A CYTOLOGICAL STUDY OF ARTIFICIAL PARTHENOGENESIS IN THE SEA URCHIN *ARBACIA PUNCTULATA*

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ABSTRACT

Eggs of the sea urchin *Arbacia punctulata* were artificially activated with hypertonic seawater. The artificially activated eggs undergo the cortical reaction which is not distinguished by a wavelike progression as in the case of inseminated eggs. The cortical granules are released at random loci at the surface of the egg and result in spaces separated by large cytoplasmic projections. Unreacted cortical granules and ribosomes are found within the matrix comprising the large cytoplasmic projections. No "fertilization cone" is formed. The subsequent release of additional cortical granules results in the formation of a continuous perivitelline space, 15 min following activation. 85 min postactivation, an organization of annulate lamellae, endoplasmic reticulum of the smooth variety, and microtubules around a centriole is observed prior to nuclear division. Before the breakdown of the nuclear envelope a streak stage is formed. The streak is composed of a central core of annulate lamellae and is encompassed by endoplasmic reticulum and vesicular components. Condensation of chromatin is followed by the establishment of the mitotic apparatus. Centrioles were not found in the mature egg; however, they are present after activation prior to the first nuclear division, in the four-cell embryo, multicellular embryo, and at blastula. Artificially activated eggs have been observed to develop to the pluteus stage in more than 50% of the eggs treated.

INTRODUCTION

Natural parthenogenesis was first described by Greef in the Echinoderm *Asterias glacialis* (starfish) (18) and is now known to occur in many organisms. On the other hand, artificial parthenogenesis has attracted the attention of researchers since the Hertwigs (37) first gave an account of the basic features of this phenomenon by utilizing chloroform and strychnine as stimulating agents in the sea urchin, *Paracentrotus lividus*. Morgan (68) used various salt solutions including sodium, potassium, and magnesium chloride to artificially activate eggs of the sea urchin *Arbacia punctulata*. It was Loeb (54), however, who was the first to obtain parthenogenetic plutei of *Arbacia punctulata* by using magnesium chloride. Development has been stimulated by physical means such as application of heat or cold (35, 63, 64) and by the utilization of a variety of chemical means, e.g. sodium chloride (33, 54, 55, 64, 68), acids (33, 45, 55), strychnine (69), sucrose (55, 71), saponin (70), and many others (see 31). Cytological studies have been made of events associated with fertilization (2, 57, 58, 65, 67, 88, 94); however, few studies are available concerning those events associated with artificial parthenogenesis at the ultrastructural levels of observation

(9, 59). The present study deals with artificially activated eggs of the sea urchin *Arbacia punctulata* and calls attention to (*a*) the cortical reaction, (*b*) streak formation, and (*c*) nuclear replication. These events are compared with those occurring in the inseminated egg.

MATERIALS AND METHODS

Arbacia punctulata were obtained from The Marine Biological Laboratory at Woods Hole, Massachusetts, during the months of June, July, and August. They were induced to spawn by applying a 10v alternating current across the oral surface (30, 31). The eggs were collected according to the recommendation of Costello et al. (4). Eggs were artificially activated by placing them in seawater made hypertonic by the addition of 30 g of sodium chloride/liter of seawater (44). The time of activation was considered to be the moment the eggs were placed in the hypertonic solution. The eggs were allowed to remain in the hypertonic seawater (19–22°C) for 20 min and were subsequently transferred to fresh seawater. Egg samples were taken at the following intervals: 30 sec, 1, 3, and 5 min, and successive 5-min intervals until 95 min or the initiation of cleavage. Some of the cleaving eggs were permitted to develop to the pluteus stage. Activated eggs, cleaving stages, and plutei were studied by both phase-contrast optics and electron microscopy. The activated eggs from each of the above-mentioned times and the initial cleavage stage were prefixed for 2 hr in a 2% glutaraldehyde-seawater solution or in the glutaraldehyde-paraformaldehyde mixture of Karnovsky (48). After fixation, the specimens were washed in seawater, postfixed for 1 hr in a 1% solution of osmium tetroxide dissolved in seawater, rapidly dehydrated in a graded series of ethanol, infiltrated, and embedded in Epon (62). 1 μ sections, cut on a Porter-Blum MT-2 ultramicrotome, were stained according to the recommendation of Ito and Winchester (43). Thin sections were also obtained with the MT-2 ultramicrotome and stained with uranyl acetate followed by lead citrate (91), and were examined in an RCA EMU-3H electron microscope.

Eggs collected in the manner indicated above were inseminated with the "dry sperm" diluted with seawater (45). The inseminated eggs were fixed for

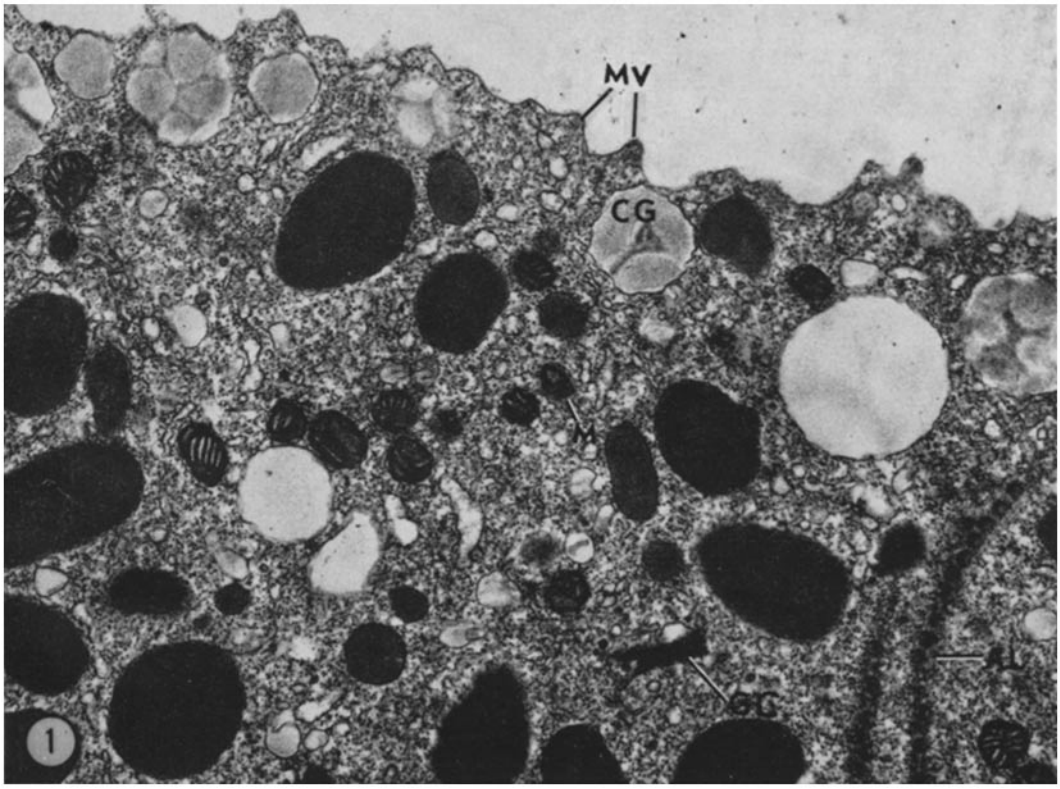

FIGURE 1 An electron micrograph depicting the untreated egg. *MV*, microvilli; *CG*, cortical granule; *AL*, annulate lamellae; *M*, mitochondria; *GC*, Golgi complex. × 8,000.

light and electron microscopy (see above) or were observed with phase-contrast optics at 10-min intervals for 70 min through the initial cleavage.

OBSERVATIONS

Unactivated Egg

The morphology of an unactivated egg is shown in Fig. 1. The oolemma is projected into short microvilli (*MV*). Immediately beneath the oolemma is a population of cortical granules (*CG*) embedded in a matrix of free ribosomes and some vesicles. The ooplasmic components such as yolk droplets, annulate lamellae (*AL*), endoplasmic reticulum, Golgi complexes (*GC*), rod-containing vesicles, and pigment granules are randomly dispersed; the majority of mitochondria (*M*) are randomly distributed, but some are closely associated with lipid droplets.

Activated Egg

CORTICAL CHANGES: When the eggs are treated with hypertonic seawater they undergo a cortical reaction. Figs. 2, 5, 6 show the cortical region of the egg at 1, 5, and 10 min after being exposed to the activating medium. All of the cortical granules that are closely associated with the inner aspect of the oolemma do not fuse with the oolemma simultaneously when activated (Fig. 2, *RCG*); however, they fuse at random loci (Fig. 2 and Fig. 2 *inset*, see arrow). The artificially activated egg produces an activation calyx (Fig. 5, *AC*) and does not produce a protrusion reminiscent of an entrance cone like that of the inseminated egg (2, 57). The fusion of the membrane encompassing the cortical granule with the oolemma produces vesicular structures (Figs. 5, 6, *V*) over the contents of the cortical granules. Upon the completion of membrane fusion, the contents of

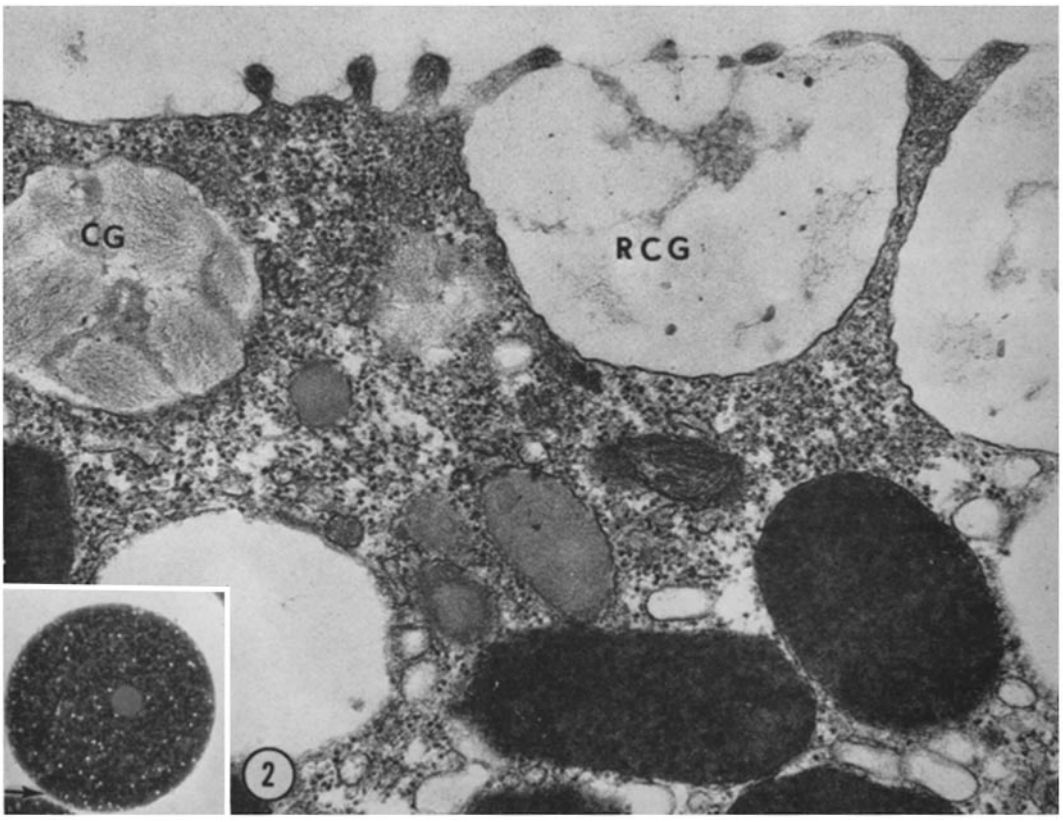

FIGURE 2 The surface of an egg, 1 min following activation with hypertonic seawater. *CG*, cortical granule; *RCG*, released cortical granule. × 25,800. *Inset* is a photomicrograph of an egg 1 min post-activation, demonstrating the release of cortical granules. × 400.

the cortical granules are released, initiating the formation of the perivitelline space (Fig. 6, *PS*). The release of the contents of all the cortical granules does not occur simultaneously, for "pillars" of unreacted cortical ooplasm which contain cortical granules, free ribosomes, and occasional pigment bodies are commonly found (Figs. 5, 6, *P*). The further release of the cortical granules results in the formation of a continuous perivitelline space limited by the "chorion" and the oolemma (Fig. 7, *PS*). In the artificially activated egg, the perivitelline space is smaller than that of the inseminated egg. As demonstrated for the inseminated egg (2), not all cortical granules are released during the initial reaction to the hypertonic seawater.

Within the relatively small perivitelline space, 30 min postactivation, the hyaline layer (Fig. 7, *HL*) may be observed directly beneath the "chorion" (Fig. 7, *C*) (also see 1). It is composed of a mat of fine, electron-opaque, filamentous material. By 65 min, the hyaline layer increases in thickness. Beneath the microvilli the cortical ooplasm is now composed of an accumulation of pigment bodies (Fig. 9, *PB*), few mitochondria, and dense yolk bodies. The cortical ooplasm contains numerous rod-containing vesicles. As in the inseminated eggs, the rods are released subsequent to the release of the contents of the cortical granules. The rodlike structures become associated with the components of the hyaline layer (Fig. 9, *R*).

When cytokinesis is initiated (1½–4½ hr postactivation), the periphery of the embryo is characterized by a well-developed hyaline layer (Fig. 9, *HL*), long microvilli (Fig. 8, *MV*), and an almost continuous stratum of pigment bodies (Fig. 9, *PB*) immediately beneath the plasma membrane.

OOPLASM: The mitochondria show an inter-

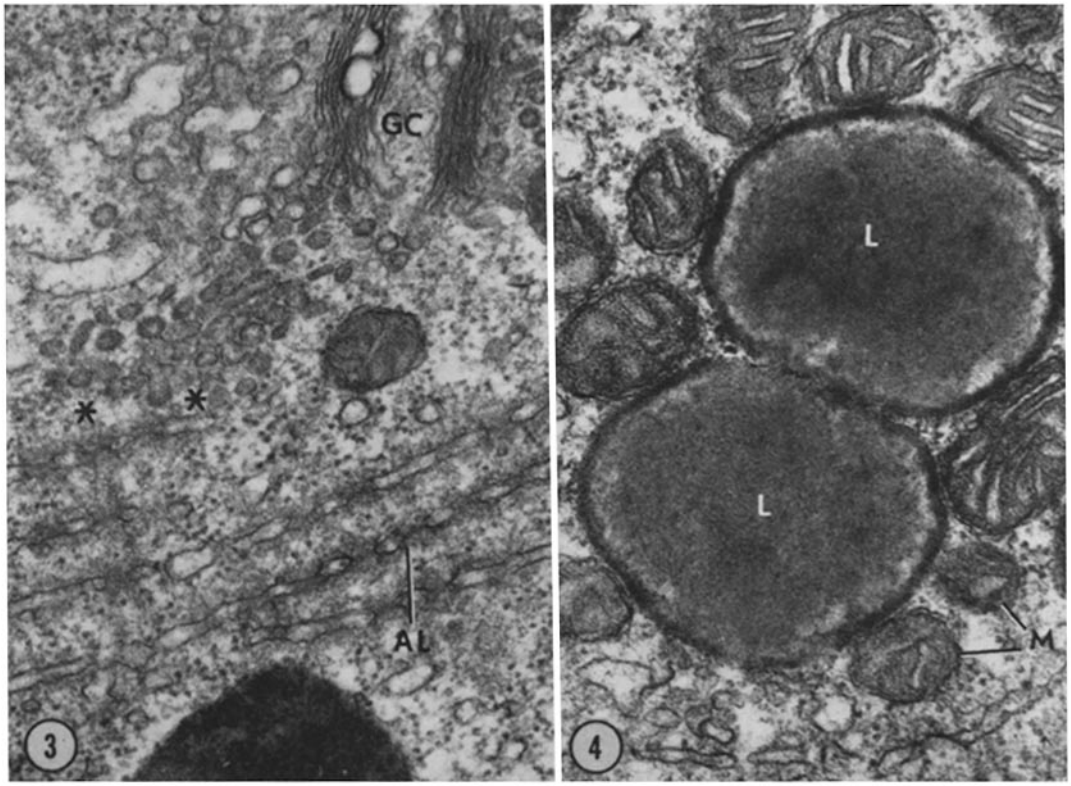

FIGURE 3 An electron micrograph of an egg in hypertonic seawater for 1 min, showing a Golgi complex (*GC*) associated with coated vesicles (**) closely associated with annulate lamellae (*AL*). × 25,000.

FIGURE 4 An electron micrograph of an egg 1 min following activation, depicting mitochondria (*M*) clustered around lipid droplets (*L*). × 38,000.

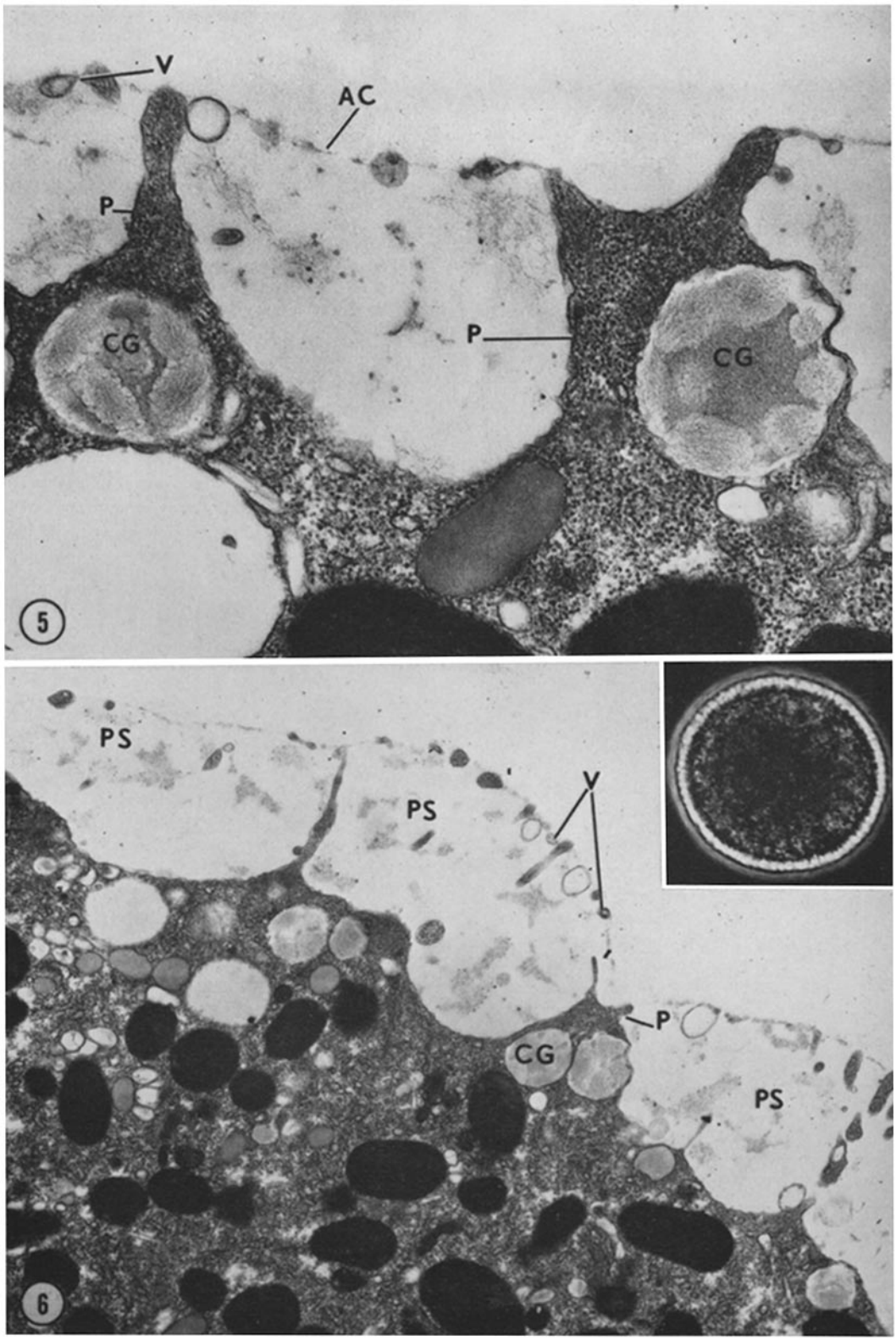

FIGURE 5 An electron micrograph of the surface of an egg, 5 min following activation. *P*, pillars of unreacted ooplasm containing cortical granules (*CG*); *V*, vesicles; *AC*, activation calyx. × 21,000.

FIGURE 6 An electron micrograph of the surface of an egg, 10 min following activation, showing the reduction in number and thickness of the pillars (*P*) which contain cortical granules (*CG*). *V*, vesicles; *PS*, incomplete perivitelline space. × 17,000. The phase-contrast photomicrograph (*inset*) of a living *Arbacia* egg depicts the appearance of the egg at the light microscope level. Note the striated appearance of the perivitelline region. × 450.

nal configuration similar to that of the unactivated egg. At 1 min postactivation, there appears to be a close spatial association between mitochondria (Fig. 4, *M*) and lipid droplets (Fig. 4, *L*). There also appears an intimate association between the Golgi complex (Fig. 3, *GC*) and its associated coated vesicles (Fig. 3, *) and the annulate lamellae (Fig. 3, *AL*) similar to that observed during pronuclear development in the rabbit (58). Centrioles have not been described in the unfertilized egg (2, 92) and have not been observed in the artificially activated eggs until the formation of the aster (9, 80).

FORMATION OF THE STREAK STAGE: According to Harvey (31), in inseminated eggs, a monaster is formed after the fusion of the male and female pronuclei. Subsequently "The rays disappear and the centrosome (probably) divides forming a curved disk over the nucleus . . .". Harvey (31) defines this stage as the *streak stage*. In the case of eggs treated with hypertonic seawater, a streak stage is also formed. Closely associated with the nuclear envelope (Fig. 11, *NE*), prior to the formation of the streak stage (60 min postactivation), are stacks of annulate lamellae (Figs. 10–12, *AL*). Occasionally, one sees intranuclear annulate lamellae (Fig. 11, *IAL*). Concomitant with the organization of the annulate lamellae, centrioles (one, two, or three) may be observed (Figs. 15, 16, 17, *C*) associated with microtubules (Fig. 17, *MT*) and endoplasmic reticulum (Fig. 15, *ER*). Together, the latter organelles form an aster (Figs. 15, 18, *inset a*, *AS*) which is similar to that reported for the sperm (57, also see 26, 27).

The annulate lamellae (Fig. 13, *AL*) become dispersed from their circumnuclear configuration, initiating the elongation of the aster and the formation of the streak. The inset of Figs. 13 and 14 is a phase-contrast photomicrograph of a streak (*ST*) stage 85 min postactivation. The streak is characterized by annulate lamellae (Fig. 14, *AL*) arranged in parallel array encompassed by endoplasmic reticulum and vesicular components. Occasionally, one finds mitochondria amongst the annulate lamellae comprising the streak; however, protein-carbohydrate yolk bodies (Fig. 14, *Y*) are

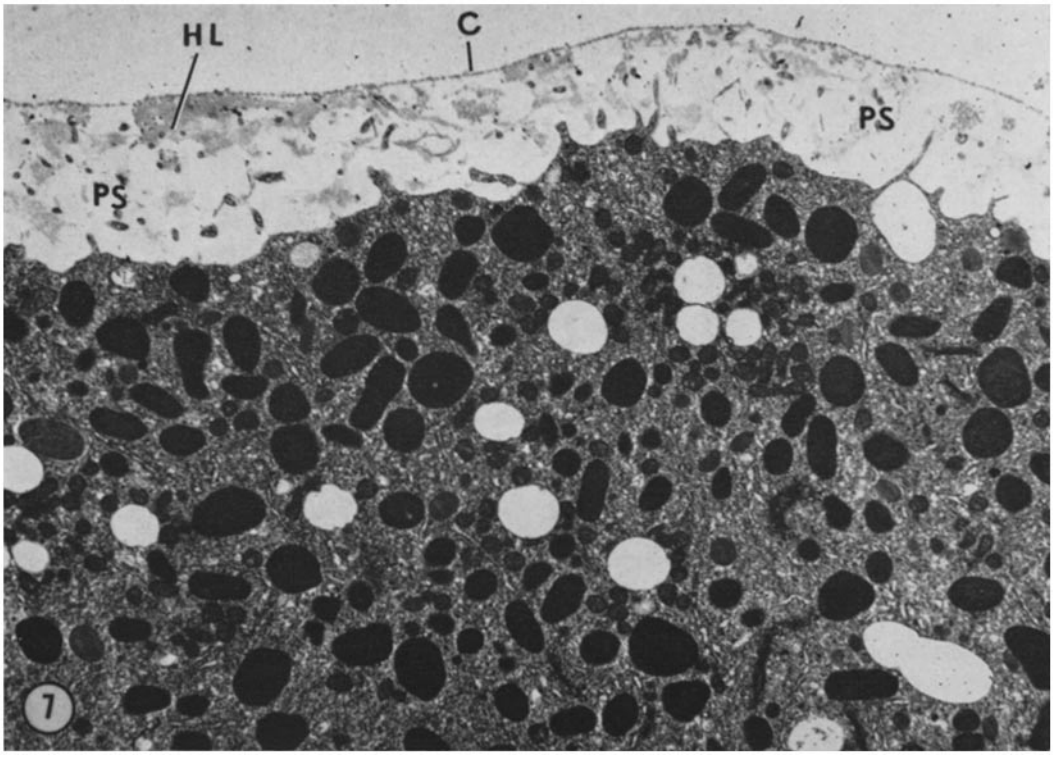

FIGURE 7 An electron micrograph of the surface of an egg 30 min postactivation, *C*, "chorion"; *HL*, hyaline layer; *PS*, perivitelline space. × 6,500.

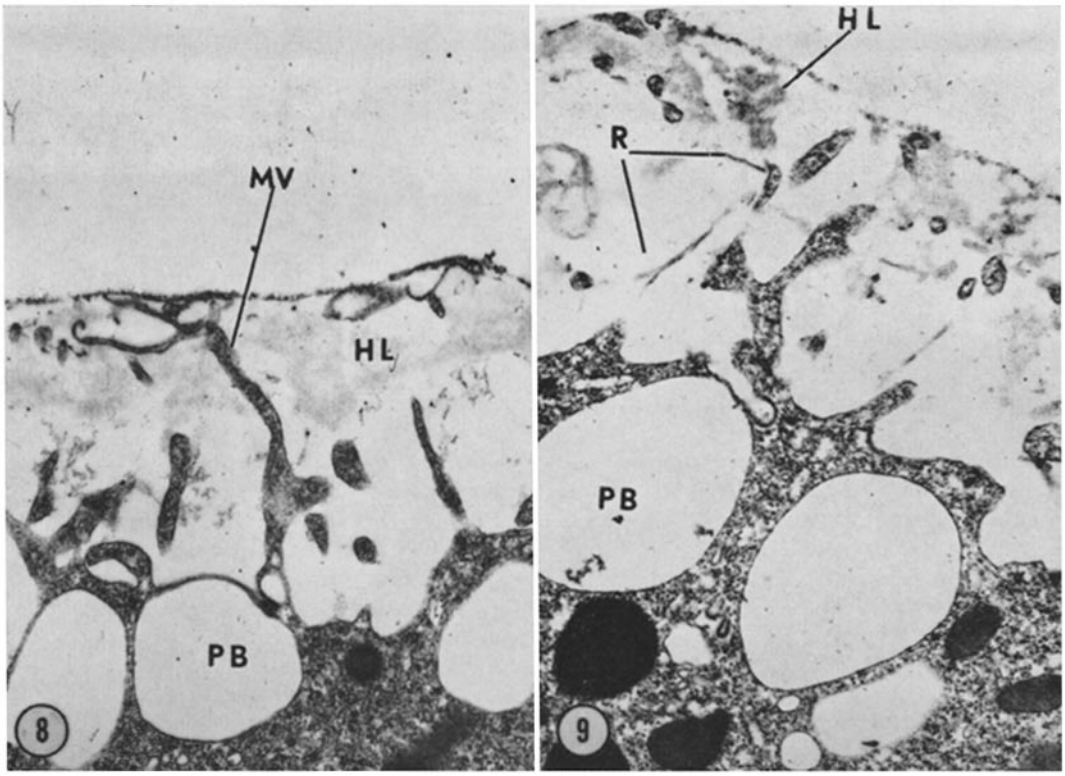

FIGURE 8 The surface of an egg at the time of cytokinesis, depicting the hyaline layer (*HL*), microvilli (*MV*), and pigment bodies (*PB*). × 16,000.

FIGURE 9 A section of an artificially activated egg 65 min postactivation. *HL*, hyaline layer; *R*, rodlike structures; *PB*, pigment bodies. × 25,000.

excluded. The conformation of the annulate lamellae (streak) extends from the tips of the longitudinal axes of the now elliptical nucleus toward the plasmalemma. We have observed that eggs artificially activated with hypertonic seawater may remain in the streak stage for 1–3 hr before dividing. Inseminated eggs remain in the streak stage for only approximately 25 min.

NUCLEUS: The pronucleus contains a granular nucleoplasm in which are suspended nucleolus-like structures (Figs. 10, 15, *NL*). The pronucleus is surrounded by a perforated nuclear envelope. At 85 min postactivation, the pronucleus elongates with a concomitant condensation of its chromatin followed by a breakdown of the pronuclear envelope (Fig. 18, *CH*, and *inset a*).

At metaphase (Fig. 18, *inset b*) and anaphase (Figs. 18, 19, *inset c*) the chromosomes appear as dense masses of granular material embedded within a matrix of ribosomes (Fig. 18, *MR*). The mitotic apparatus (Fig. 18, *inset b* (*SA*), *c*; Fig. 19) is composed of predominantly microtubules (Fig. 19, *MT*) with some endoplasmic reticulum (*ER*) and ribosomes (see 26, 27, 57). At the periphery of the mitotic apparatus may be found mitochondria (Fig. 19, *M*) and yolk bodies.

At telophase, the chromosomes (Fig. 20, *CH*) are elongated in the direction of the centrioles (Fig. 20, *C*) and are often found in intimate association with nucleolus-like bodies (Fig. 20, *NL*). Microtubules (Fig. 20, *MT*), mitochondria, and annulate lamellae are also found among the chromosomes. A perforated envelope forms around the chromosomes, establishing chromosome-containing vesicles (karyomeres) (Fig. 20, *inset*) (see 94). Fusion of the chromosome-containing vesicles and subsequent dispersal of the chromatin results in the formation of two nuclei prior to cytokinesis (Fig. 21). Each of these nuclei contains some dense chromatin material (*CH*).

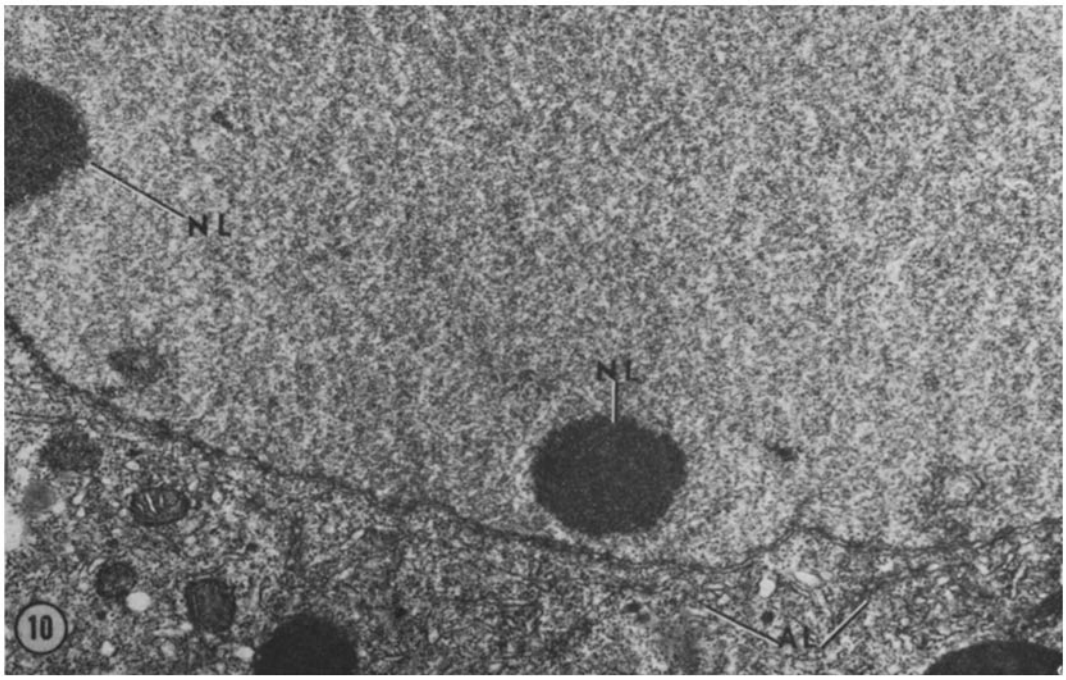

FIGURE 10 A section through the nucleus of an egg 50 min following activation. *NL*, nucleolus-like bodies; *AL*, annulate lamellae. × 22,000.

Development to the Pluteus Larva

First cleavage of artificially activated eggs occurs between 1½ and 4½ hr and results in the two-cell embryo (Fig. 22). Ensuing cleavages result in various multicellular stages (Figs. 23–26). The cells of the multicellular embryo (of which the electron micrographs are not included) are spherical and contain the regularly occurring organelles including centrioles, microtubules, mitochondria, smooth and rough forms of endoplasmic reticulum. Large quantities of yolk are present, but there is a reduction in the amount of lipid. The cells of the morula stage are often found associated by tight junctions.

The blastula contains elongated polarized cells with apically situated nuclei. The cells of the ciliated blastula contain organelles similar to those described for the multicellular stage. The pluteus appears, at the light microscope level, to be identical with that formed from the inseminated egg.

DISCUSSION

Cortical Reaction

Evidence obtained during this study suggests that the complex cortical reaction of the eggs of the sea urchin, *Arbacia*, brought about by treatment with hypertonic seawater, is different from that initiated by insemination (see 2, 13). Artificially activated eggs do not demonstrate the wavelike propagation of cortical granule release seen in inseminated eggs (65). The perivitelline space formed as a result of the cortical reaction is smaller in the artificially activated egg, although the mechanism by which the cortical granule reaction occurs, i.e. fusion and vesiculation as discussed by Anderson (2), appears to be the same for the artificially activated egg and the inseminated egg.

The fact that an increase in tonicity, via the addition of 3% sodium chloride to seawater, induces the cortical reaction suggests that this reaction is due to a change in the water and/or ion content of the egg. Loeb (55) wrote that "It appeared to me that nothing would more clearly demonstrate the sovereign role that electrolytes play in the phenomena of life than by causing, if possible, with their help, unfertilized eggs to develop into larvae." In subjecting *Arbacia* eggs to a hypertonic sodium chloride solution, the actual activating agent in the solution could be a change in water flow, the sodium or chloride ions, or, possibly, a change in the surface of the egg. We choose

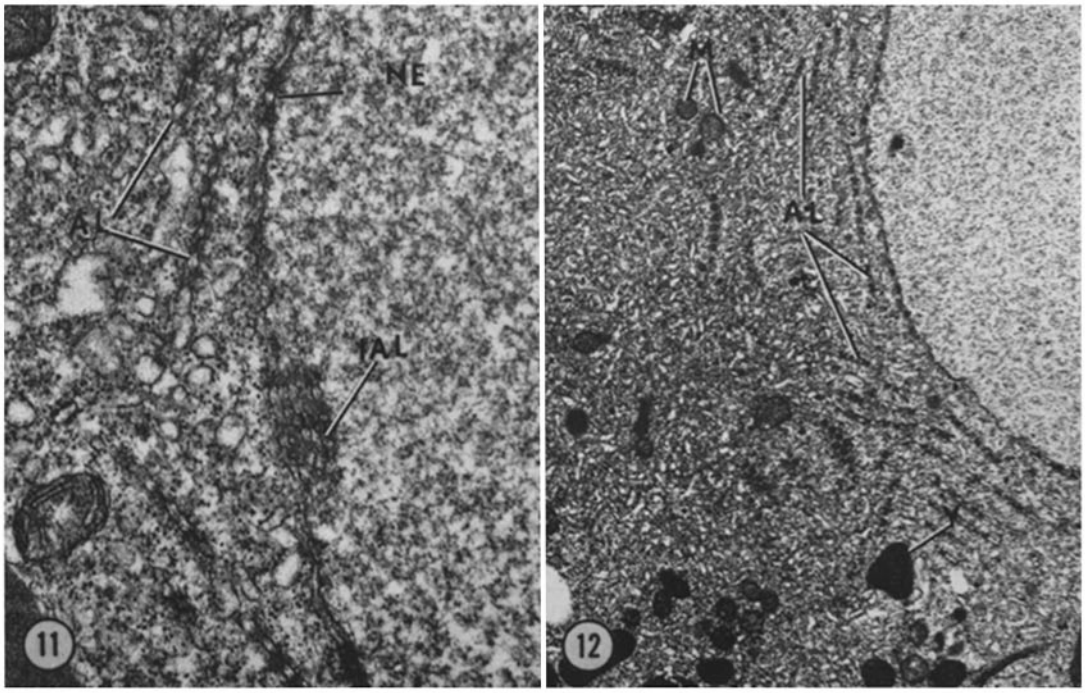

FIGURE 11 An electron micrograph of an artificially activated egg 60 min following activation, depicting annulate lamellae (*AL*) and intranuclear annulate lamellae (*IAL*). Note the nuclear envelope (*NE*). × 29,000.

FIGURE 12 An electron micrograph taken of an egg 75 min postactivation showing annulate lamellae (*AL*) circumferentially located around the nucleus. Note mitochondria (*M*) and yolk (*Y*). × 12,000.

to discuss the possibility of a change in water flow or sodium ion as the activating agent. Discussion favoring the concept that water flow, causing either a decrease or increase in the amount of water in the egg, is responsible for the initiation of the cortical reaction is based largely on the fact that the oolemma acts as a selectively permeable membrane (33, 61, 64). Eggs subjected to hypotonic solution maintain their shape, although there appears to be an increase in volume. When returned to seawater the eggs undergo shrinkage, indicating that the moiety passing through the plasma membrane is water and that the salt content in the egg probably remains constant (61). Many investigators consider that the loss of water from an egg placed in hypertonic seawater is the factor causing what they refer to as the "explosion" of the cortical ooplasm (33, 40, 54, 55, 68). Heilbrunn (33, 34) suggested that treatment with various agents including sodium chloride causes a marked change in the viscosity of the cortical cytoplasm. Recent investigation by Anderson (unpublished data) has demonstrated that there is a change in the position of the cortical granules when treated with sodium chloride or urethane and then centrifuged at high speeds (also see 29). In eggs so treated, the cortical granules abandon their peripheral position and form a stratum. The change in the cortical granule membrane-oolemma relationship does not explain, however, what would dittiate the fusion and subsequent vesiculation process associated with the cortical reaction.

The concentration of the sodium chloride and the exposure time of the eggs to the hypertonic medium are both critical if development is to ensue. Examinations of swelling and shrinkage are only crude indicators of water flow. It would be must fruitful to have the techniques of (*a*) diffusion tracing and (*b*) bulk flow brought to bear on the unactivated and initially activated *Arbacia* egg in order to establish, quantitatively, the actual rate and amount of water flow at activation. These techniques, used on artificial membranes, have demonstrated the ability to measure water flow

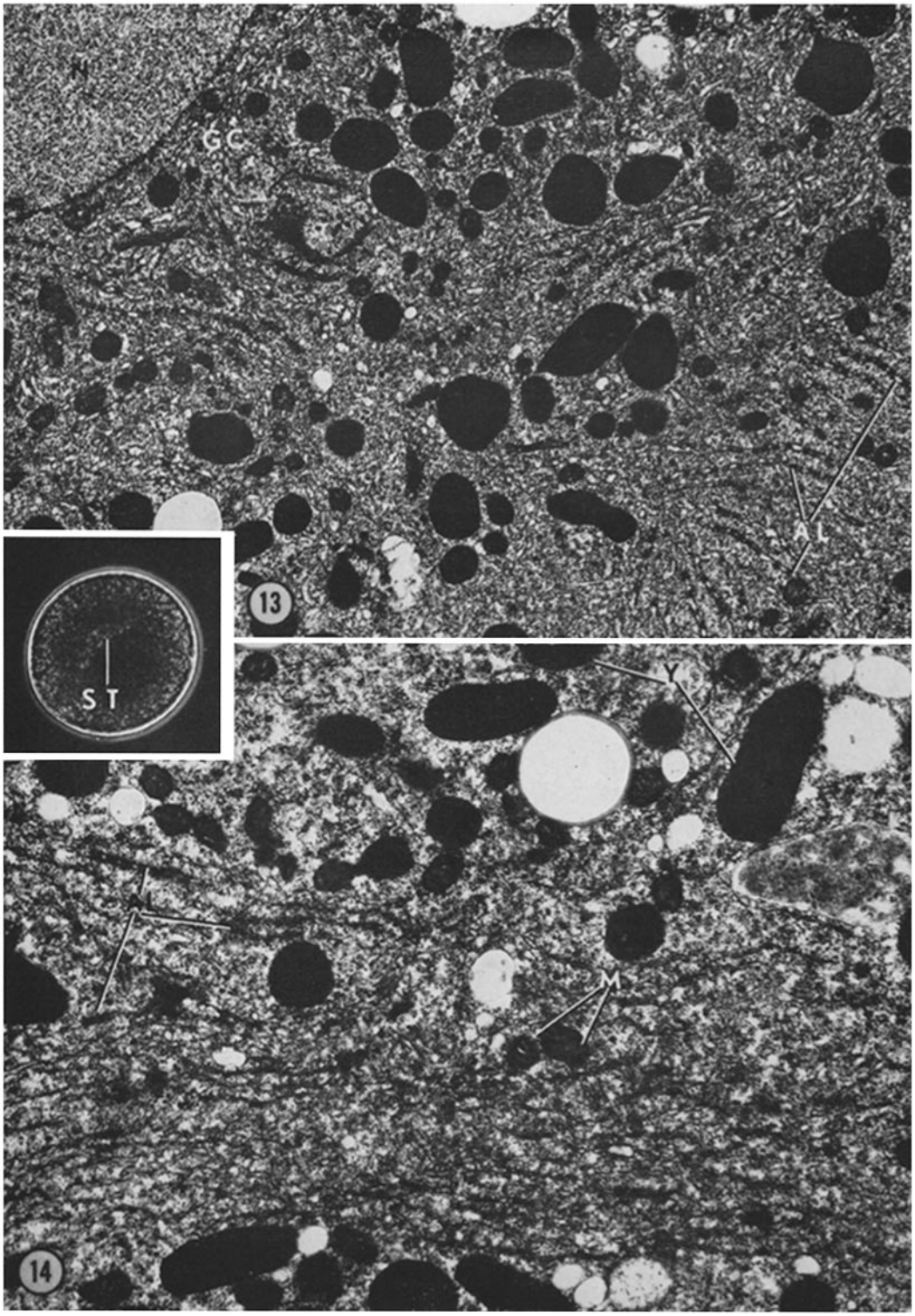

FIGURE 13 A section of an egg 65 min postactivation, showing the initiation of streak formation. *N*, nucleus; *AL*, annulate lamellae; *GC*, Golgi complex. × 11,000.

FIGURE 14 An electron micrograph depicting the components of the streak stage. *AL*, annulate lamellae; *M*, mitochondria; *Y*, yolk. × 11,660. The *inset* is a phase-contrast photomicrograph of the living egg illustrating the streak (*ST*). × 300.

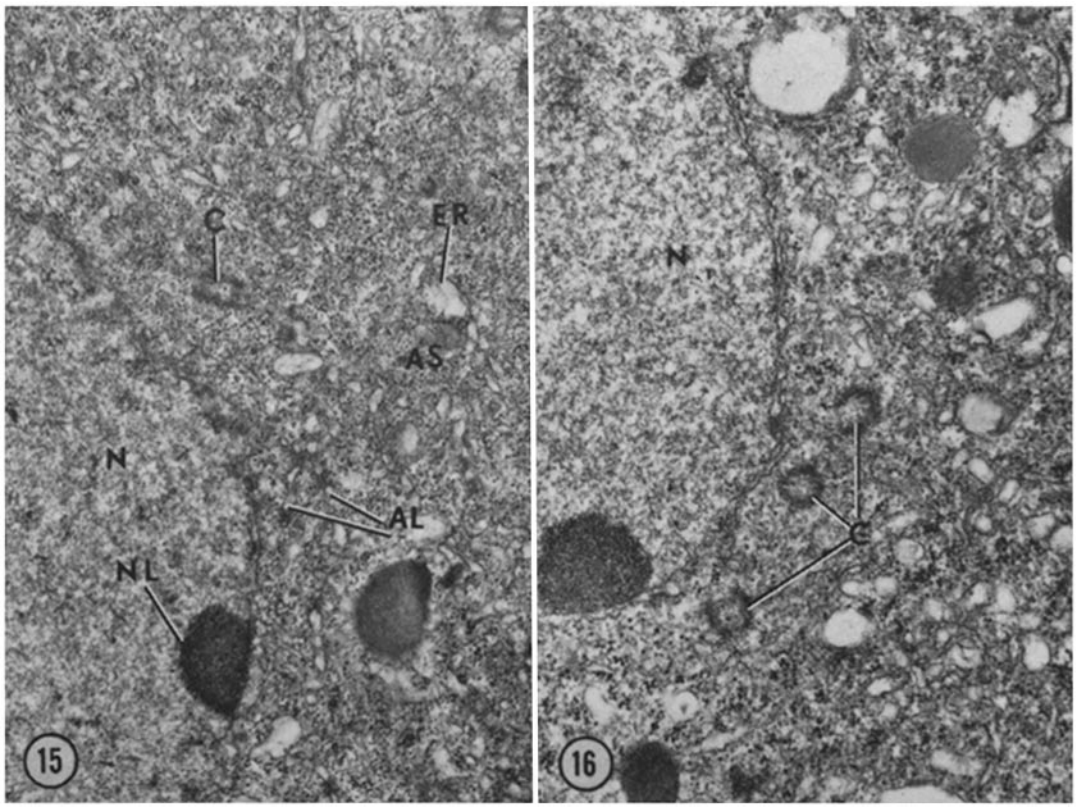

FIGURE 15 A section through the aster (*AS*) (60 min postactivation), which consists of endoplasmic reticulum (*ER*), annulate lamellae (*AL*), and a centriole (*C*). Note the nucleolus-like body (*NL*). × 19,000.

FIGURE 16 An electron micrograph showing three centrioles (*C*) adjacent to the nucleus (*N*). 60 min postactivation. × 18,000.

(16, 22, 38, 72). They have been applied to oocytes of the frog *Rana* and have led investigators to believe that cytoplasmic resistance to water flow is a factor to be considered as well as the physical state of the membrane (7, 55, 60, 76). This is especially significant in many eggs, where the ooplasm is filled with a variety of inclusions, all of which may act as a type of endogenous buffer. This may help to explain the ability of the unactivated egg to maintain its shape when placed in an anisotonic-activating solution.

An alternative hypothesis would be that there is an ion flow into the egg initiated by the increase of Na^{++} and Cl^{-} ions in the hypertonic seawater. In fresh seawater, the sodium ion concentration outside the *Arbacia* egg far exceeds that found in the ooplasm (67, 73, 79, 89). This information, coupled with the findings of Ohnishi (75) that there is an ATP-dependent active transport system in the oolemma, suggests that the oolemma may be not simply a passive barrier to sodium entrance, but instead is equipped to maintain the transmembrane potential via active transport. Ussing (90) and others (6, 36, 51, 86, 93) have stated that active transport, in various cells and tissues responsible for the maintenance of transmembrane potential, can be virtually eliminated by introducing the cells to an osmotic gradient, resulting in a change in the volume of the cell. Thus, when *Arbacia* is subjected to our hypertonic solution the shrinkage may lead to an influx of sodium. The possible relationship of this influx to the initiation of the cortical granule release is unclear.

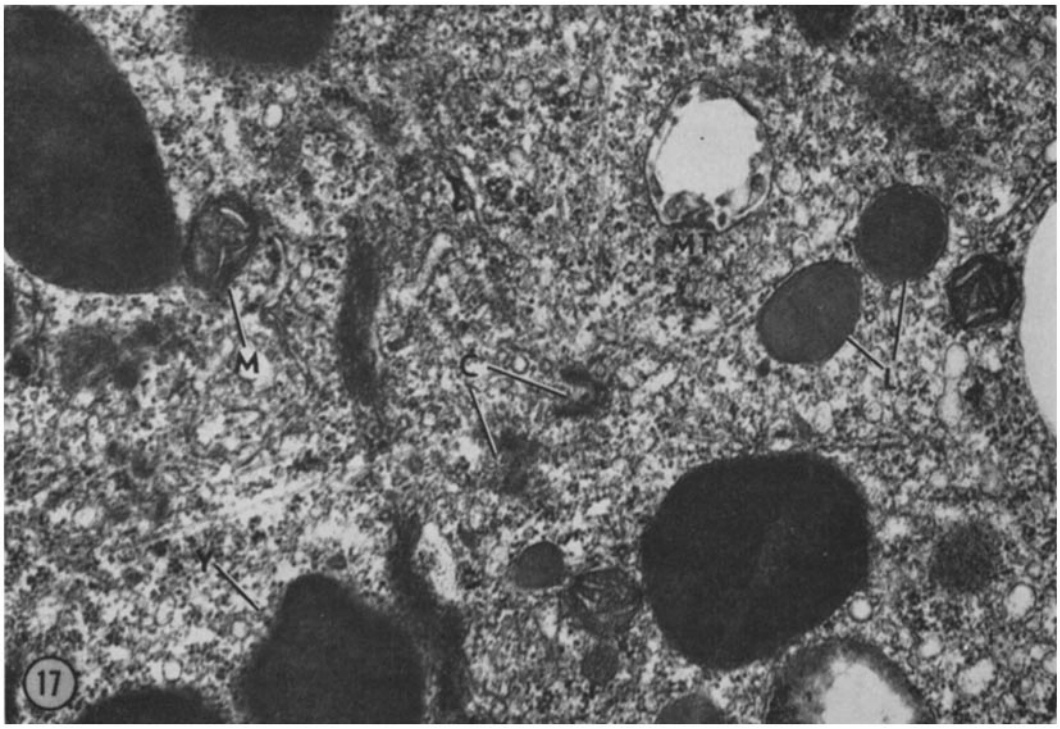

FIGURE 17 A section through an egg 60 min after activation. *C*, centrioles; *MT*, microtubules; *L*, lipid; *Y*, yolk; *M*, mitochondria. × 38,000.

Nucleus

We have seen that the pronucleus (containing the haploid number of chromosomes) can be activated to go through periods of development leading to its division. Division eventually leads to the formation of a pluteus larva. Development, upon activation with hypertonic seawater, proceeds, in a very large percentage of eggs, to the streak stage. Often there is a temporary arrest of the nuclear activity at the streak stage in development, e.g. approximately 1–3 hr. This temporary arrest suggests to us that, in order for development to continue, an "activation" of the nucleus must occur.

In connection with the activation of the nucleus in other systems, some investigators have indicated that an informational transfer between cytoplasm and nucleus results in a change in nuclear activity, for example, DNA replication, RNA synthesis, and protein synthesis (14, 15, 23–25, 39, 47, 49, 74). In our study, the activation of the nucleus could be a direct result of ion or water flow from cytoplasm to nucleus, or possibly an indirect effect of a change in the ion constituency in the cytoplasm, inducing macromolecular synthesis which, in turn, transfers information to the nucleus.

A number of studies have demonstrated a definite relation between the effect of ion and water shifts between the nucleus and the cytoplasm and the activation of nuclear activity. In Dipteran salivary glands, Loewenstein et al. (56) have demonstrated an appreciable resistance of the nuclear envelope. Furthermore, the permeability of the nuclear envelope undergoes changes during development (42; also 41). The change in permeability is accompanied by a change in total DNA content and total protein and is related to the effect of ecdysone. The relationship between the changes mentioned, and the action of the chromosomal puffs, may well be dependent upon ion flow into the nucleus (49, 50, 52, 56). In this connection, Kroeger (49) states that the "genetic loci activated in vitro by ions are also activated by these ions in normal development and that ecdysone exerts it effect on the puffing pattern by stimulating the sodium pump; the consequential change in the internal ion balance of the cell activates the re-

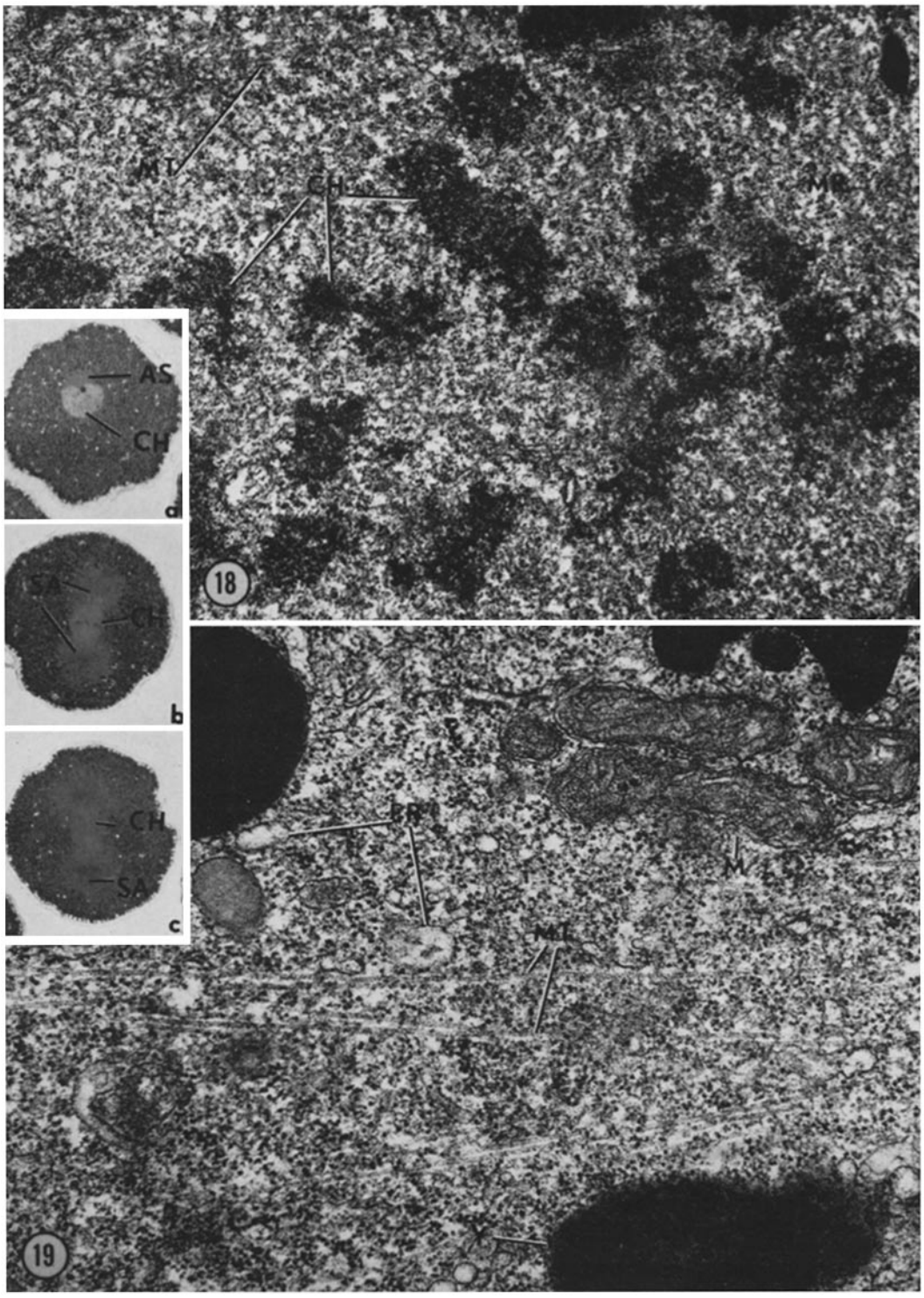

FIGURE 18 An electron micrograph of an egg at early anaphase, depicting the chromosome (*CH*), associated with microtubules (*MT*) and ribosomes (*MR*), 110 min postactivation. × 27,000.

FIGURE 19 A section through a mitotic figure at anaphase, 110 min postactivation, showing mitochrondria (*M*), endoplasmic reticulum (*ER*), yolk (*Y*), microtubules (*MT*). × 34,240.

Insets a, b, c Photomicrographs of artificially activated eggs at 95 minutes (*a*) prophase, 100 min (*b*) metaphase, and 105–115 min postactivation (*c*) anaphase. *CH*, chromosome; *AS*, aster; *SA*, spindle apparatus. *a*, *b*, *c*, × 400.

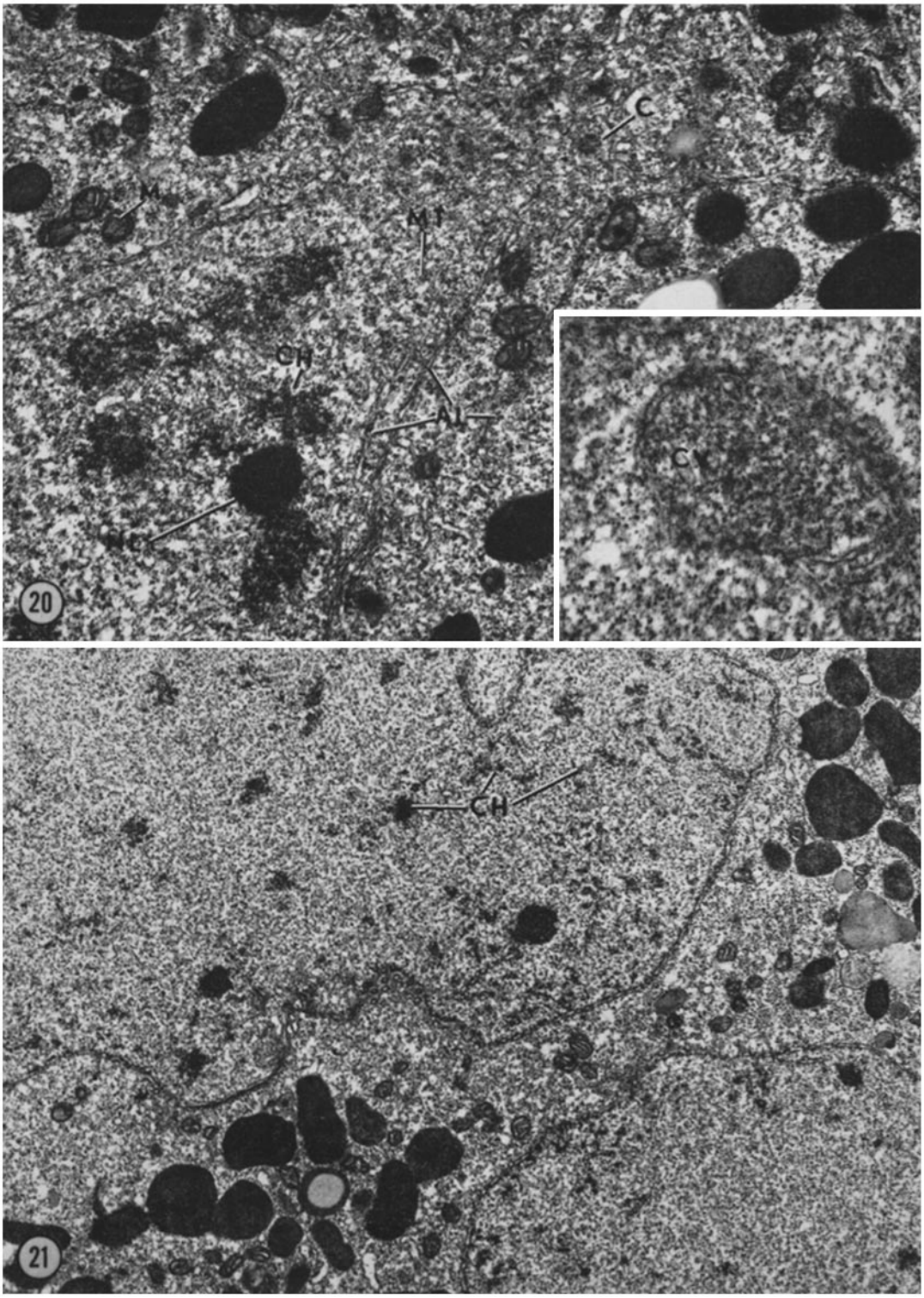

FIGURE 20 An electron micrograph through telophase stage of mitosis of an embryo (110–120 min postactivation). *CH*, chromatin; *C*, centriole; *MT*, microtubules; *AL*, annulate lamellae; *NL*, nucleolus-like body; *M*, mitochondria. *Inset* is an electron micrograph demonstrating the perforated membrane-bounded, chromosome-containing vesicle (*CV*). Fig. 20, × 10,600; *Inset*, × 34,000.

FIGURE 21 A section through an embryo (120 min postactivation) illustrating the membrane-bounded nuclei and chromatin (*CH*). × 17,000.

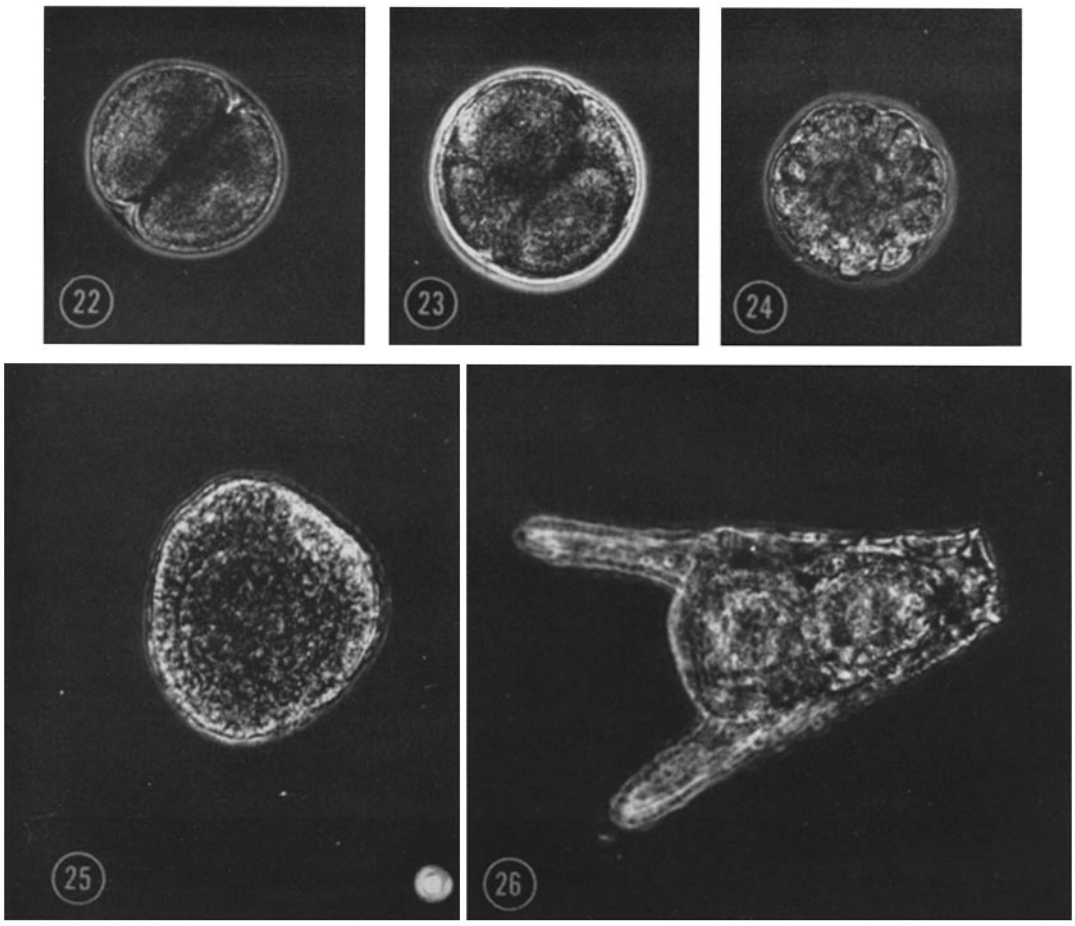

FIGURE 22 A phase-contrast photomicrograph of a two-cell stage, living embryo (3½ hr following activation with hypertonic seawater). × 250.

FIGURE 23 A phase-contrast photomicrograph of a four-cell stage, living embryo (4½ hr following activation with hypertonic seawater). × 400.

FIGURE 24 A phase-contrast photomicrograph of a living multicellular embryo (10–12 hr following activation). × 300.

FIGURE 25 A phase-contrast photomicrograph of a living early gastrula (24–30 hr postactivation). × 500.

FIGURE 26 A phase-contrast photomicrograph of a living, swimming pluteus larva (48 hr following activation). × 500.

spective genes." Further evidence of the effect of ion and water changes in the nucleus on the initiation of nuclear activity have been described by Harris (23–25). In hybrid cells in which erythrocyte nuclei become associated with HeLa cell cytoplasm, Harris (23–25) notes changes in nuclear volume and chromatin dispersion. He suggests that the nuclei are affected by shifts of water and electrolytes across the nuclear envelope. He has found these effects in hybrid cells regardless of species differences. Another example of the possible effects of egg cytoplasm in inducing a change in the nuclear volume and concomitant nuclear activity is in the nuclear transplantation studies. Swelling of the transplanted nuclei is often observed (19, 20, 21, 84) and is found to precede a period of DNA synthesis. The swelling may directly affect the chromosomes or, as Gurdon (20) sug-

gests, may induce the transplanted nuclei to be susceptible to a cytoplasmic factor which thus results in DNA synthesis.

It is not unusual for physical agents, such as ion and water balance, light, heat, etc, to control cellular activity. The ability to artificially synchronize cellular activity in many organisms is a demonstration of the effect of a change in the physical environment of the cell that results in a regulation of cellular activity. Synchronization represents a specialized form of intracellular communication between nucleus and cytoplasm, resulting in a dramatic demonstration of the interdependence of these two cellular compartments.

In many cases, the eggs of some organisms remain diploid after artificial activation. Presumably the diploid condition is achieved by the retention of the second polar body in the final division of maturation (see 12). In the case of *Arbacia*, the egg is shed in the haploid pronuclear stage. We have not been able to ascertain the ploidy state of the nuclei of the artificially activated eggs. It would be of great interest to analyze the replication of DNA prior to first cleavage and in ensuing cleavages to determine if and when the diploid amount of DNA is restored.

Centrioles

The aster in the artificially activated egg is composed of a multitude of vesicular structures embedded in a matrix which also contains microtubules and annulate lamellae oriented around a centriole.

In their investigations, Anderson (2), Longo and Anderson (57), Harris (28), and Verhey and Moyer (92) did not report centrioles in the unactivated egg of *Arbacia*. The possibility exists that centrioles are present in the unactivated egg (see 94), but the alternative possibility, i.e. that one is organized from submicroscopic precursors, is strongly suggested. Evidence for centriole morphogenesis has been demonstrated in the work of Dingle and Fulton (8) and Schuster (81) on the protozoan *Naegleria*.

The artificially activated egg has been demonstrated to contain a centriole prior to the first cleavage (9, 80). The present study illustrates the presence of centrioles before and during the first cleavage, as well as in the four-cell stage embryo, multicellular embryo, and blastula.

In the artificially activated egg, the presence of centrioles and their replication may provide the catalyst for the organization of monomers into those microtubules that constitute the spindle apparatus. Microtubules have been seen emanating from the lateral surface of centrioles, or satellites associated with centrioles (5, 77, 85). Moreover, when the cell is treated with colchicine, low temperature, or hydrostatic pressure, or when the cell is not in mitosis (78), pieces of microtubules may be found associated with the centriole (87). Thus, both the centrioles and microtubules, owing to their consistency in the form they assume and in their temporal appearance within the cell, lend themselves to the assembly theory. The temporal and spatial relationship strongly suggests that the centriole does orient microtubular assembly in the formation of the aster. We have observed, in some artificially activated eggs, the presence of many cytasters (10). We were not able, however, to determine if all these cytasters were centered around centrioles. The fate of these eggs with multiple cytasters was not determined.

Voluminous studies on the origin of basal bodies (possessing centriolar architecture) indicate that procentrioles develop further into basal bodies of cilia (11, 17, 46, 82, 83). The procentriolar structure has also been discussed in the formation of centrioles in the sperm of the water fern *Marsilea* (66). The aforementioned study lends further support to our suggestion that centriole formation in the artificially activated egg is a process of assembly in various steps to produce what may be then morphologically identifiable as a centriole.

Parthenogenesis

The production of an embryo from a female gamete without any genetic contribution from a male gamete, or parthenogenesis (natural or artificial), is found in many phyla, including Echinodermata, Mollusca, Annelida, Arthropoda, Rotifera and Chordata (3, 32). The ubiquity of this phenomenon, together with the fact that in many cases the embryo produced is functionally similar to that produced as a result of fertilization, emphasizes the egg's capability to support development. In the present study, "normal"-appearing plutei developed upon artificial stimulation. The development to the pluteus larva does not assure one that development to the adult sea urchin will follow, but it does suggest that within the machinery of the mature egg the potential for all of the events necessary for larval development is present.

Tyler (88) has stated that "While the discovery

of artificial parthenogenesis did not bring the realization of the early hopes that problems of fertilization would be readily solved, it has greatly enlarged the scope of the attack on the problem of activation by substituting relatively simple chemical and physical agents for the spermatozoon."

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