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Ultrastructural analysis and chemical inhibition studies were carried out on first cleavage of Mytilus zygotes to determine possible relationships between the mitotic apparatus and the cortex during polar lobe formation.

The stages of mitosis correlated with the appearance of the polar lobe were first determined by light microscopy. The polar lobe does not appear until anaphase of first cleavage. The early polar lobe is symmetrical in position at the vegetal pole, but at late anaphase it shifts position towards the side of the future CD blastomere. During cytokinesis the furrow at the vegetal pole is displaced by the polar lobe resulting in an unequal division.

Ultrastructural study at anaphase shows astral microtubules penetrating the polar lobe. At the animal pole microtubules reach almost to the cortex. When furrowing begins cortical granules move

into the furrow region at both the animal and vegetal poles. An amorphous electron dense area is present just below the plasma membrane in all cell furrows. This region may correspond to a contractile band of microfilaments.

Cell division inhibitors were used to analyze the role of microtubules and microfilaments in Mytilus cleavage. Prior to anaphase, mercaptoethanol, an inhibitor of microtubule function, halts cell division in Mytilus. Before the mitotic apparatus is formed, mercaptoethanol also prevents polar lobe formation. Polar lobe formation can occur in the inhibitor once the mitotic apparatus has begun to form. Cytochalasin B which destroys microfilaments inhibits both polar lobe formation and cytokinesis in Mytilus. Once the polar lobe has appeared, it is resorbed when the cells are placed in cytochalasin.

It is suggested that both microtubules and a contractile band of microfilaments play a role in first cleavage. Initial polar lobe formation could be triggered by microtubules of the mitotic apparatus penetrating the vegetal pole whereas final constriction of the definitive polar lobe and the cleavage furrow may be caused by microfilaments.

An Ultrastructural Study of Early Cleavage in *Mytilus*

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AN ULTRASTRUCTURAL STUDY OF EARLY CLEAVAGE IN *MYTILUS*

I. INTRODUCTION

The cell lineages of many molluscs and annelids are initiated by unequal cleavage divisions. Among these forms the most extreme examples of unequal cell division include the formation of a polar lobe accompanying early cleavage. The polar lobe is a large bleb of cytoplasm set apart during mitosis. It can be as large as the AB blastomere and is connected to the rest of the CD cell by a fine bridge of cytoplasm so that following first cleavage, at trefoil, the egg appears to be partitioned into three cells (Clement, 1971).

The presence of polar lobe cytoplasm is essential for normal development. Removal of the polar lobe results in larvae lacking definite structures (Clement, 1952; Rattenbury and Berg, 1954; Wilson, 1904b). Although evidence suggests that the polar lobe is necessary for normal larval development, the presence of unique structural elements in the polar lobe is variable (Humphreys, 1964; Pucci-Minafra *et al.*, 1969; Reverberi, 1958).

The actual mechanism of polar lobe formation is of interest for understanding the nature of cell division. Little information on this mechanism is available. Early studies on cell lineage were carried out on the developmental role of the polar lobe, but techniques were

not available for determining the way the polar lobe formed. Thus Wilson (1904a) in his extensive study on Dentalium notes the appearance and role of the polar lobe only.

Studies on the more general case of unequal cleavage suggest that the position of the mitotic apparatus determines where the cleavage furrow will form. Lillie (1901) in his study of Unio observed that the spindle is formed in the center of the egg. Just prior to metaphase, it moves towards one side until one aster almost comes in contact with the cortex. This aster then becomes smaller and flattens on the distal side. He concludes that the position of the spindle is controlled by the cytoplasm. Similarly Kawamura's work on insect neuroblast cells (1960a, b) demonstrates that astral rays on the ganglion side diminish, while astral rays in the neuroblast enlarge, causing a shift of the spindle towards the ganglion side. Since the furrow forms midway between the asters, the resulting ganglion cell is smaller than the neuroblast. Until the process of cytokinesis is actually underway, it is possible to affect the location of the furrow by shifting the position of the spindle.

Techniques for isolating the mitotic apparatus have been used to study unequal cleavage. Isolation of the mitotic apparatus of sea urchin micromere divisions proved that unequal cells result from unequal asters, with the larger cell forming from the larger aster (Dan et al., 1952; Dan and Nakajima, 1956). In Spisula the first

cleavage is unequal but no polar lobe is formed. Not only does the AB cell result from a smaller aster, but this smaller aster is flattened on the distal side (Dan et al., 1952; review Dan, 1960). In this case the size difference becomes apparent at metaphase. In Adula which forms a polar lobe, it was similarly found that the asters are initially equal, but as division continues, one aster becomes larger (Harris and Strelou, 1966).

Several reviews on cell division offer possible mechanisms of polar lobe formation. Raven (1958) states that the asters are originally equal in divisions in which a polar lobe is formed. If the polar lobe is removed, the zygote undergoes equal cleavage. From centrifugation studies on Ilyanassa it was found that the polar lobe always formed at the vegetal pole regardless of the cytoplasmic inclusions located there (Morgan, 1933). In the same study it was also found that the isolated polar lobe continues to undergo rhythmic activity related to that of the zygote. Raven concludes that the determining factors of polar lobe formation are cortical rather than cytoplasmic. Wolpert (1960) in his statement of the astral relaxation theory of cell division feels that the polar lobe forms where the membrane relaxes. His theory would imply the presence of microtubules within the polar lobe to cause relaxation of the cell membrane. Mazia (1961) suggests protrusion of the polar lobe is a way of segregating unequal amounts of cytoplasm around equal asters.

Existing theories of cell division imply a role for the mitotic apparatus in determining the site of the cleavage furrow (review Wolpert, 1960). Another line of evidence suggesting the important role of the cell cortex comes from studies by Hiramoto (1956) on sea urchin eggs. Using a micropipette he was able to completely remove the spindle. By mid-anaphase the cleavage plane was established and furrowing could occur without the mitotic apparatus.

Further interest in cytokinesis has led to the description of microfilaments in the cleavage furrow of dividing cells of jellyfish (Schroeder, 1968; Szollosi, 1970) and squid (Arnold, 1969). Furthermore, microfilaments can be induced to form in new positions following pressure centrifugation of egg components in sea urchin (Tilney and Marsland, 1969). The new area of the cortex where the microfilaments are translocated may then furrow. Cytochalasin B which destroys these filaments halts cytokinesis in sea urchin (Schroeder, 1969 and 1972) and HeLa cells (Schroeder, 1970) and stops other developmental processes (review Wessells et al., 1971). Recent experiments using cytochalasin stop polar lobe formation in Ilyanassa (Conrad, 1971; Raff, 1972). No inhibition of polar lobe formation was found using colchicine, an inhibitor of microtubule function.

Ultrastructural observations are necessary to resolve some of the questions concerning the role of the mitotic apparatus and the cortex in unequal cell division. In Mytilus and other polar lobe-forming

genera, fine structural analysis has not been carried out during the whole mitotic cycle. Longo and Anderson (1969a, b) have described early events accompanying fertilization including meiosis through pronuclear association in Mytilus. Other studies on the polar lobe have been confined to trefoil at which stage the nuclei have already re-formed. Humphreys (1962 and 1964) using osmium fixation has studied the ultrastructure of the Mytilus egg before and after fertilization as well as at trefoil. He found no preferential localization of cytoplasm in the polar lobe and showed the polar lobe partitioned from the CD cell by a sheet of vesicles.

The present study was undertaken to observe the ultrastructure of polar lobe formation in Mytilus. Glutaraldehyde-containing fixatives were employed for this purpose. The stages of mitosis associated with the appearance of the polar lobe were first determined by light microscopy. The ultrastructure of the mitotic apparatus, cell cortex, and cleavage furrows was studied. An experimental study was begun utilizing inhibitors of mitotic apparatus function (mercaptoethanol) and cytokinesis (cytochalasin B) in an attempt to show relationships between the mitotic apparatus and the cortex.

II. MATERIALS AND METHODS

Two species of mussel, Mytilus californianus (Conrad) and Mytilus edulis (Linnaeus), were used in this study. Since the gonads of the two species mature at different times of the year, it is possible to obtain gametes during many months of the year. Medium sized M. californianus were collected from late winter to spring at Yaquina Head or Boiler Bay on the central Oregon coast. Large M. edulis were obtained from boat docks in Yaquina Bay, Oregon, during the spring through early fall. All animals were stored in aerated seawater tanks prior to induction of spawning.

Spawning in both species was induced by the following method suggested by Breese (1970). Animals were stored dry overnight in a cold room at 9.5°C and the next morning placed in KCl solution (2g/l seawater) for at least one hour. Sometimes animals spawned in the KCl solution, but these gametes were not used. To prevent contamination, it was found necessary to administer the KCl to individual mussels in separate fingerbowls. The KCl was removed and the mussels were then covered with fresh seawater. Spawning was often immediate under these conditions.

Eggs were passed through course bolting silk to remove debris and were washed three times in filtered seawater of salinity near 25 °/oo for M. edulis and 33 °/oo for M. californianus. Active sperm

were filtered through course bolting silk and a dilute suspension made in the appropriate filtered seawater. A trial fertilization run was always made to check the condition of the gametes. Only batches of eggs showing at least 95% polar body formation were subsequently used in experimental procedures. Fertilization was accomplished by thorough mixing of the gametes at 12°C (M. californianus) or 15°C (M. edulis). Eggs were permitted to develop with constant aeration by stirring and were monitored with the light microscope.

Phase micrographs were taken of living cells as well as material fixed in 3% formalin in seawater. To stage mitotic events, specimens were collected every four minutes beginning one hour after fertilization, fixed in Bouin's fixative, and embedded in paraffin. Sections were stained with Mayer's hematoxylin and eosin.

Several fixatives were used for electron microscopy. Osmolarity and pH were varied in order to determine the best fixative for each species. M. edulis was fixed using the following: (1) Karnofsky's (1965) formaldehyde-glutaraldehyde high osmolarity fixative made with 0.2 M phosphate buffer at pH 6.4 for up to two and three-quarter hours; (2) 3% glutaraldehyde buffered with 0.2 M cacodylate buffer at pH 6.8 for one and one-half hours; and (3) 3% glutaraldehyde buffered with 0.2 M cacodylate at pH 6.8 containing 0.15 M NaCl for two and one-half hours. In this last case subsequent washes and postfixation contained increasing NaCl concentrations as suggested by Schroeder

(1968). M. californianus was fixed using 3% glutaraldehyde buffered with 0.2 M phosphate at pH 7.2 containing 0.45 M sucrose for three hours.

Fixation was carried out at room temperature. In some experiments specimens were collected at 10 and 15 minute intervals and preserved in Karnofsky's fixative so that the time course of division could be studied. The longest fixation time reflects the time for the earliest samples. Initial fixation was followed by the appropriate buffer rinses and postfixation in buffered 1% osmium tetroxide for one hour. The samples were rapidly dehydrated in an ethyl alcohol series and embedded according to the Araldite method of Luft (1961) or the Epon method of Spurr (1969). The embedding media were Araldite 502 or 6005 (Ladd Research Industries, Burlington, Vt.) and Spurr (Polysciences, Inc., Harrington, Pa.).

Sections were cut with a Porter-Blum ultramicrotome. Thick sections were cut to monitor appropriate material and stained with methylene blue and toluidine blue according to Richardson et al. (1960). For electron microscopy thin sections were stained with uranyl acetate for 15 minutes followed by lead citrate for three minutes (Venable and Coggeshall, 1965) and observed with the RCA-EMU 3H or 2D microscopes.

Investigations using the cell division inhibitors 2-mercaptoethanol (Polysciences, Inc., Rydal, Pa.) and cytochalasin B (Imperial

Chemical Industries Limited, England) were carried out on M. edulis zygotes. At five different times corresponding to different mitotic stages of first cleavage, 1 ml of mercaptoethanol was added to 9 ml of egg suspension in Stender dishes giving a final concentration of 0.08 M (Harris and Strelou, 1966; Mazia and Zimmerman, 1958). The effect of the inhibitor on each stage was determined by counting the number of cells undergoing polar lobe formation and cytokinesis out of 200 cells after the seawater controls had completed first cleavage. The results from all experiments were then averaged for each of the five times cells were treated. Both control and treated cells were fixed in 3% formalin in seawater for photography.

Since cytochalasin B (CB) is not water soluble, stock solutions were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg CB/ml DMSO. The stock solution was stored under refrigeration; just before use it was diluted to concentrations of 0.1 μg CB/ml seawater (2.08×10^{-7} M) to 10.0 μg CB/ml seawater (2.08×10^{-5} M). Cytochalasin was administered at two different stages during first division by centrifugation and resuspension of the cells in 10 ml of solution. DMSO controls were made by diluting the DMSO with seawater to give final concentrations of 0.01-1.00% which correspond to the concentrations of DMSO in the diluted CB solutions. Untreated seawater controls were also monitored. The effect of the inhibitor was quantified in the same manner as in the mercaptoethanol experiments and cells were fixed for light microscopy.

III. RESULTS

Fixation

Glutaraldehyde-containing fixatives varying in pH and osmolarity were used to fix zygotes of both species. The addition of 0.45 M sucrose to 3% glutaraldehyde at pH 7.2 gave adequate preservation of the cytoplasm of M. californianus. If the sucrose was omitted much swelling of membranes occurred. Some swelling occurred, however, between the bounding membranes of the mitochondria. The structures of the mitotic apparatus were well preserved with this fixative, but the tubular nature of the microtubules was somewhat distorted. This damage to microtubules could be caused by the addition of sucrose to the fixation medium, since sucrose has been used by Hiramoto (1965) to dissolve the mitotic apparatus.

M. edulis was treated with several different fixatives, none of which preserved all cell structures well. Karnofsky's fixative at pH 6.4 gave variable results perhaps dependent upon the seasonal condition of the eggs. In some fixation attempts the cytoplasm was well preserved whereas extraction occurred in others. The centrosphere of the mitotic apparatus often contained swollen vesicles and empty areas. Chromosomes were preserved adequately with Karnofsky's fixative.

The other fixatives used on M. edulis included glutaraldehyde buffered with cacodylate at pH 6.8 both with and without the addition of 0.15 M NaCl. If the salt was omitted, membrane-bound systems including endoplasmic reticulum, mitochondria, and cortical granules were swollen. This fixative did preserve an amorphous-appearing substance around cleavage furrows. When salt was added, the cytoplasm was well preserved with no swelling of membranes, but microtubule structure was damaged and the chromosomes somewhat extracted.

Timetable of Cleavage Events

Starting one hour after fertilization, batches of eggs were fixed every four minutes and embedded in paraffin for sectioning and staining in order to determine the sequence of mitotic events. The mitotic events could then be related to the morphological events observed on living material (Table 1). The timetable of cleavage events given applies to M. edulis, although the same correlation of cell shape and mitotic stages also exists in M. californianus. Phase micrographs of living and fixed material demonstrate the cell shape changes associated with first cleavage in both species (Figures 1-10).

At the time of pronuclear association, the basic shape of the zygote is round (Figure 1). A distinct ruffling of the vitelline coat at the vegetal pole is apparent at this time. The zygote remains

Table 1. Timetable of cleavage events in M. edulis determined from light microscopy. Cells were grown at 15°C in 25 °/oo S.

Minutes after fertilization	Stage
15	First Polar Body
35	Second Polar Body
45	Migration of Pronuclei
60-65	Pronuclear Association Breakdown of Pronuclei Aster Formation
70	Metaphase
75	Anaphase
80	Cleavage Beginning Reconstitution of Nuclei
85	Trefoil
90	Polar Lobe Incorporated into CD Cell; Cleavage Completed
120	Second Cleavage

symmetrical in shape through metaphase. Concurrent with anaphase there is a definite bulge at the vegetal pole which constitutes the beginning of the formation of the first polar lobe (Figures 2 and 7). In order to observe the mitotic apparatus it was necessary to compress the cells slightly. In Figure 7 the mitotic apparatus does not occupy a central position due to the presence of the polar lobe. At first the polar lobe is symmetrically positioned at the vegetal pole, but at late anaphase it shifts towards the side of the future CD cell (Figures 3 and 8).

During late anaphase to early telophase cytokinesis begins (Figure 9). As cleavage continues the furrow at the vegetal pole is obviously displaced by the polar lobe, resulting in an unequal cleavage (Figures 4 and 10). The mitotic apparatus appears somewhat eccentric in position, perhaps contributing to the formation of a larger CD cell. Further furrowing between the polar lobe and the CD cell results in the trefoil appearance (Figure 5). At trefoil, the furrow almost completely separates the cytoplasm of the polar lobe from that of the CD blastomere. This condition is present for only a few minutes until the temporary furrow separating the polar lobe and CD cell disappears and the cytoplasmic contents of the two are united.

Several aspects of the trefoil stage can be seen in a low power electron micrograph (Figure 11). Due to the plane of sectioning the polar lobe appears as an entirely separate entity although part of its

connection to the CD cell may be the bleb of cytoplasm to the lower left of the micrograph. The chromosomes have decondensed and the nuclei have re-formed. The nuclei are confined to the blastomeres proper and are never found in the polar lobe. The furrowing process has not completely separated the AB from the CD cell nor is it possible to distinguish the cells since the polar lobe connection is not present. One of the polar bodies can be seen at the animal pole. Direct observation reveals no unique components in the polar lobe cytoplasm. Lipid, yolk, and mitochondria along with cortical granules in the cortex are found in all portions of this early two-cell stage. Microvilli with their adhering amorphous vitelline coat surround all portions of the zygote, but the microvilli have lost their connection with the oolemma at furrow regions.

Following trefoil, the polar lobe is incorporated into the CD blastomere. The cells flatten along their adjoining surfaces and cleavage is complete (Figure 6).

Structure of the Mitotic Apparatus

The mitotic apparatus is composed of asters, centrioles, and the spindle with its associated chromosomes. The asters of the mitotic apparatus are quite large with each aster measuring up to 30 μ in diameter. From observations on both living and fixed material, neither aster has appeared definitely larger. Figure 7 shows

displacement of the asters away from the center of the cell towards the animal pole once the polar lobe has formed. Low power electron micrographs (Figures 12-14) demonstrate the proximity of the microtubules to the cortex at the animal pole. In this region microtubules come to within 5 μ of the oolemma. Long microtubules also penetrate into the polar lobe at the vegetal pole (Figures 12 and 13).

The asters are comprised of radiating microtubules surrounding a less organized central region, the centrosphere (Figures 14 and 16). The centrosphere contains a pair of centrioles (Figure 15); Golgi bodies and multivesicular bodies may also be present (Figures 16 and 18). Large cell inclusions, among them lipid, yolk, and mitochondria, are excluded from the centrosphere and often appear at its periphery ordered in rows between the microtubules.

Microtubule orientation within the centrosphere is less organized than in the astral rays where bundles of microtubules radiate together (Figure 16). In material preserved with Karnofsky's fixative, microtubules are absent in the astral center where much swelling and extraction seems to have occurred (Figures 14 and 15). In more favorably preserved material a vesicular component is present in the asters and has the appearance of endoplasmic retiulum (Figures 17 and 18). At anaphase, rod-shaped dense structures are occasionally seen near the astral center (Figures 17 and 18). These structures appear membrane-bound, but their exact nature is not known.

Chromosome Structure and Nucleus Formation

It was not the purpose of this study to observe chromosome structure, but several incidental observations were made. The appearance of the chromosomes varied with the fixation method employed, although a species difference may also be present. The chromosomes of M. californianus fixed with glutaraldehyde containing sucrose are extremely electron dense and stand out in marked contrast to the surrounding cytoplasm (Figures 17-19). In M. edulis preserved with Karnofsky's fixative the chromosomes appear less dense. When fixed with glutaraldehyde containing salt, which is known to extract chromosomal material at neutral pH (Harris, 1962b), the chromosomes have a similar density to the cytoplasmic background (Figure 20).

Figure 19 shows an anaphase subtelocentric chromosome. The attached microtubules indicate the kinetochore region. Two rows of material less electron dense than the chromosome proper are evident, but a substructure cannot be seen. In adjacent sections, only one less dense region occurred at this location.

It has already been mentioned that much membranous material is found within the asters. At late anaphase to early telophase as the chromosomes approach the poles, the endoplasmic reticulum is present between the chromosomes. At this time double membranous

elements containing annuli adhere to the chromosomes. As telophase progresses these individual chromosomal vesicles coalesce, eventually reconstituting a single nucleus in each blastomere (Figures 21 and 22).

The Cortex

The cortex of the fertilized egg of Mytilus contains a layer of cortical granules. Unlike the activation process in some invertebrate eggs, the cortical granules are not discharged at fertilization. Only occasionally is a granule seen which appears to be discharging its contents. The cortical region containing these granules is approximately 2 μ wide. In M. edulis the cortical granules consist of two components: a granular electron dense component and an array of parallel tubules as described by Humphreys (1967). The entire structure is surrounded by a unit membrane (Figure 23). Cortical granules measure up to 1 μ in diameter, and the tubular structures extend another 1 μ towards the oolemma.

The cortex of M. californianus contains spindle-shaped electron dense bodies surrounded by a unit membrane (Figure 24). Another type of granule less dense in appearance and containing a substructure is also present. The parallel array of tubules so evident in M. edulis has not been observed in this species. No other unique components have been observed in the cortex, although mitochondria and

endoplasmic reticulum may be found here as well.

Regularly spaced microvilli embedded in an amorphous vitelline coat project from the surface of the egg (Figure 23). At the tips of the microvilli fibrous elements arise and these constitute the jelly coat.

Furrow Formation

The cell furrow bisects the zygote between the asters of the mitotic apparatus. As the cleavage furrow advances during telophase, an area of increased electron density appears just below the plasma membrane (Figure 25). This area is not found in the cortex prior to furrowing. In all the micrographs presented this region appears amorphous probably a result of the plane of sectioning. In more advanced furrows later in telophase this region of electron opacity is confined to the advancing portion of the furrow. Cortical granules are present along the margins of the furrow (Figure 26) as well as at the advancing tip. Prior to furrow formation, cortical granules are seldom seen within the cell interior. The microvilli in furrow regions are widely separated from the cell surface.

As the furrow advances at the animal pole, a prominent furrow also begins to form at the vegetal pole. This furrow was present earlier when it delimited the polar lobe at anaphase, but it becomes more pronounced at telophase. Along with the furrow formed in the

CD cell, it separates the polar lobe at trefoil. For convenience these last furrow components will be called polar lobe furrows. Figure 27 shows an advanced furrow at the vegetal pole. Along much of its length and especially at the tip an amorphous fuzzy area can be seen. Close inspections reveal this area to be granular or almost dot-like in appearance. Its structure is identical to that of the cleavage furrow advancing from the animal pole fixed under the same conditions. Cortical granules are again in close proximity to the furrow.

Figure 28 shows a high magnification micrograph of the polar lobe furrow within the CD cell. The electron dense area is confined to part of the furrow only as in other cell furrows, but the dense band is narrow relative to that in Figure 27. Differences in the appearance of this region may be due to the short life of this furrow.

Experimental Work

Investigations were begun using two inhibitors of cell division, mercaptoethanol and cytochalasin B, to study possible interactions of the mitotic apparatus and the cell cortex. All experiments were carried out on M. edulis zygotes.

1. Mercaptoethanol

Cells were transferred into 0.08 M mercaptoethanol at 25, 45, 60, 65, and 75 minutes after fertilization. The corresponding mitotic

stages were completion of meiosis I, pronuclear migration, pronuclear association, metaphase, and anaphase. The results were monitored at 90 minutes after fertilization when the controls had completed first cleavage, and the cells were fixed in 3% formalin in seawater. Table 2 summarizes the results of the mercaptoethanol experiments.

Table 2. The effect of 2-mercaptoethanol on first cleavage in M. edulis. Cells were transferred into 0.08 M mercaptoethanol at the times indicated and remained in the inhibitor until monitored at the time the controls had completed first cleavage (90 minutes).

	Time Treated	% First Cleavage
Mitotic Stage	Minutes after fertilization	
1. First Polar Body	25	0
2. Pronuclear Migration	45	0 (68% "pear-shaped")
3. Pronuclear Association	60	0 (95% "pear-shaped")
4. Metaphase	65-70	2 (88% polar lobe)
5. Anaphase Polar Lobe Formation	75	95
6. Controls-no treatment	(90)	99

Approximately half of the cells treated at 25 minutes after fertilization went on to form the second polar body, but subsequent development was halted. The cells remained round in appearance and gave no indication of polar lobe formation (Figure 29).

Treatment of cells at 45 minutes after fertilization resulted in a "pear-shaped" appearance in 68% of the cells. Similarly, cells

blocked at the time of pronuclear association when the mitotic apparatus is beginning to form showed 92% "pear-shaped". The "pear-shaped" appearance is a very early indication of polar lobe formation, but this reaction was weak and the "pear-shaped" form disappeared in the fixative (Figure 30).

Cells treated at metaphase showed 88% polar lobe formation, and these polar lobes remained more obvious after fixation (Figure 31). Once the polar lobe formed in these mercaptoethanol-treated cells, it remained evident and was not resorbed while the cells were in the inhibitor. A small percentage of the metaphase-treated cells underwent abortive cleavages.

When cells were treated at anaphase after the polar lobe had already formed, 99% of them underwent first cleavage (Figure 32). The blastomeres produced in the mercaptoethanol were round and widely separated from one another whereas control blastomeres are flattened along their adjoining surfaces (Figure 33).

Two hours after fertilization the cells treated at 25 minutes were washed twice in fresh seawater and allowed to recover for one hour. "Normal cleavage" preceded by polar lobe formation occurred in 71% of the cells (Figure 34). By 24 hours these cells had formed trochophore larvae.

2. Cytochalasin B

Cells were transferred into cytochalasin at metaphase before polar lobe formation and at anaphase after polar lobe formation. They were monitored at 90 minutes after fertilization when the controls had completed first cleavage and fixed at two hours when the controls had undergone second cleavage. Table 3 summarizes the results of the cytochalasin experiments.

At metaphase, addition of cytochalasin at concentrations of 0.1, 1.0, and 10.0 μg/ml completely prevented both polar lobe formation and cytokinesis. Figure 35 shows a cell treated with 1.0 μg CB/ml. Although slightly ovoid, the cell lacks the characteristic "pear-shaped" appearance of the polar lobe. DMSO controls of 0.01%, 0.10%, and 1.00% corresponding to the concentration of DMSO found in the cytochalasin gave high percentages of cytokinesis preceded by polar lobe formation (Figure 36).

When cytochalasin was added at anaphase after the polar lobe had already formed, the polar lobe was resorbed and cytokinesis prevented (Figure 37). The DMSO controls underwent the first two cleavages normally (Figure 38). None of the DMSO controls differed in appearance or time of division from the untreated seawater controls (Figure 39).

At both times during mitosis that cells were treated, karyokinesis continued even though cytokinesis was prevented. The

Table 3. The effect of cytochalasin B on first cleavage in M. edulis. Cells were transferred into cytochalasin made from a cytochalasin-DMSO stock solution at the times indicated and were observed at the time the controls had completed first cleavage (90 minutes). DMSO controls at the same concentration contained in the cytochalasin were also run.

Time Treated			
Mitotic Stage	Minutes after fertilization	Concentration (μgCB/ml) (% DMSO)	% First Cleavage
		A. Cytochalasin	
1. Metaphase	65-70	0.1	0
		1.0	0
		10.0	0
		controls-no treatment	96
2. Anaphase	75	0.1	0
		1.0	0
		10.0	0
		controls-no treatment	97
		B. DMSO Controls	
1. Metaphase	65-70	0.01	98
		0.10	96
		1.00	91
2. Anaphase	75	0.01	98
		0.10	96
		1.00	97

resulting cells were found to contain from two to eight nuclei when fixed almost three hours after fertilization. Figure 40 shows a cytochalasin-treated cell with two nuclei that was fixed two hours after fertilization. The vitelline coat appears ruffled at the animal pole. Distortion of the cell surface was more obvious in cells treated with 10.0 μg CB/ml, and the inhibition was not reversible. Cells treated with 1.0 μg CB/ml can recover if washed twice in seawater.

IV. DISCUSSION

In the present study the earliest indication of the polar lobe coincided with anaphase of first cleavage. Few other observations have been made correlating the appearance of the polar lobe with mitotic events. In his study of Ilyanassa, Clement (review 1971) documents the formation of three separate polar lobes prior to the completion of first cleavage. The first two are associated with the formation of the two polar bodies, and the third appears at late prophase just before the pronuclear membranes disappear. This polar lobe then enlarges to prominence during metaphase. Other stages at which formation of the polar lobe is said to occur include pronuclear association in Mytilus (Field, 1922), "about metaphase" in Chaetopterus (Lillie, 1906), and late anaphase or early telophase in Dentalium (Wilson, 1904a).

Neither light nor electron micrographs show either aster of the mitotic apparatus to be larger. This possibility cannot be eliminated, however, and the presence of microtubules within the polar lobe would suggest that late in anaphase one of the future blastomeres may contain more mitotic apparatus material. The possible enlargement of one aster may coincide with the shift of the polar lobe to the side of the future CD blastomere. Isolation of asters of unequal size from Adula supports this idea (Harris and Strelou, 1966).

The observations on the structure of the mitotic apparatus are in accord with studies on other dividing marine eggs. The mitotic apparatus is composed of microtubules, membranes, and centrioles. The presence of microtubules in the mitotic apparatus was first demonstrated by Harris (1962b) in Strongylocentrotus using osmium fixation in combination with low pH or the divalent cations of seawater. This method appears optimal for fixation of microtubules but not necessarily for preservation of other cytoplasmic structures. The orienting of non-mitotic components including yolk and multivesicular bodies by microtubules has been documented by Rebhun (1960) in Spisula, and similar arrangements are seen in Mytilus.

The membranous component of the mitotic apparatus has also been described by Harris (1961) in sea urchin material. These vesicles or lamellae were found at metaphase and anaphase at the periphery of the spindle region. At late anaphase the membranous elements condense on the surface of individual chromosomes to form a double membrane containing pores. This process appears the same in Mytilus at first cleavage and has been documented during the polar body divisions as well (Longo and Anderson, 1969a).

In mammalian cell lines Robbins and Gonatas (1964) found vesicles present at prophase which disappear by mid-metaphase. They also report that the Golgi apparatus disappears at metaphase and does not reappear until telophase. In Mytilus the Golgi apparatus was an

obvious component during anaphase, especially in the region of the poles. In a further study on HeLa cells Robbins and Jentzsch (1969) found that as the cell approaches anaphase, the pericentriolar microtubules fragment and become encapsulated by a unit membrane. The authors hypothesize that endoplasmic reticulum may store depolymerized microtubule protein. Some of the structures they identify look similar to the rod-shaped dense structures seen in the asters of Mytilus at anaphase. A relationship between microtubules and membranes has been advanced on morphological grounds by Sandborn et al. (1965) and chemical composition by Mazia and Ruby (1968), but such ideas are highly speculative.

The observations on the kinetochore differ somewhat from those reported previously on dividing marine eggs. In both Strongylocentrotus (Harris, 1965) and Urechis (Luykx, 1965) fixed in osmium, the kinetochore appears more electron dense than the chromosome proper. In M. californianus fixed in glutaraldehyde-sucrose, the kinetochore appears less dense than the chromosomes. Less dense kinetochore regions are often found in mammalian cell strains fixed with glutaraldehyde (review Brinkley and Stubblefield, 1970). In M. edulis the kinetochore appears to have the same density as the chromosome. This difference may result from fixation or perhaps reflect a species characteristic.

The most important new observation presented here involves penetration of microtubules into the polar lobe. In light of this observation the role of mercaptoethanol as an inhibitor of microtubule function provides insight into the process of polar lobe formation. On the fine structural level mercaptoethanol is known to disrupt the structure of the mitotic apparatus and to affect the position of centrioles (Harris, 1962a). Since the effect is known to be primarily on the mitotic apparatus, mercaptoethanol can be used as a tool for separating the process of mitosis from cytokinesis. A possible effect on the furrowing capacity of cells cannot be overlooked, however (Zimmerman, 1964; Zimmerman et al., 1968).

The mitotic apparatus may function in polar lobe formation. If the mercaptoethanol is applied early before any indication of the mitotic apparatus, no polar lobe is formed. Treatment of cells once the pronuclei are evident at any time up to pronuclear association stopped mitosis but did not entirely prevent polar lobe formation although the indication was very slight. By metaphase, when the asters are fully developed, a more definite polar lobe formed in mercaptoethanol-treated cells. Moreover, the polar lobe was not resorbed while metcaptoethanol was administered. Once the cells had entered anaphase and formed a polar lobe, they were able to undergo cleavage in the inhibitor indicative of a "point of no return" (Mazia et al., 1960).

Experiments blocking the cells at a very early stage would indicate that polar lobe formation is not solely a cortical reaction programmed to occur at a given time. The results of a similar study on Ilyanassa (Conrad, 1971) using colchicine as an inhibitor of microtubule function demonstrate that the polar lobe can form in all concentrations that inhibit mitosis, but the time of application is not given. Raff (1972) also found that the polar lobe could form in colchicine. In both of these studies the polar lobe was not resorbed in colchicine-inhibited zygotes, and the authors hypothesize that microtubules play a role in this function. The reverse hypothesis could be just as valid. If microtubules play a role in resorption of the polar lobe, they could also initiate its formation. It must be recalled that Ilyanassa forms three polar lobes prior to first cleavage. The idea of the mitotic apparatus of the meiotic divisions affecting polar lobe formation in this organism may not be realistic. In Ilyanassa, however, there is a successive increase in the prominence of each polar lobe with the third being the most obvious and long-lived.

The cortex, including the structure of the cortical granules, has been well described for M. edulis by Humphreys (1967). The present observations on this species are in accord with his work. Some discrepancy, however, exists concerning the cortex of M. californianus. Since this is the first ultrastructural study on this species, these differences will be discussed here. The spindle-shaped structures

in the cortex were originally considered to be mitochondria in a light microscopic study by Worley (1944). Recently it has been suggested that Worley might have actually been observing the microvilli surrounding the egg (Reverberi, 1971). Humphreys (1967), however, states that Worley was observing the cortical granules. Upon re-examination of Worley's light micrographs, it is obvious that he is indicating cortical structures. These structures are considered cortical granules in the present study. The lack of the parallel arrays of tubular material may indicate a species characteristic.

The observation that the polar lobe can be prevented from forming by treatment with cytochalasin links this process to the more general one of cytokinesis. Once formed, the polar lobe is resorbed when treated with cytochalasin. Similar studies have recently been reported on the effect of cytochalasin on polar lobe formation in Ilyanassa (Conrad, 1971; Raff, 1972). In both of these studies polar lobe formation was inhibited by cytochalasin, but only Conrad found resorption of the polar lobe once it had formed. Conrad also found that the third polar lobe could still be inhibited by concentrations that permitted cytokinesis. Similarly the second polar lobe could be prevented at concentrations in which the third polar lobe could still form. Differences may exist in the degree of furrowing required for each of these processes.

The concentrations of cytochalasin found to inhibit polar lobe formation differ in Ilyanassa and Mytilus. Raff (1972) found inhibition from 5.0-10.0 μg CB/ml, whereas in Mytilus inhibition occurs from 0.1-10.0 μg CB/ml and possibly at lower concentrations. The inhibition is not reversible, however, in cells treated with 10.0 μg/ml. The large size of the egg of Ilyanassa or differences in the handling of the cytochalasin could account for the concentration differences.

The ultrastructural observations presented here are the first implicating cortical microfilaments in the formation of the polar lobe. These structures are also present in the cleavage furrow proper. The only other observation on the fine structure of polar lobe formation in Mytilus or other organisms was the presence of a row of vesicles demonstrated by Humphreys (1964) to separate the polar lobe from the CD cell. It should be recalled that his material was fixed with osmium which is known to cause vesicularization of membranes (Franzini-Armstrong and Porter, 1964). In Mytilus zygotes fixed with osmium, this author has observed the cleavage furrow as well as the polar lobe furrows to appear vesicular.

Location of cortical granules in the cleavage furrow of Mytilus supports the idea of a contractile process in furrowing. Studies on the movements of pigment granules during cleavage in Hemicentrotus and Arbacia demonstrate the presence of granules along the entire surface of the two blastomeres as soon as cytokinesis is completed (Dan and

Dan, 1940; Dan, 1960). Following cleavage the granules slowly retreat from the furrow area so that they come to line the free margins of the blastomeres at the time the cells bulge out from one another. Dan feels the movement of the granules is a reflection of the movement of the cortex as a whole in which the granules are embedded. More recently it has been shown that cytochalasin treatment, which halts cytokinesis in Arbacia, results in a concentrated band of pigment granules arrested in the presumptive furrow cortex (Belanger and Rustad, 1972).

It is interesting that cortical granules can be detected in the polar lobe furrows as well as in the cleavage furrow because Dan and Dan (1942) have claimed two different processes are at work during cytokinesis and polar lobe formation in Ilyanassa. By following kaolin particles attached to the egg surface they identified an initial shrinkage phase followed by stretching at the animal pole. Events at the vegetal pole were attributed to a stretching of the cortex. The authors feel the furrow separating the AB cell from the CD cell behaves much like the furrow at the animal pole but the initial shrinkage phase is lacking. Since cortical granules and a dense layer which may contain microfilaments are found in all cell furrows in Mytilus, it is difficult to believe that these furrowing processes differ in mechanism.

Evidence linking the mitotic apparatus and cortex in cell division has been presented by Sakai (review 1968) who has isolated a protein

from the cortex of sea urchin eggs that forms artificial contractile fibers. Sakai hypothesizes that contraction of this protein is triggered by an interaction with microtubule protein of the spindle whereby a transition from S-H to S-S occurs during contraction in the cortex. The reverse reaction occurs in the mitotic apparatus explaining why no net change is found within the cell. The maximum amount of sulfhydryl groups in the protein thread occurs at metaphase and anaphase.

Belief in Sakai's idea depends upon chemical or direct contact between the mitotic apparatus and the cortex. Many investigators have claimed physical contact of the mitotic apparatus and the cortex (Conklin, 1917; Kawamura, 1960a), but this contact has never been shown on the ultrastructural level. In the present study the microtubules extend within 5 μ of the oolemma. The exact diameter of the cortex is not known. Functionally the cortex is considered to be the distance housing the cortical granules which doesn't move during centrifugation. Estimates of the width of the cortex based upon centrifugation and measurement of sea urchin eggs vary from 1.5 μ (Mitchison, 1956) to 6 μ (Marsland and Landau, 1954).

V. SUMMARY

Observations on the ultrastructure and chemical inhibition of first cleavage in Mytilus imply a role for microtubules and microfilaments in unequal cleavage in which a polar lobe is formed.

1. The polar lobe in Mytilus does not form until anaphase of first cleavage. Initially the polar lobe is symmetrically positioned at the vegetal pole, but at late anaphase the polar lobe shifts position towards the side of the future CD blastomere.
2. The asters of the mitotic apparatus are composed of microtubules, membranes, and centrioles. The astral microtubules definitely penetrate the polar lobe, and it is suggested that one aster may be larger.
3. Cell furrows form between the asters of the mitotic apparatus with the vegetal pole furrow displaced to one side by the polar lobe. At the time of furrow formation, the cortical granules move into the furrows indicative of a contractile process. During cytokinesis and polar lobe formation, an area of increased electron density is present below the plasma membrane in all furrows. This area may correspond to a band of microfilaments which functions as a contractile ring.
4. Mercaptoethanol treatment prior to aster formation stops mitosis as well as polar lobe formation. Once the mitotic apparatus

begins to form, polar lobe formation can occur in the inhibitor. The degree of polar lobe formation is greater the later the cells are placed in mercaptoethanol. Polar lobes do not retract while in the mercaptoethanol. It is suggested that the mitotic apparatus exerts a causative influence on polar lobe formation.

5. Cytochalasin B treatment of cells prior to polar lobe formation prevents the appearance of the polar lobe and cytokinesis as well. If the polar lobe formed before addition of cytochalasin, it was resorbed and cytokinesis halted. These experiments suggest that the polar lobe forms by a contractile process similar to cytokinesis.

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APPENDIX

Figure 1. Round living M. edulis cell at the time of pronuclear association. Note the ruffling of the vitelline coat at the vegetal pole (arrows).

Figure 2. Polar lobe formation in M. edulis cell preserved in 3% formalin in seawater. The polar lobe is essentially symmetrical in position.

Figure 3. Asymmetrical polar lobe in M. edulis cell preserved in 3% formalin in seawater. The indentation (arrow) indicates where the furrow will separate the blastomeres.

Figure 4. Cytokinesis in M. edulis cell preserved in 3% formalin in seawater. The blastomeres are separated from one another by a furrow.

Figure 5. Trefoil in M. edulis cell preserved in 3% formalin in seawater. A furrow separates the polar lobe from the CD cell.

Figure 6. Cleavage completed in M. edulis cell preserved in 3% formalin in seawater.

Phase contrast. 1, 370 X. 7.3 μ = 1 cm.

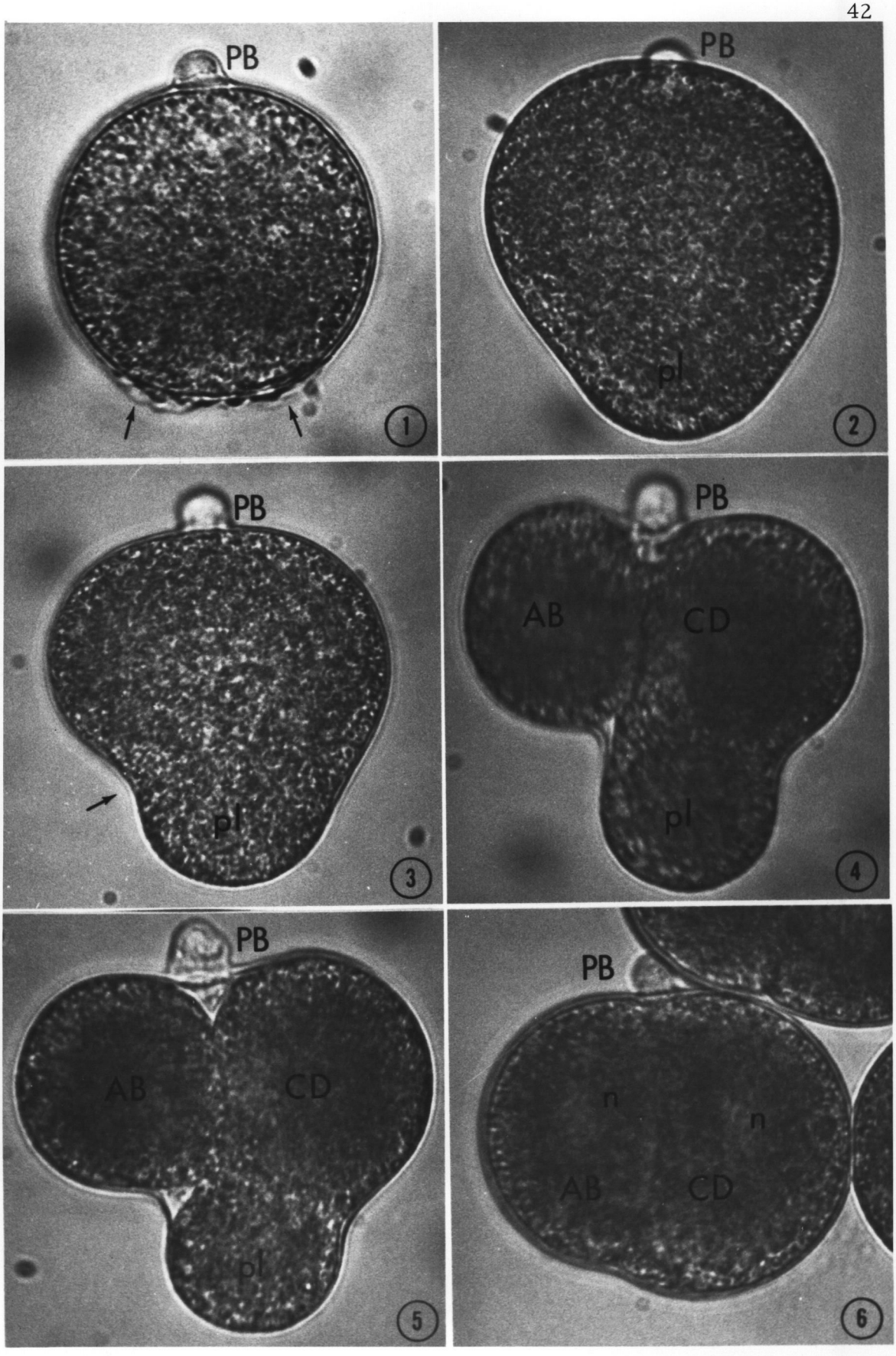
AB - AB blastomere

CD - CD blastomere

n - nucleus

PB - polar body

pl - polar lobe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1
PB
pl
2
PB
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3
PB
AB
CD
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4
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AB
CD
pl
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AB
CD
6

Figure 7. Polar lobe formation in living M. californianus cell. Note the lighter astral regions (a) towards the animal pole.

Figure 8. Asymmetrical polar lobe in living M. californianus cell. The polar lobe has shifted towards the side of the future CD blastomere.

Figure 9. Early cytokinesis in living M. californianus cell. A furrow has appeared at the animal pole and a more definite constriction (arrow) separates the polar lobe from the AB cell.

Figure 10. Cytokinesis in living M. californianus cell. Note the position of the furrow between the asters.

Phase contrast. 1,370 X. 7.3 μ = 1 cm.

AB = AB blastomere

CD - CD blastomere

PB - polar body

pl - polar lobe

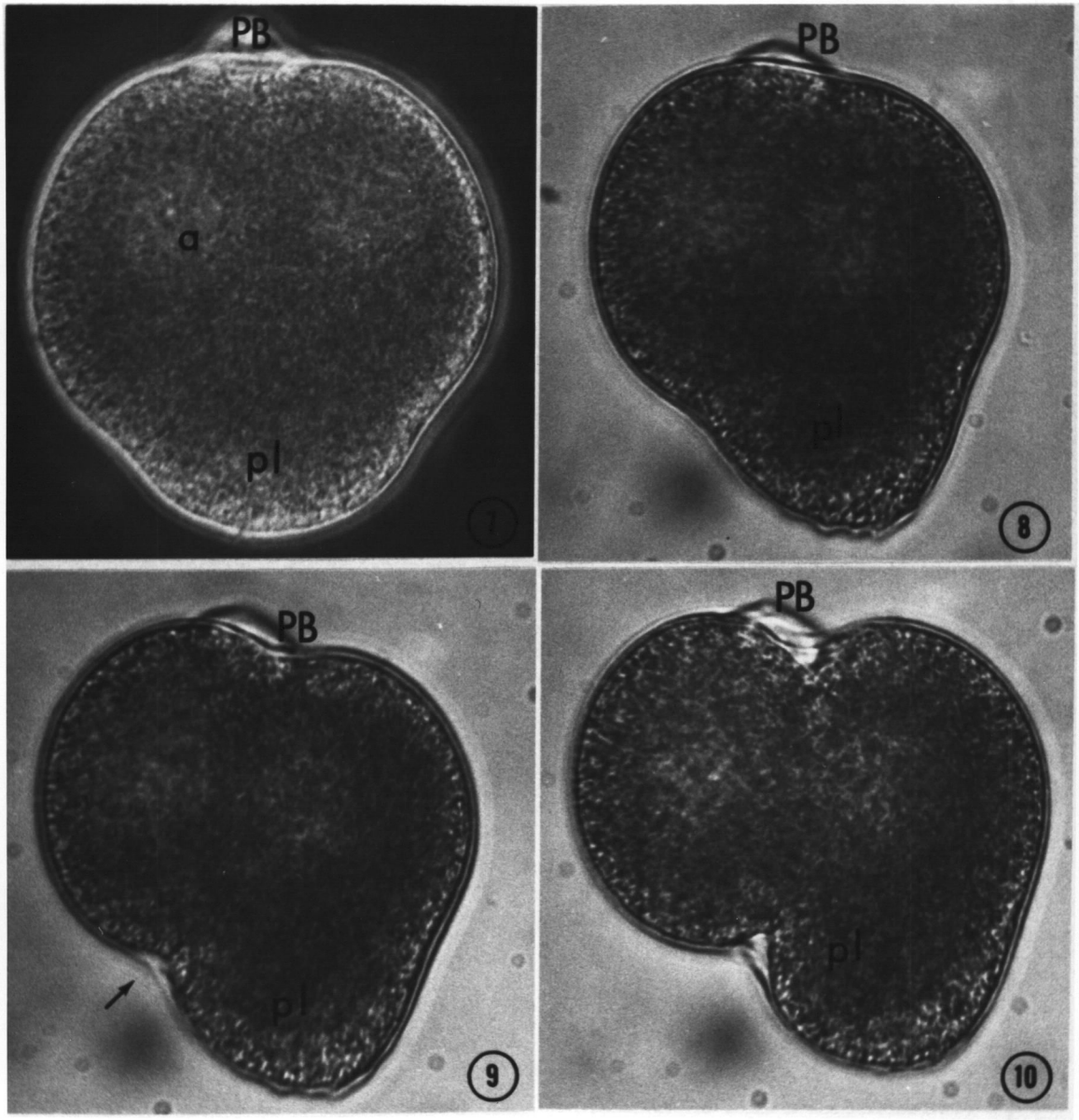
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Figure 11. Trefoil in M. edulis cell preserved in Karnofsky's fixative at pH 6.4. 2,910 X. 3.44 μ = 1 cm.

l - lipid droplet

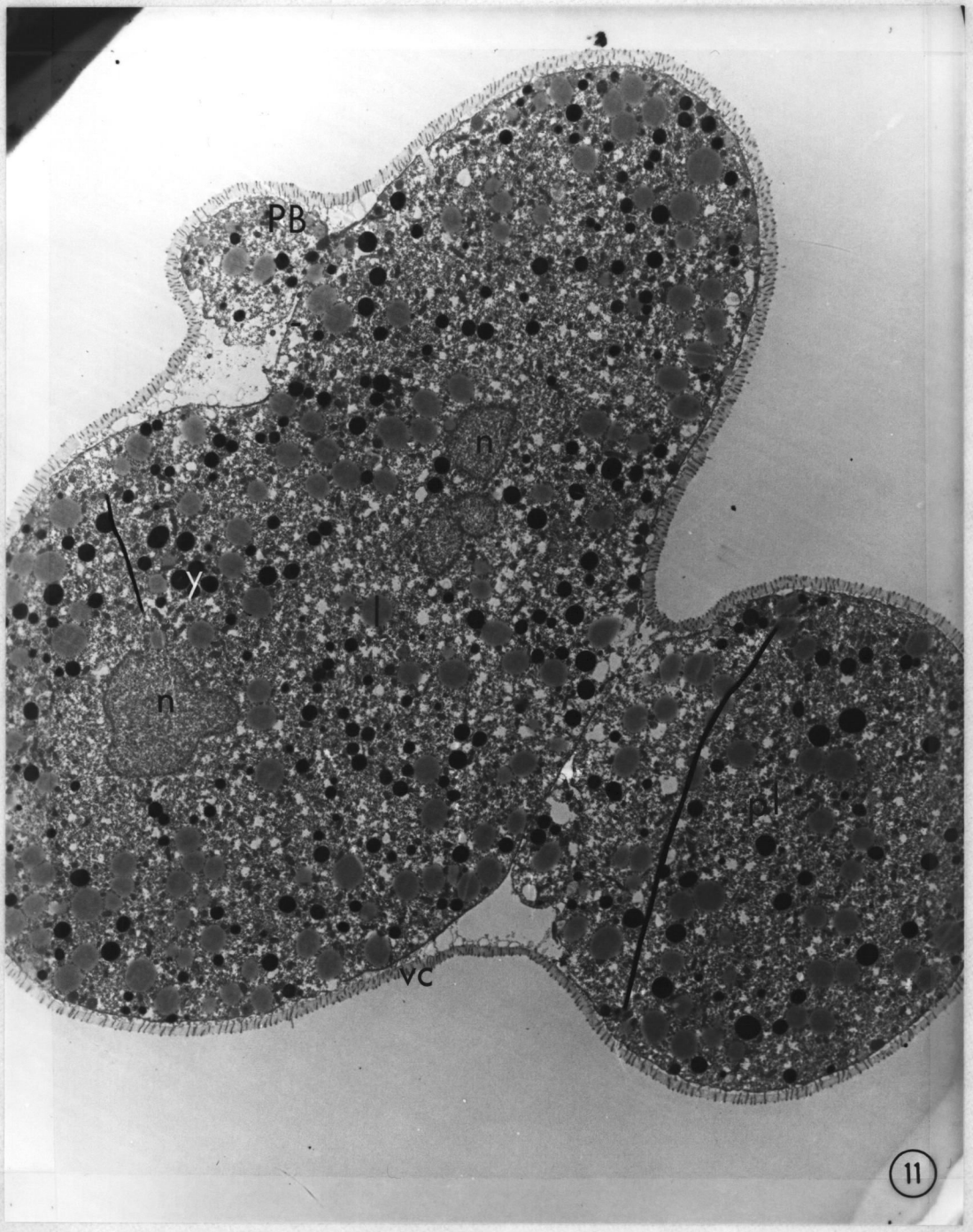
n - nucleus

PB - polar body

pl - polar lobe

vc - vitelline coat

y - yolk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11

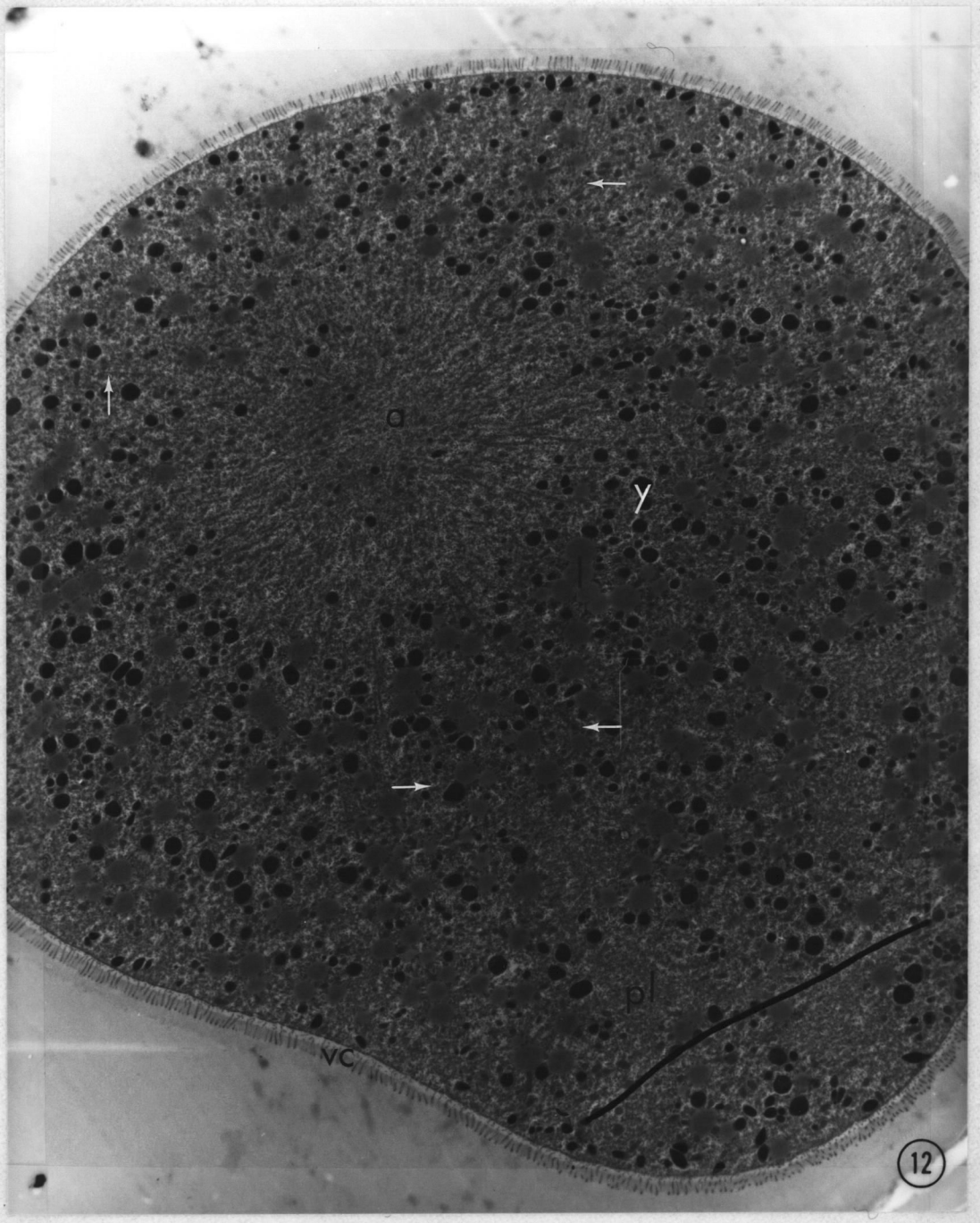
Figure 12. Aster (a) and polar lobe (pl) of anaphase M. californianus cell fixed in glutaraldehyde-sucrose at pH 7.2
2,910 X. 3.44 μ = 1 cm.

l - lipid droplet

vc - vitelline coat

y - yolk

arrows indicate microtubules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12

Figure 13. Aster (a) and polar lobe (pl) of anaphase M. californianus cell showing the long distance penetrated by microtubules (arrows). Glutaraldehyde-sucrose fixation at pH 7.2. 2,610 X. 3.83 μ = 1 cm.

ch - chromosome

l - lipid droplet

n - nucleus

PB - polar body

vc - vitelline coat

y - yolk

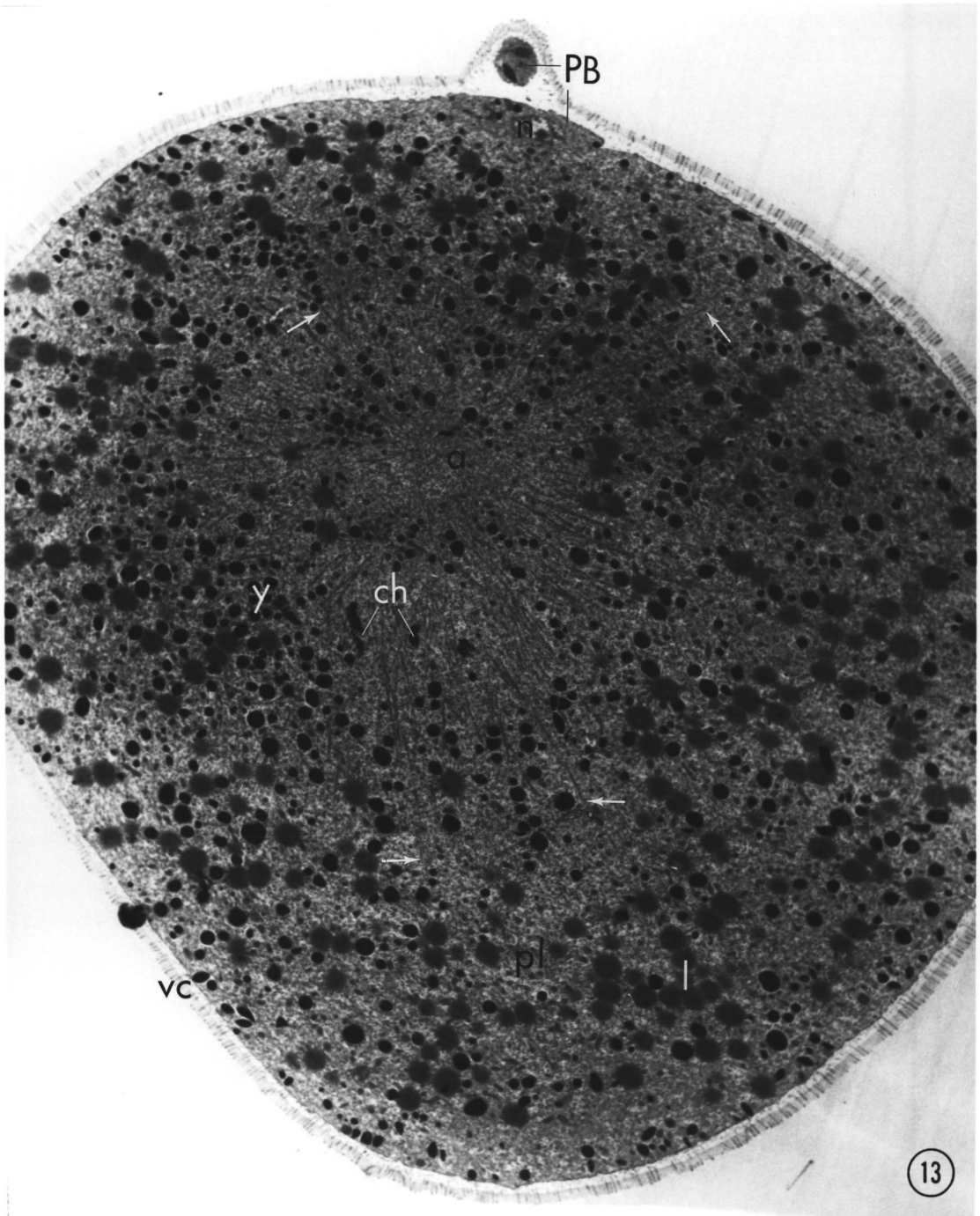
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Figure 14. Asters of anaphase M. edulis cell showing the centrosphere (cs) surrounded by bundles of astral microtubules (mt). Some extraction and enlargement of vesicles (arrows) has occurred within the centrosphere. Karnofsky's fixative at pH 6.4. 4,620 X. 2.17 μ = 1 cm.

Figure 15. Centriole pair (c) of anaphase M. edulis cell preserved in Karnofsky's fixative at pH 6.4. 6,000 X. 1.67 μ = 1 cm.

ch - chromosome

l - lipid

y - yolk

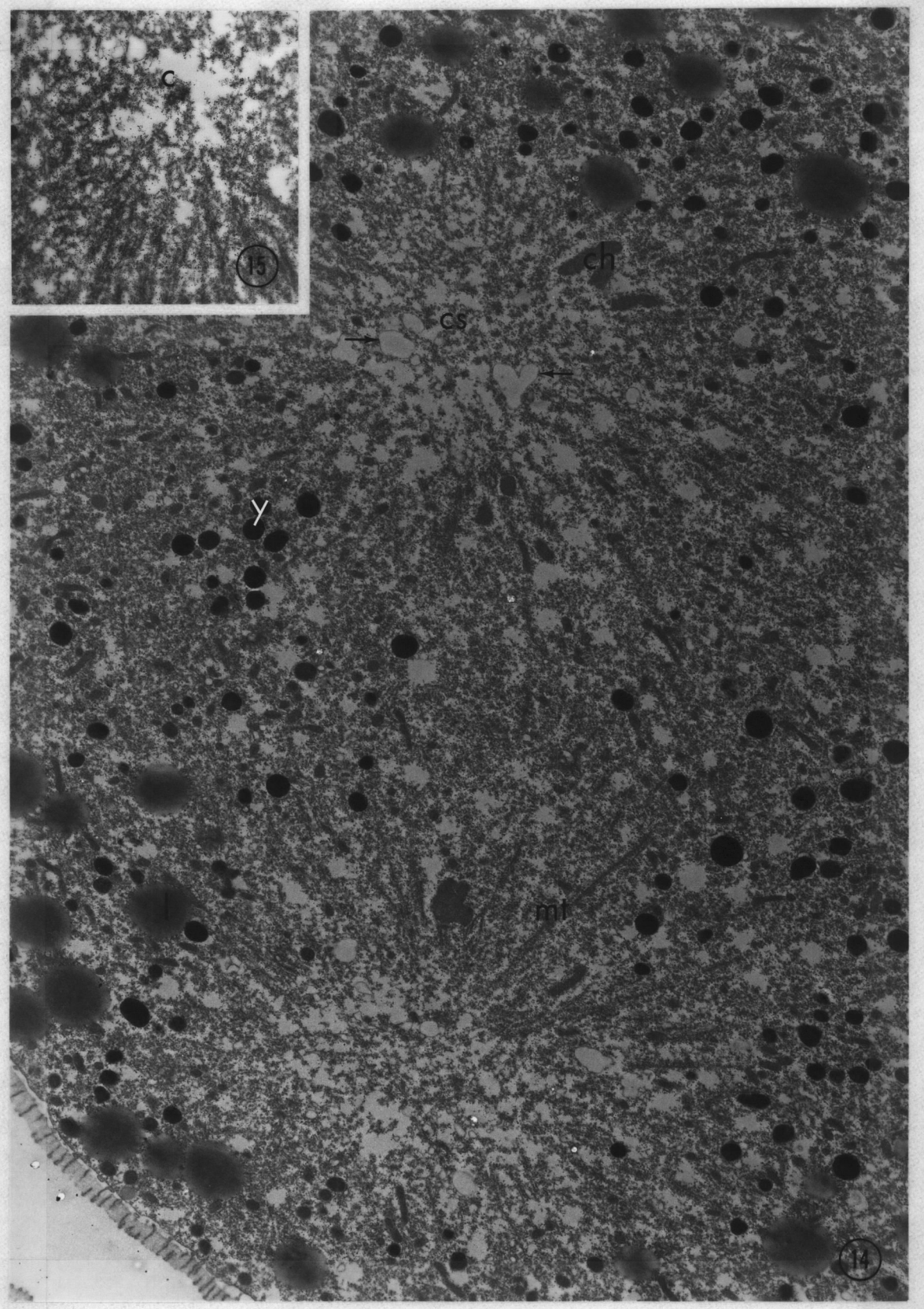
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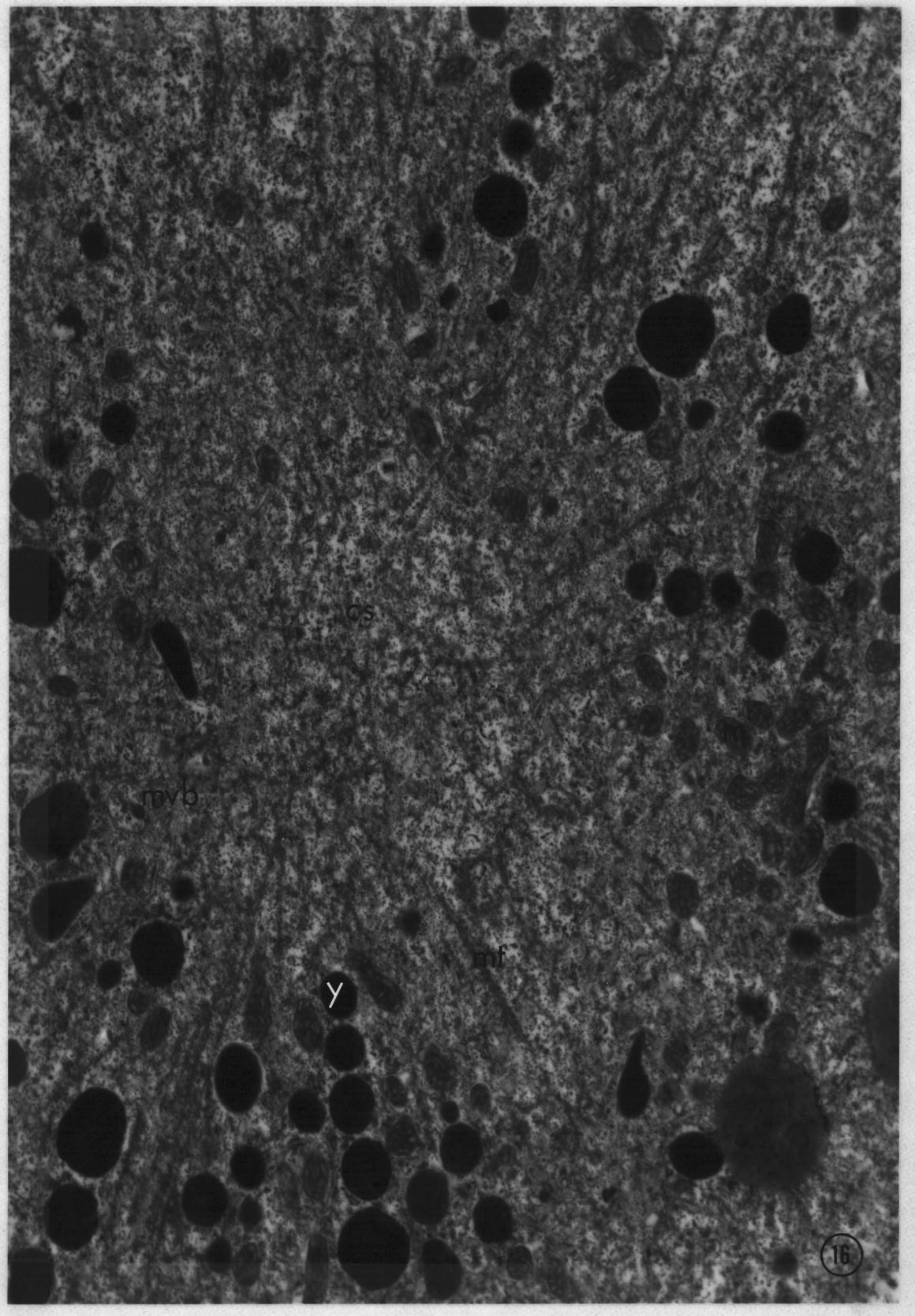
Figure 16. Enlargement of the aster of the anaphase M. californianus cell shown in Figure 13. The mitochondria (m) and yolk (y) are aligned by microtubules (mt). Glutaraldehyde-sucrose fixation at pH 7.2. 15,800 X. 0.64 μ = 1 cm.

cs - centrosphere

l - lipid droplet

mvb - multivesicular body


cs
myb
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y
16

Figure 17. Aster of anaphase M. californianus cell fixed in glutaraldehyde-sucrose at pH 7.2. 31,500 X. 0.32 μ = 1 cm.

ch - chromosome

mt - microtubule

r - rod-shaped dense structure

v - vesicle

y - yolk

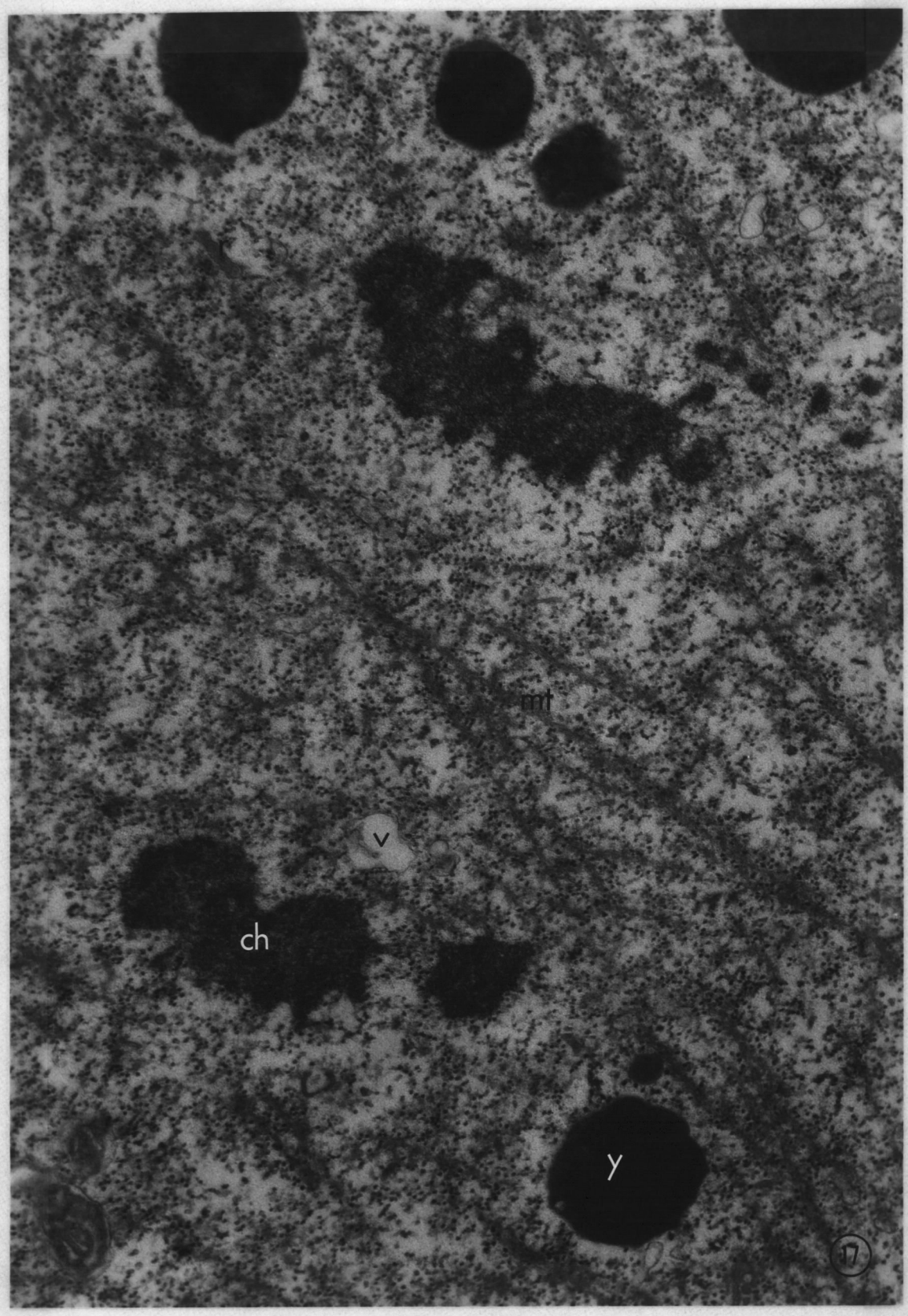

mt
v
ch
y
17

Figure 18. Aster of anaphase M. californianus cell fixed in glutaraldehyde-sucrose at pH 7.2. 31,500 X. 0.32 μ = 1 cm.

Figure 19. Subtelocentric chromosome (ch) of anaphase M. californianus cell fixed in glutaraldehyde-sucrose at pH 7.2. 31,500 X. 0.32 μ = 1 cm.

G - Golgi complex

k - kinetochore

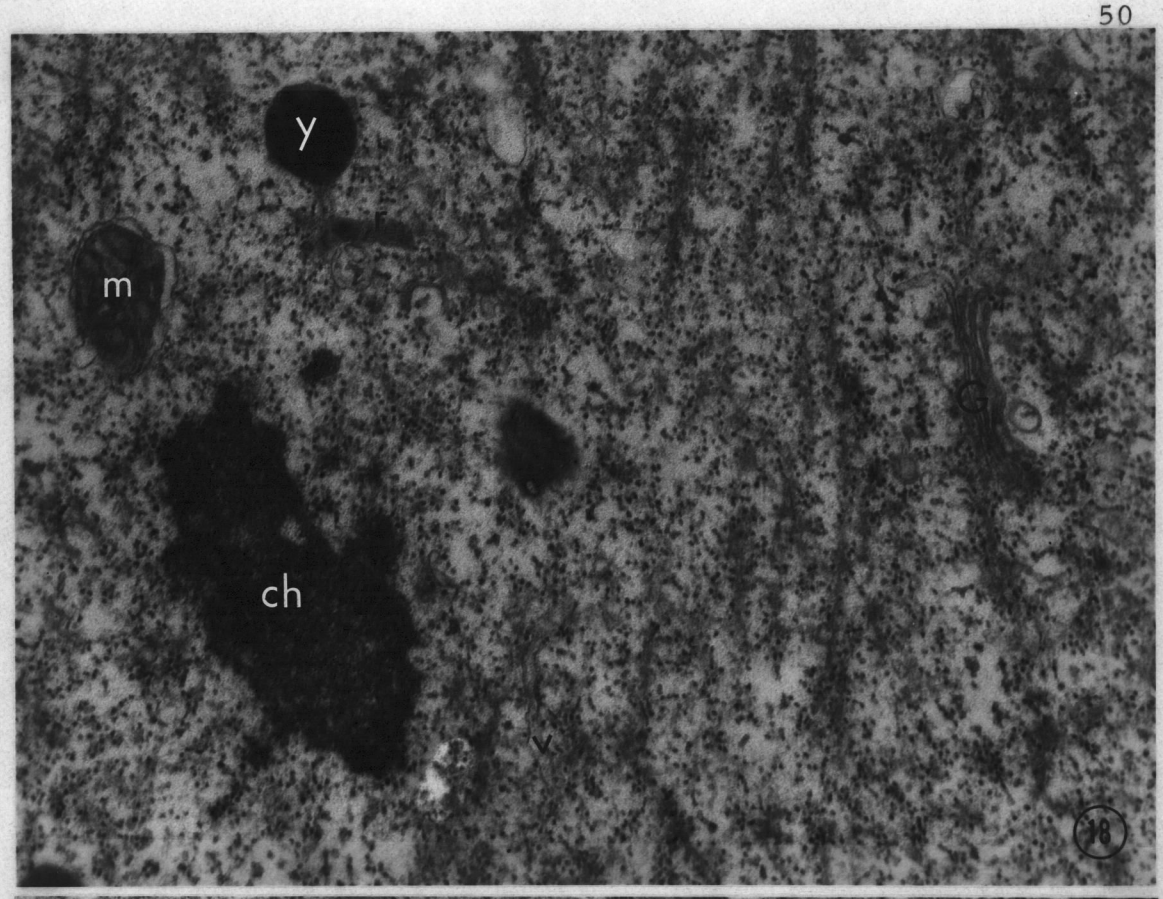
m - mitochondrion

mt - microtubules

r - rod-shaped dense structure

v - vesicle

y - yolk


y
m
ch
v
18

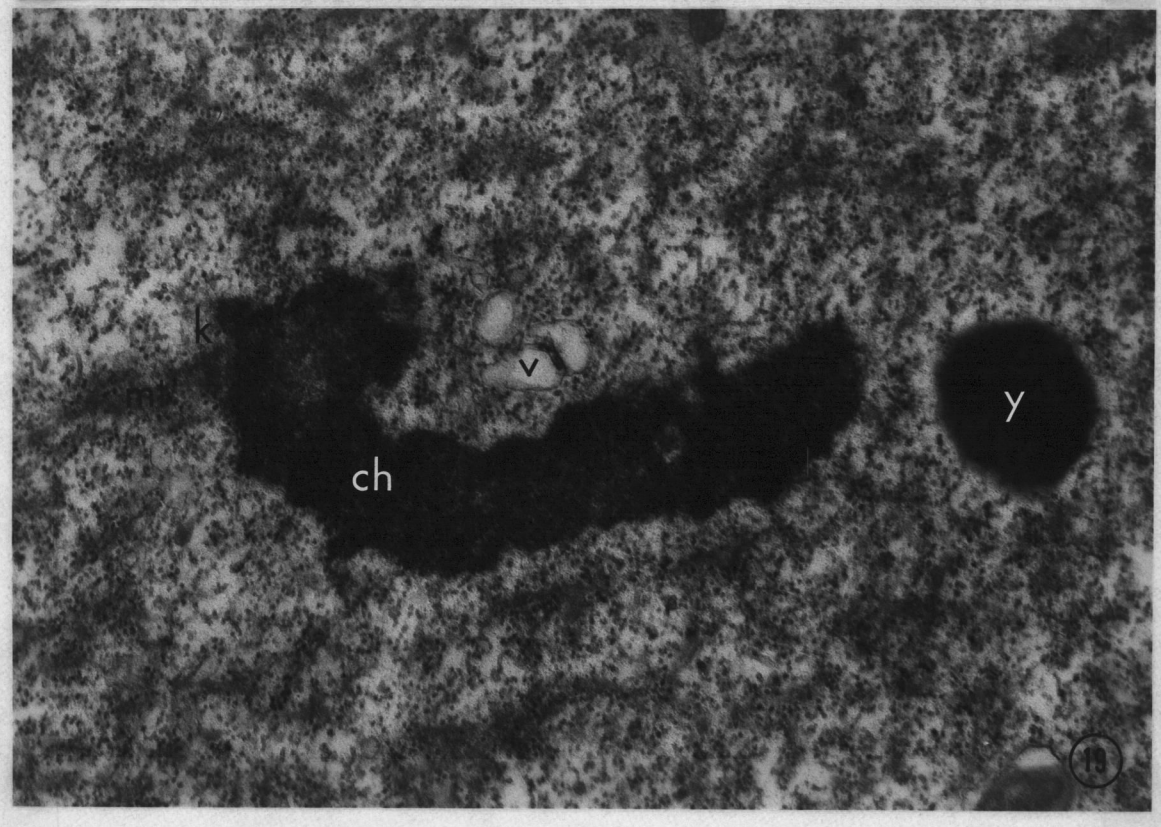

k
v
y
ch
19

Figure 20. Membranes surrounding chromosomes at telophase in M. edulis cell. Separate membranes resembling endoplasmic reticulum (er) are also present. Glutaraldehyde-salt fixation at pH 6.8. 14,900 X. 0.67 μ = 1 cm.

Figure 21. Nuclear reconstitution at telophase in M. edulis cell fixed in glutaraldehyde-salt at pH 6.8. 14,900 X. 0.67 μ = 1 cm.

c - centriole

ch - chromosome

cv - chromosomal vesicle

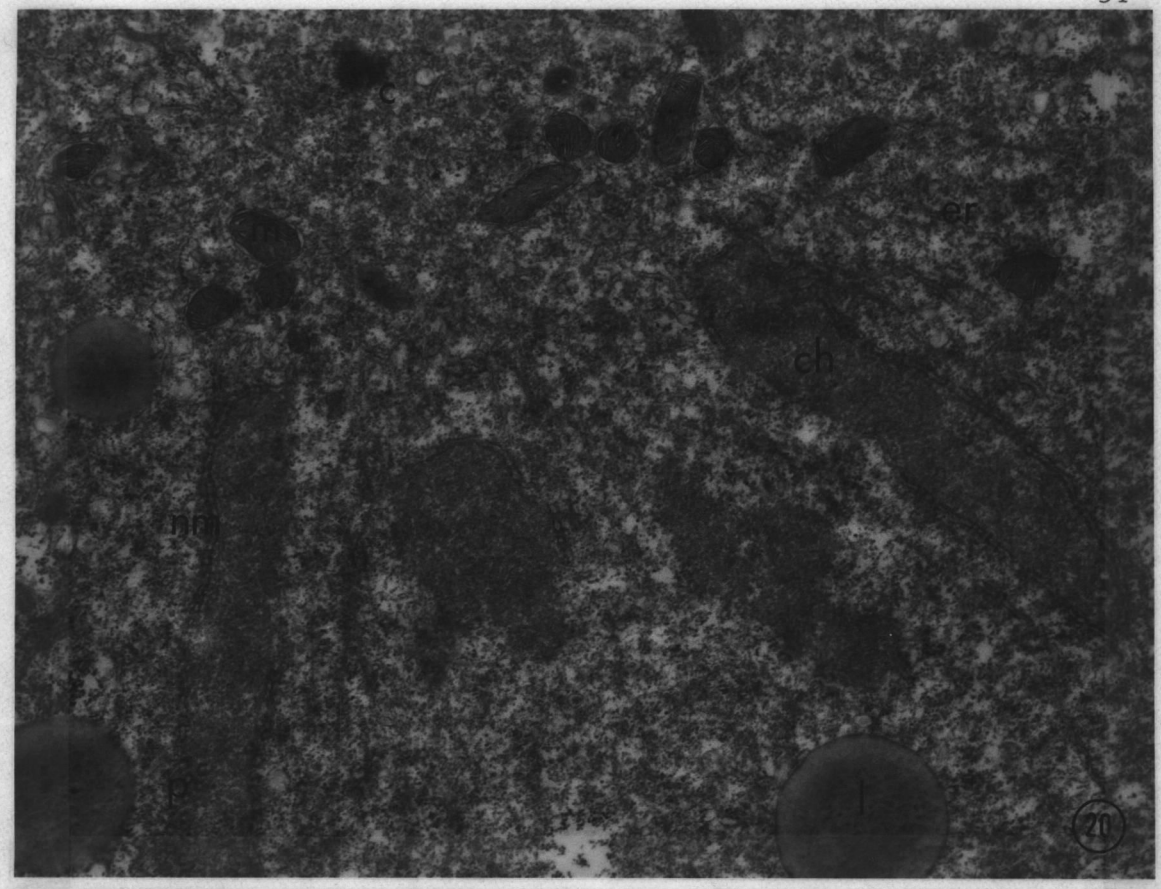
l - lipid droplet

m - mitochondrion

nm - nuclear membrane

p - nuclear pore

r - rod-shaped dense structure


er
ch
nm
l
20

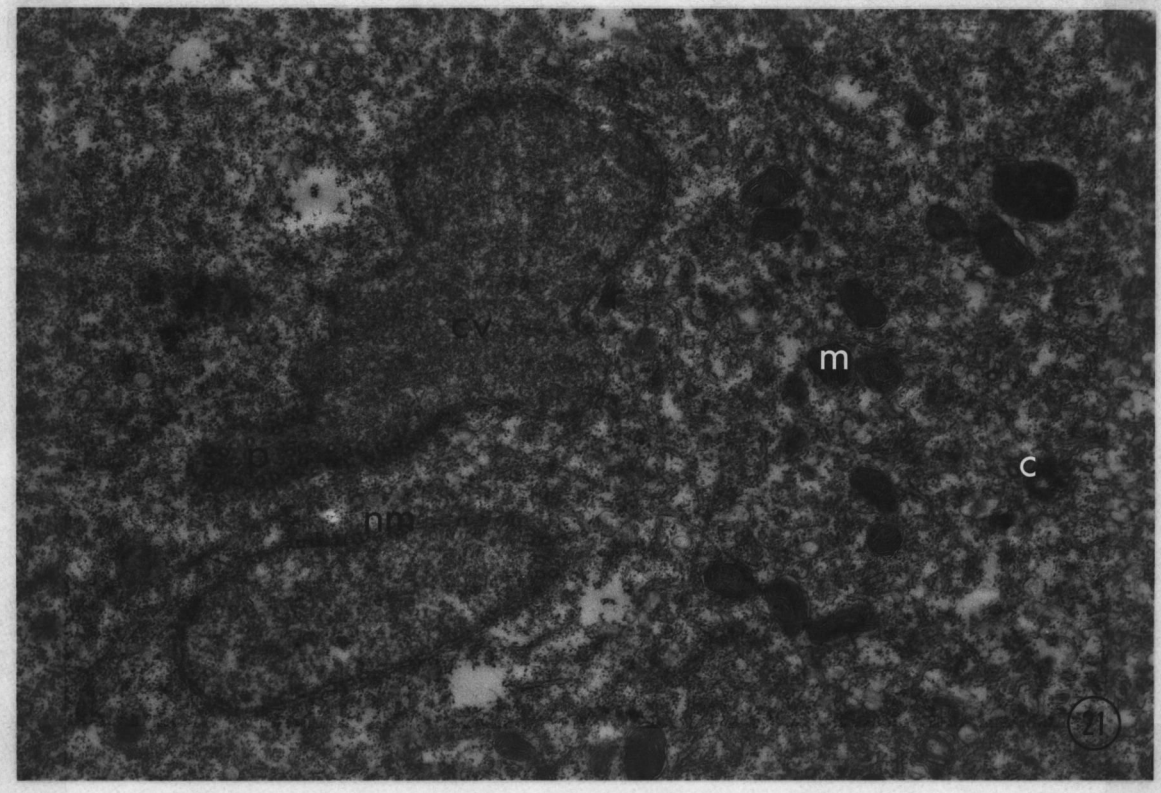

cv
m
c
nm
21

Figure 22. Coalescence of chromosomal vesicles (cv) at late telophase in M. edulis cell preserved in Karnofsky's fixative at pH 6.4. Micrograph taken with the RCA EMU-2d microscope. 21,200 X. 0.47 μ = 1 cm.

l - lipid droplet

m - mitochondrion

nm - nuclear membrane


CV
23

Figure 23. Cortex of M. edulis cell prior to cytokinesis. Note the layer of cortical granules (cg) beneath the oolemma. Glutaraldehyde-salt fixation at pH 6.8. 22,800 X. 0.44 μ = 1 cm.

Figure 24. Cortex of M. californianus cell prior to cytokinesis fixed in glutaraldehyde-sucrose at pH 7.2. 22,800 X. 0.44 μ = 1 cm.

cg - cortical granule

d - electron dense component of cortical granule

er - endoplasmic reticulum

g - less dense granule

jc - jelly coat

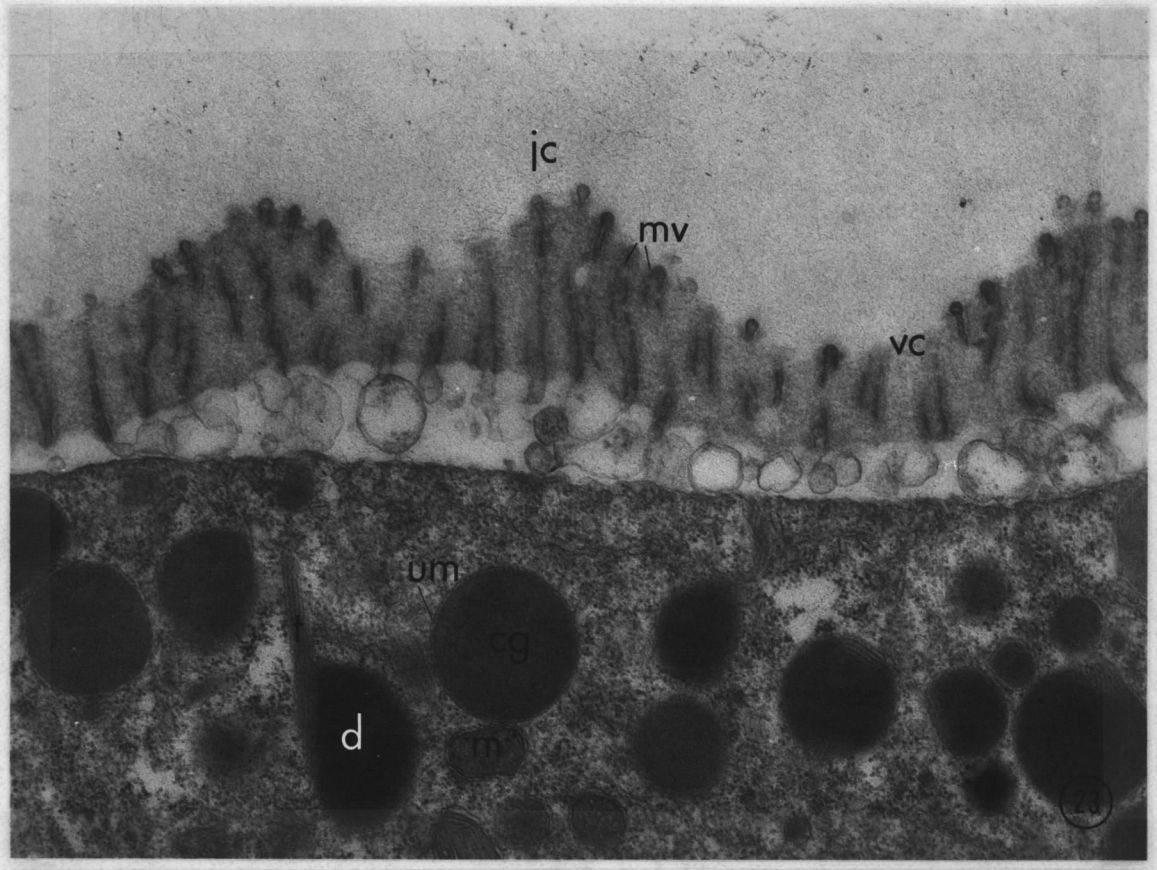
m - mitochondrion

mv - microvillus

t - tubular component of cortical granule

um - unit membrane

vc - vitelline coat


jc
mv
vc
um
cg
d
m
24

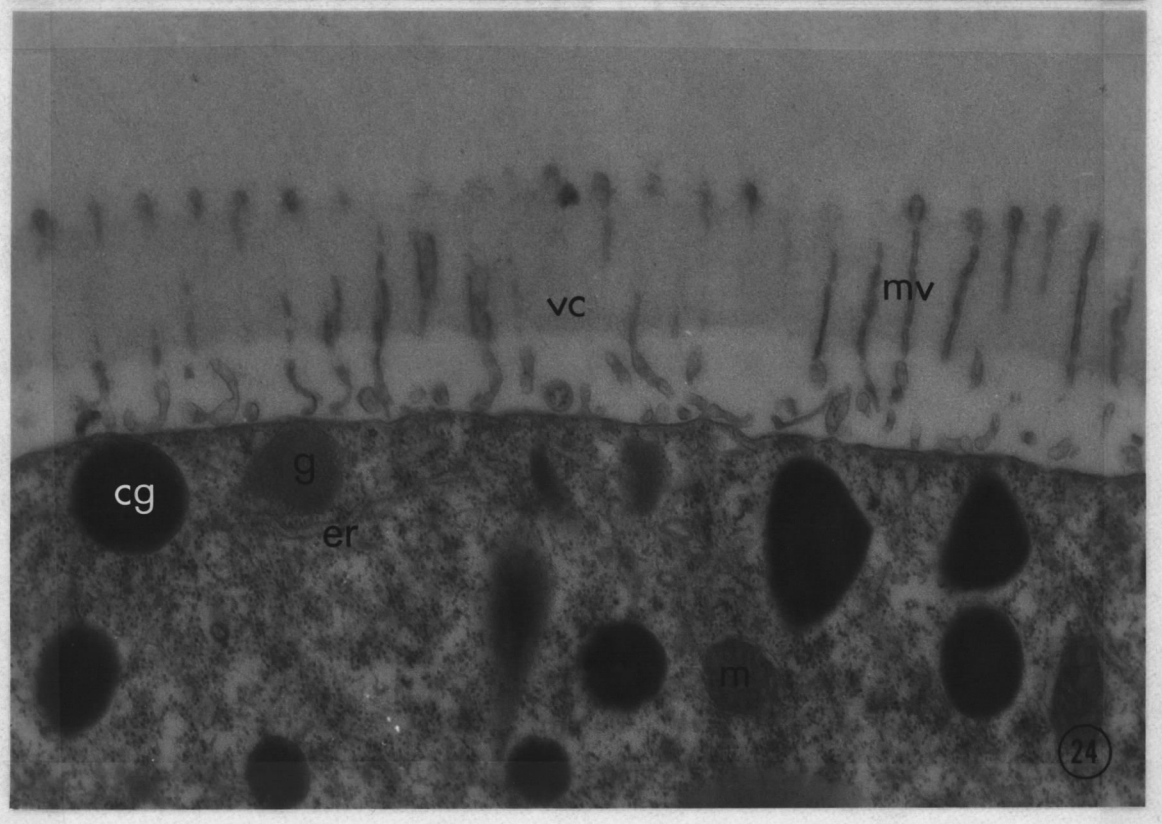

vc
mv
cg
g
er
m
24

Figure 25. Early furrow formation at the animal pole showing an electron dense region (arrows) along the advancing furrow. Cortical granules (cg) are present in the furrow region. M. edulis cell fixed in glutaraldehyde-salt at pH 6.8. 16,350 X. 0.62 μ = 1 cm.

er - endoplasmic reticulum

jc - jelly coat

l - lipid droplet

m - mitochondrion

mv - microvillus

vc - vitelline coat

y - yolk

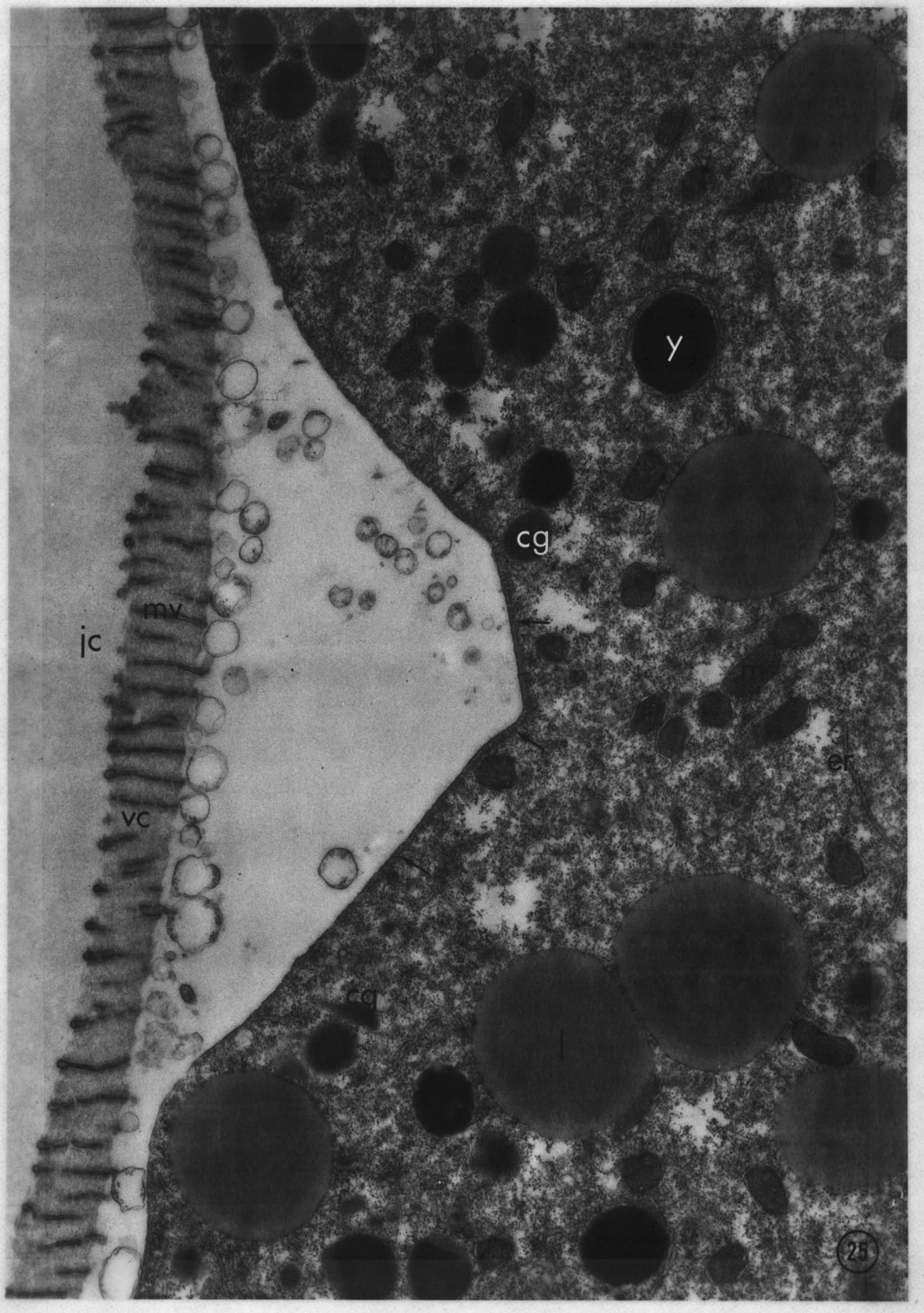

y
cg
mv
jc
vc
er
cg
l
25

Figure 26. Furrow margins near animal pole showing many cortical granules (cg) associated with the furrow. M. edulis cell preserved in Karnofsky's fixative at pH 6.4. 60,700 X. 0.17 μ = 1 cm.

m - mitochondrion

y - yolk

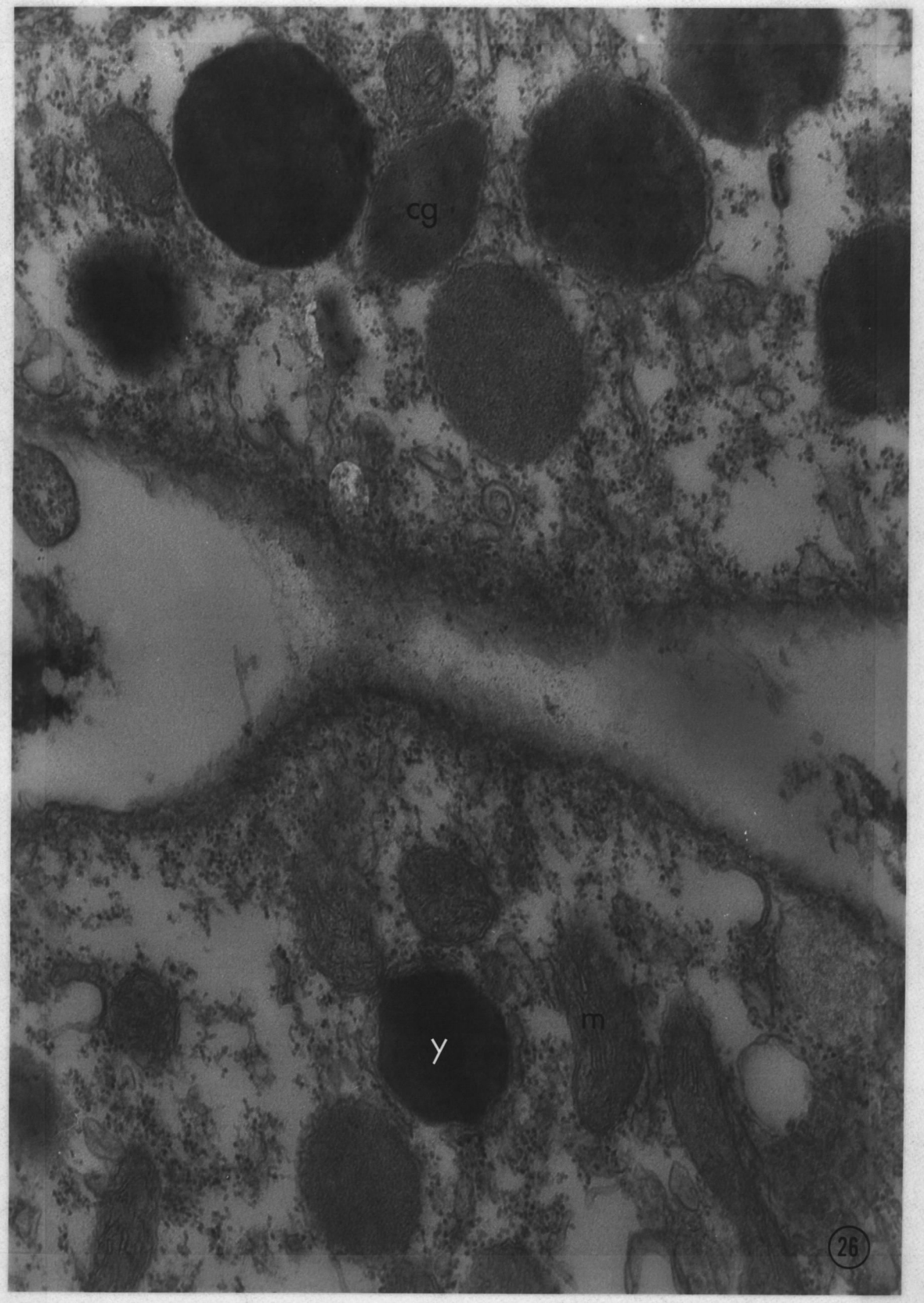

cg
y
m
26

Figure 27. Furrow at vegetal pole - polar lobe furrow. Note the extent of the electron dense region along the furrow (arrows). Much of the swelling of organelles is due to the fixation. M. edulis cell fixed in glutaraldehyde at pH 6.8. 35,300 X. 0.28 μ = 1 cm.

cg - cortical granule

m - mitochondrion

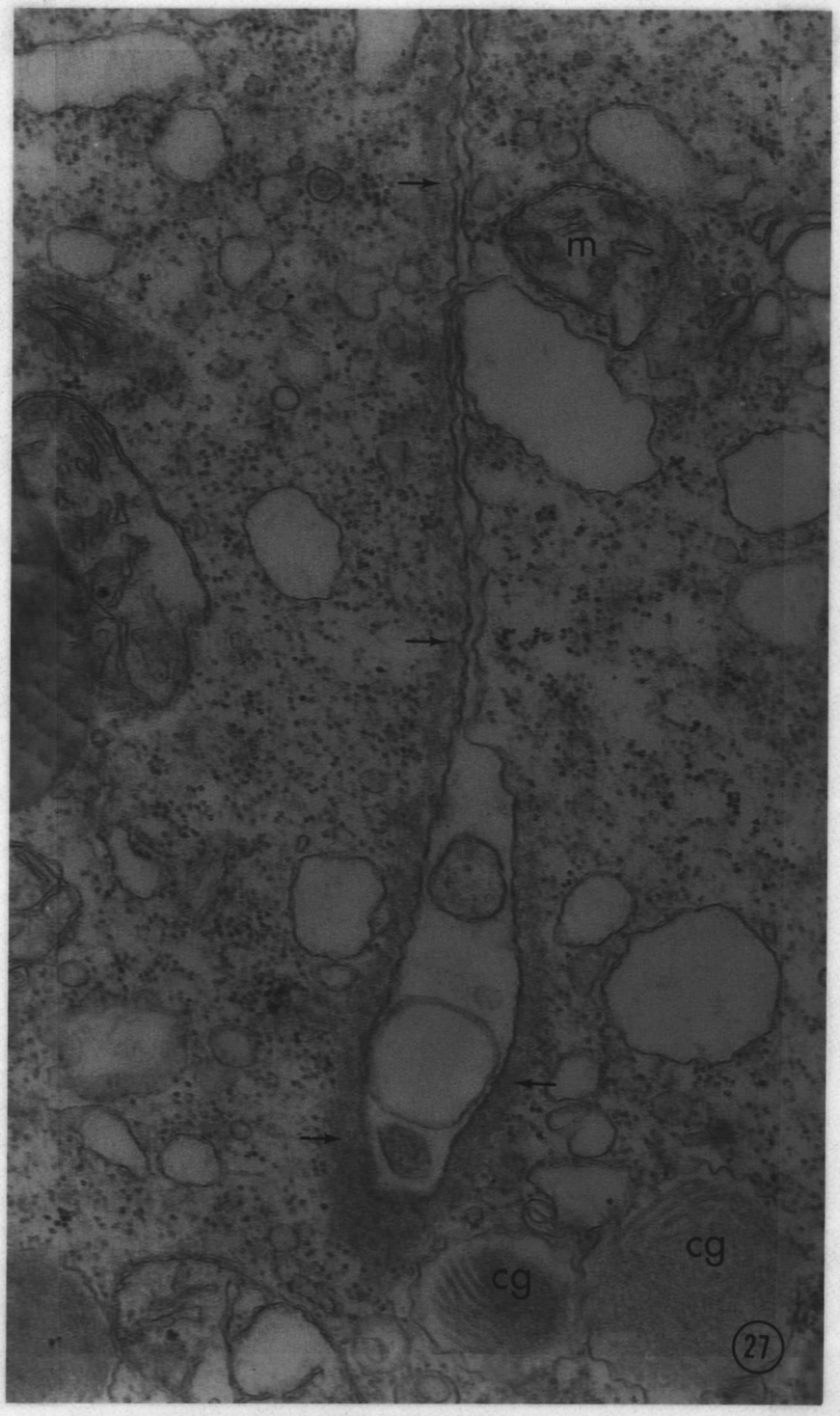

m
cg
cg
27

Figure 28. Furrow formation in the CD cell - polar lobe furrow. Note the electron dense region (arrows). M. edulis cell preserved in Karnofsky's fixative at pH 6.4. 34,700 X. 0.30 μ = 1 cm.

cg - cortical granule

l - lipid

m - mitochondrion

mv - microvillus

vc - vitelline coat

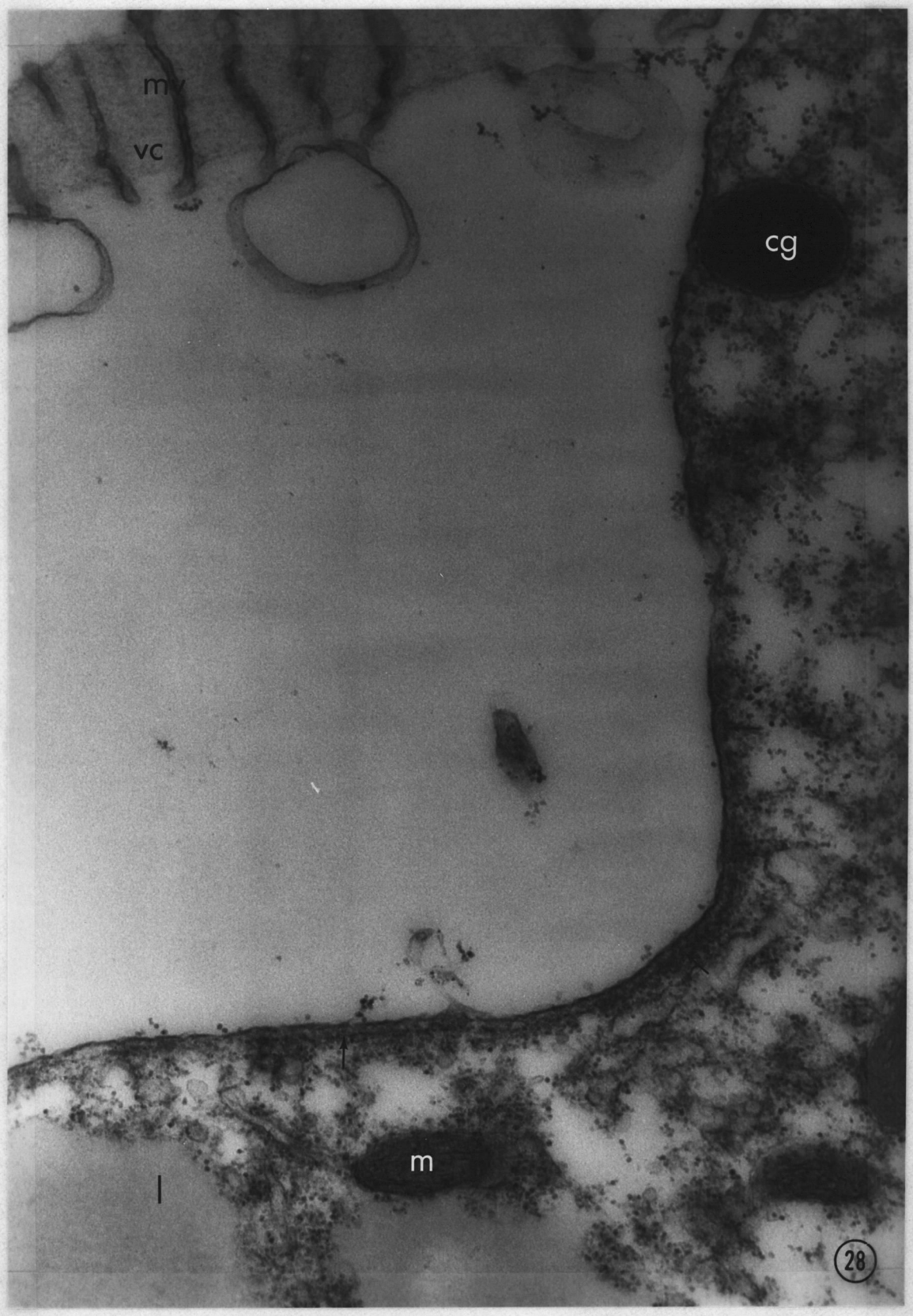

mv
vc
cg
m
l
28

Figure 29. Cell placed in mercaptoethanol at 25 minutes after fertilization and fixed at 90 minutes. The second polar body formed but the polar lobe did not appear.

Figure 30. Cell placed in mercaptoethanol at 60 minutes after ferilization and fixed at 90 minutes. The cell was "pear-shaped" in appearance when fixed but this condition was lost in the fixative.

Figure 31. Cells placed in mercaptoethanol at 65 minutes after fertilization and fixed at 90 minutes. The "pear-shaped" appearance has been retained in the fixative.

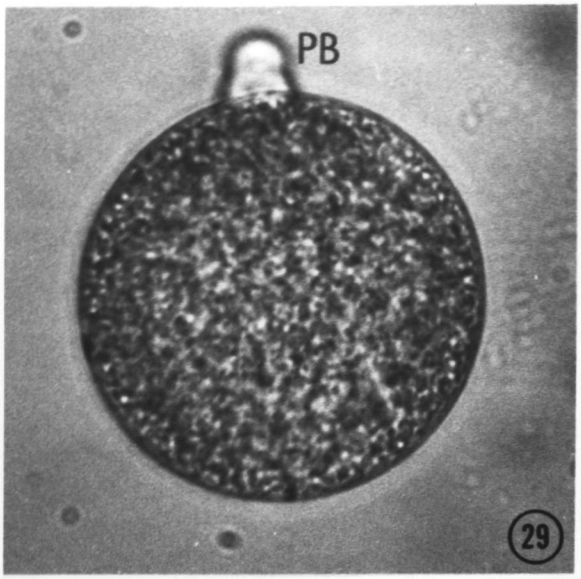
Figure 32. Cells placed in mercaptoethanol at 75 minutes after fertilization divided in the inhibitor by 90 minutes. The blastomeres are round and widely separated from one another (compare with Figure 33).

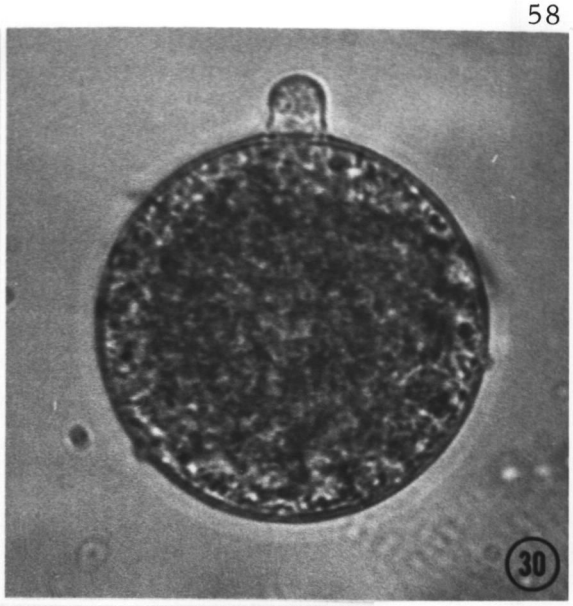
M. edulis preserved in 3% formalin in seawater. Phase contrast. 1, 370 X. 7.3 μ = 1 cm.

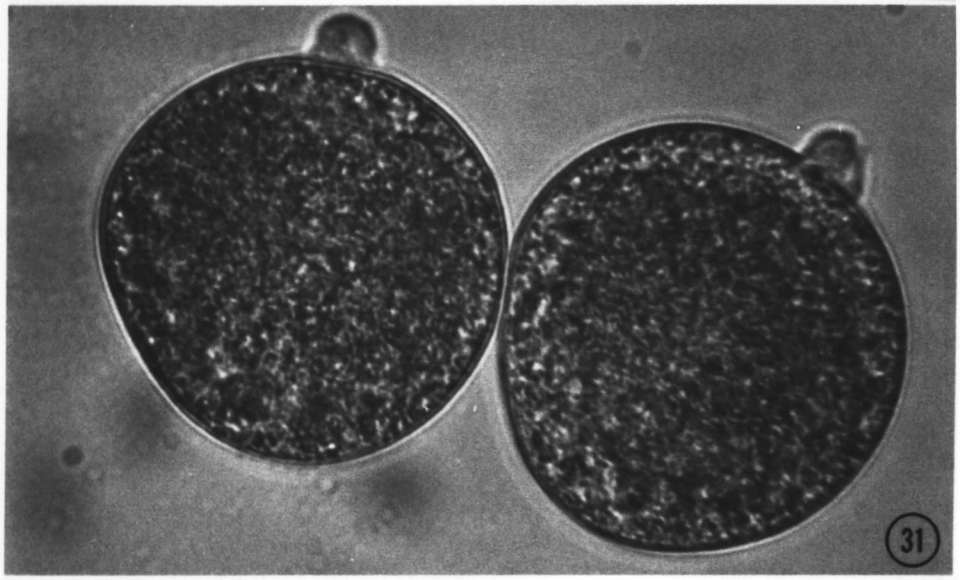
AB - AB blastomere

CD - CD blastomere

PB - polar body


PB
29


30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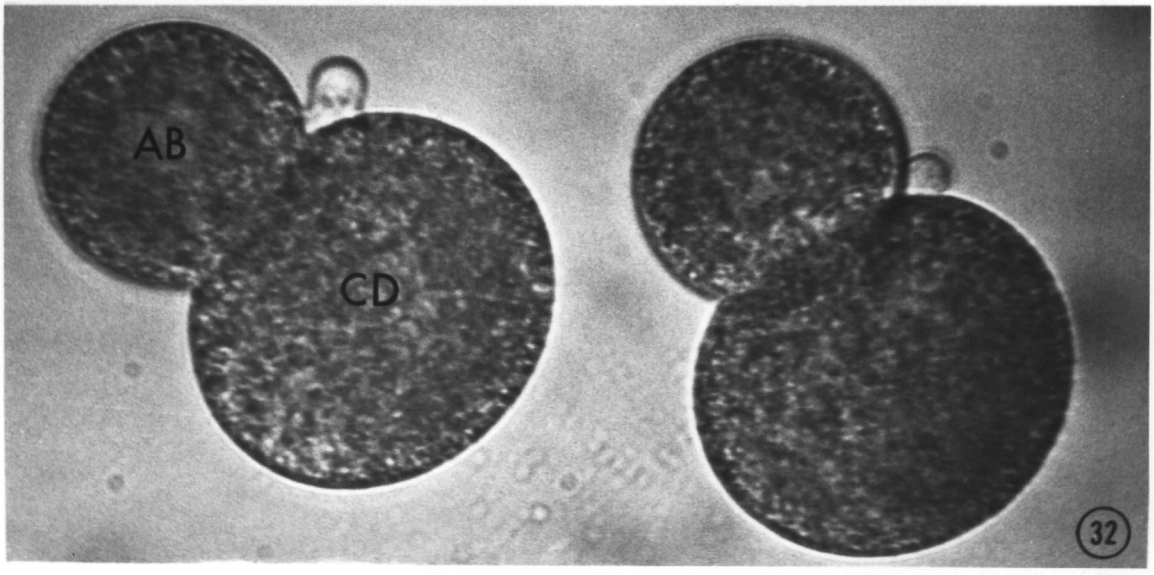

AB
CD
32

Figure 33. Control cells fixed at 90 minutes after fertilization showing the blastomeres flattened along their adjoining surfaces.

Figure 34. Cells placed in mercaptoethanol at 25 minutes after fertilization. The inhibitor was removed at two hours after fertilization and the cells recovered for one hour in fresh seawater. Cleavage appears normal.

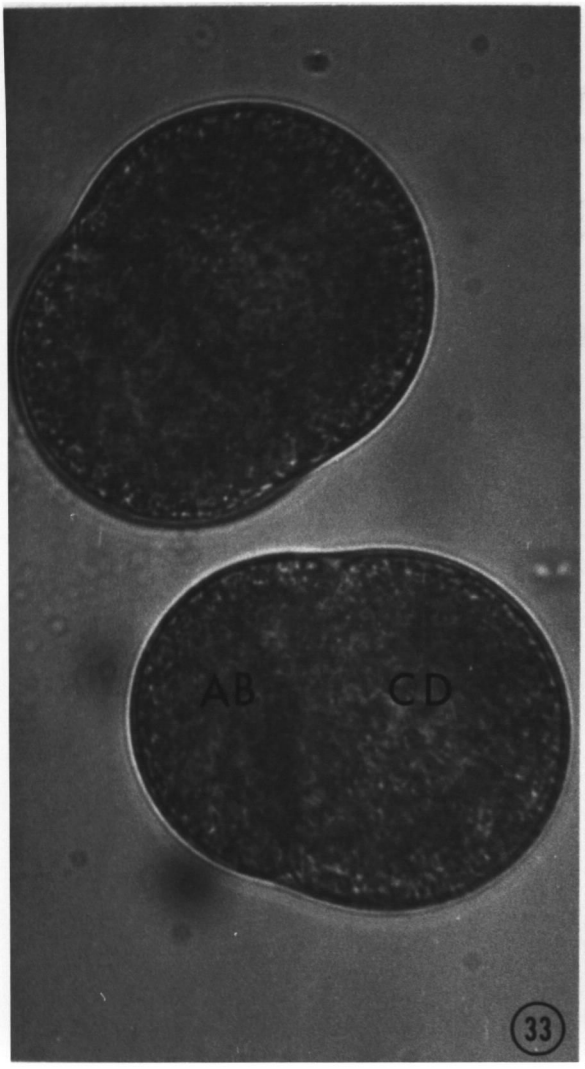
M. edulis preserved in 3% formalin in seawater. Phase contrast. 1, 370 X. 7.3 μ = 1 cm.

AB - AB blastomere

CD - CD blastomere

PB - polar body

pl - polar lobe


AB
CD
33

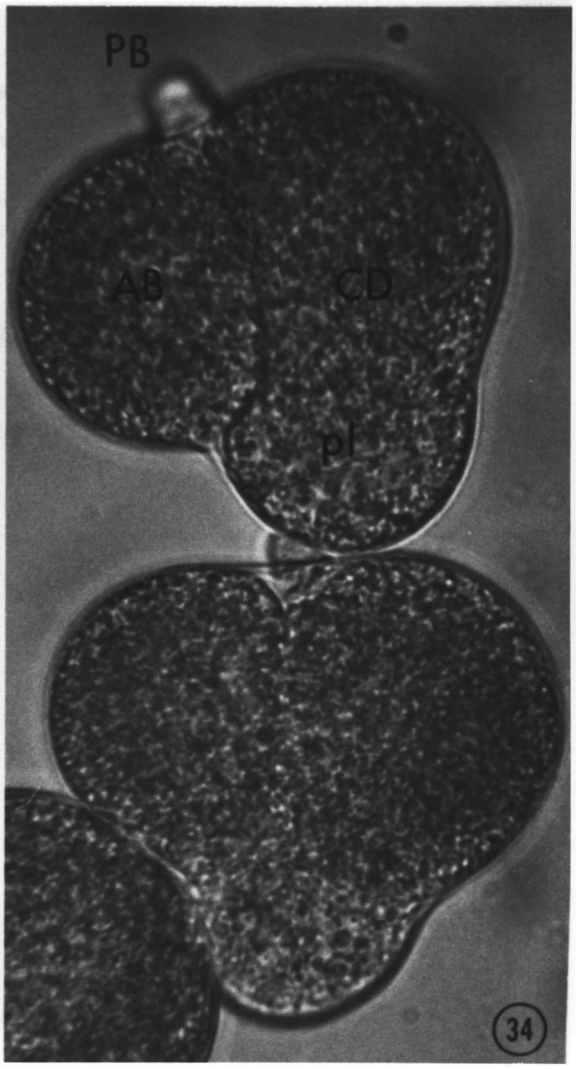

PB
AB
CD
P1
34

Figure 35. Cell placed in 1.0 μg cytochalasin/ml at 65 minutes after fertilization and fixed at two hours. The cell is slightly ovoid but lacks the characteristic "pear-shaped" morphology of the polar lobe.

Figure 36. DMSO control treated at 65 minutes after fertilization in a 0.1% solution and fixed at two hours. Note the large D blastomere.

Figure 37. Cell placed in 1.0 μg CB/ml at 75 minutes after fertilization and fixed at two hours. The polar lobe was resorbed in the inhibitor.

Figure 38. DMSO control treated at 75 minutes after fertilization in a 0.1% solution and fixed at two hours.

Figure 39. Seawater control fixed at two hours (compare with Figures 36 and 38).

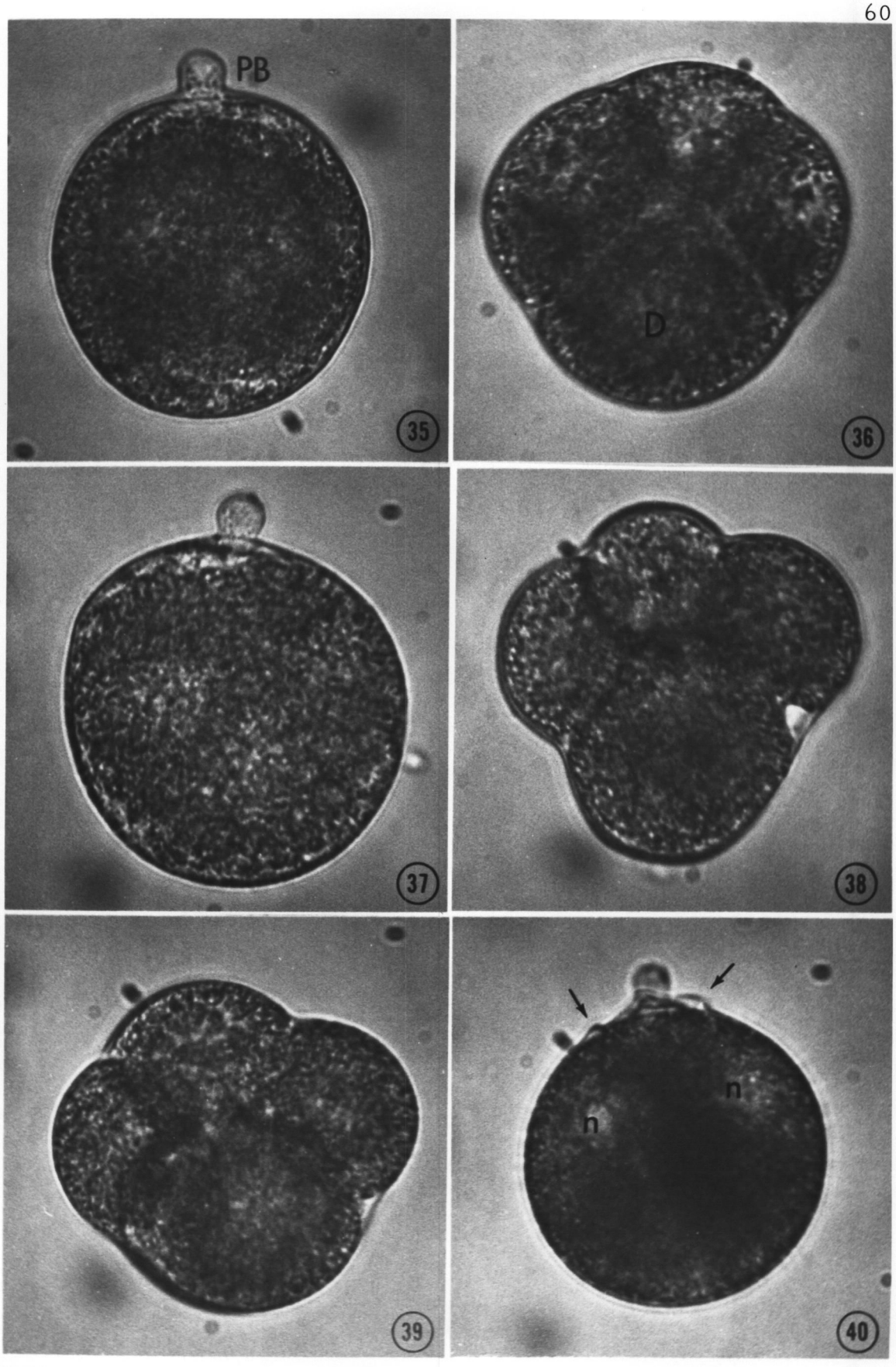
Figure 40. Cell placed in 0.1 μg CB/ml at 75 minutes after fertilization and fixed at three hours. Karyokinesis has continued in the inhibitor. The cell was not compressed in order to demonstrate the ruffling of the vitelline coat (arrows) at the animal pole.

M. edulis preserved in 3% formalin in seawater. Phase contrast. 1,370 X. 7.3 μ = 1 cm.

D - D blastomere

n - nucleus

PB - polar body


PB
35
D
36
37
38
39
n
n
40