. *J. Exp. Zool.* **164**:195.

WEINSTEIN, R. S. and R. R. HERBST. 1964. Electron microscopy of cleavage furrows in sea urchin blastomeres. *J. Cell Biol.* **23**:101A.

WOHLMAN, A. and R. D. ALLEN. 1968. Structural organization associated with pseudopod extension and contraction during cell locomotion in *Difflugia*. *J. Cell Sci.* **3**:105.

WOLPERT, L. 1960. The mechanics and mechanisms of cleavage. *Internat. Rev. Cytol.* **10**:246.

YAQUI, R. and Y. SHIGENAKA. 1963. Electron microscopy of the longitudinal fibrillar bundles and the contractile system in *Spirostomum ambiguum*. *J. Protozool.* **10**:364.

ZIMMERMAN, A. M. and D. MARSLAND. 1956. Induction of premature cleavage furrows in the eggs of *Arbacia punctulata*. *Biol. Bull.* **111**:317.

ZIMMERMAN, S. B., T. H. MURAKAMI, and A. M. ZIMMERMAN. 1968. The effects of selected chemical agents on furrow induction in the eggs of *Arbacia punctulata*. *Biol. Bull.* **134**:356.