A FINE STRUCTURAL INVESTIGATION OF SURFACE SPECIALIZATIONS AND THE CORTICAL REACTION IN EGGS OF THE CNIDARIAN *BUNODOSOMA CAVERNATA*

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ABSTRACT

Developing oocytes of the cnidarian *Bunodosoma cavernata* are located within the mesoglea of the mesenteries of the gastrovascular cavity. The cortex of the more mature vitellogenic oocytes contains numerous, electron-dense, membrane-bound, cortical granules. The surface of these oocytes possesses prominent radially projecting structures termed cytospines. Each cytospine has a core of microfilaments, 50–70 Å in diameter, that extends basally as a rootlet through the cortical layer. During spawning, ova lacking any extraneous investments are released from the enclosing gastrodermis. As a consequence of fertilization or events associated with the earliest stages of development the ova undergo a massive cortical reaction. This reaction, which occurs during or just after release of the ova, involves extensive reorganization of the cortical layer. The cortical granule membranes and egg surface membrane fuse and vesiculate resulting in the massive discharge of granule contents. This event is accompanied by the loss of vesicular remnants of cortical ooplasm and the disruption of cytospine organization. Light and electron microscope comparisons of unreacted and reacted eggs show that the reaction results in a significant decrease in egg diameter with the oolemma of the reacted egg reorganizing in a position centripetal to its original location.

INTRODUCTION

Many excellent fine structural investigations centered on the final stages of gamete development and/or gamete interaction during fertilization have been presented in recent years. With the exception of a few, these investigations are confined to those invertebrate groups in which the spermatozoon has a well-defined acrosome and the egg possesses an investment(s) that must be traversed before gametic union (see reference 6 for review).

In view of this it is interesting that several recent fine structural studies on acoelomate invertebrates reveal that the spermatozoa characteristically lack well-defined acrosomes. These include one poriferan (34) and several members each of the hydrozoans (2, 18, 19, 23, 31–33, 37), scyphozoans (3, 19), and anthozoans (13, 19). Although it is reported that the eggs of the poriferans and cnidarians in general lack egg investments other than the egg plasma membrane (27),

there is only preliminary ultrastructural evidence on the egg surface and cortex in these groups (12, 29, 32).

Furthermore, no studies deal specifically with the cortical reaction in eggs that lack investments. In those organisms having eggs with investments as well as cortical granules which undergo a distinct cortical reaction, the investments are usually affected in some way by the reaction. For example, the release of cortical material, whether during a cortical reaction in response to fertilization (5, 6, 9, 17, 20, 35, 38) or to other factors such as exposure to sea water (25, 26), is often associated with the elevation and/or structural and chemical change of the investment. These are probably several functions involved in this interaction between the investment and the released cortical material. Whatever functions are proposed, however, they should be considered in light of those animals whose eggs are limited solely by an oolemma. For this reason the examination of a more primitive condition, such as that exhibited by the cnidarian *Bunodosoma cavernata*, may lead to a clearer understanding of the significance of the cortical reaction in other organisms.

MATERIALS AND METHODS

Adult animals were collected near Port Bolivar on the Texas Gulf Coast and maintained in the laboratory in shallow aquaria. Preparations of ovary were obtained by dissecting the animal and transferring portions of the mesentery containing ovary to fixative. Fixation was carried out for 1 h at room temperature in the paraformaldehyde-glutaraldehyde (pH 7.4) mixture recommended by Karnovsky (21). Subsequently, the preparations were washed with sea water and postfixed in 1.0% osmium tetroxide in sea water for 1 h at 4°C. The tissue was then rapidly dehydrated in a graded acetone series, embedded in a low viscosity epoxy resin (30), and sectioned on a Sorvall MT-2 ultramicrotome. Thin sections were mounted on uncoated grids, stained with alcoholic uranyl acetate and lead citrate (36), and observed with an AEI EM-6B, Hitachi HS-8, or Hitachi HU-11B electron microscope. Thick sections (1 μm) for general light microscopy were stained with 0.25% aqueous toluidine blue in 0.15% sodium borate.

Animals collected in the late spring, when spawning occurs in natural populations, would occasionally exhibit a spawning reaction in the laboratory within 24 h after collection. For this study spawned eggs were obtained: (1) from the bottom of aquaria or from the oral cavity of spawning females which were (*a*) separated from males or (*b*) with males that failed to spawn and, (2) from the oral cavity of spawning females in the same aquaria with spawning males. Eggs so obtained were fixed according to the techniques already described. Those eggs used for determining any possible changes in egg diameter as a consequence of the cortical reaction were obtained from the same female, one that released both reacted and unreacted eggs (see Results). Measurements were made on whole eggs embedded in flat embedding molds.

RESULTS

The sexes are separate in *B. cavernata*. In females the developing oocytes are located within the mesoglea of the incomplete mesenteries just radial to the septal filaments (Fig. 1). The loose layer of mesoglea that separates the oocytes from the overlying gastrodermis also extends between the developing oocytes throughout the gametic region. The vitellogenic oocyte has a very large germinal vesicle with a single, discrete, electron-dense nucleolus (Figs. 1, 2). Mitochondria, lipid-like inclusions, annulate lamellae, and both membrane- and nonmembrane-bound fibrous bodies are commonly present (Figs. 2, 3). With continued growth yolk bodies fill the cytoplasm eventually occupying all but the cortical region of the oocyte (Figs. 2, 3). Compared to the cortical granules the yolk bodies are generally larger, more electron dense and less homogeneous as a result of electron-lucent inclusions. The cortical granules are spherical, homogeneous, and membrane-bound (Figs. 3–5). Although their apparent abundance at the oocyte surface depends upon the angle of section and the degree of maturation, they fill the outermost cytoplasm to a depth several times their diameter in the nearly mature oocyte and egg. Regardless of the state of maturity some cortical granules discharge their contents to the outside (Figs. 3, 5). Golgi elements involved in the packaging of the cortical granules, as well as other oocyte constituents such as yolk, are visible (Fig. 4).

The surface of the nearly mature oocyte and egg possesses striking spike-shaped projections (Figs. 3, 6, 8). These structures, perhaps best described as cytospines (see Discussion) form aggregates of 20–30 members whether the cell is confined within the ovary or free after spawning (Figs. 3, 6, 8, 10). Individual cytospines are 15 μm in length (Figs. 6, 9, 21) and possess a core of filaments, 50–70 Å in diameter (Figs. 7, 11). This core extends basally as rootlet through the cortical layer (Figs. 6, 7, 11). In describing an aggregate of cytospines the term "spine" is occasionally employed since it appears both in early

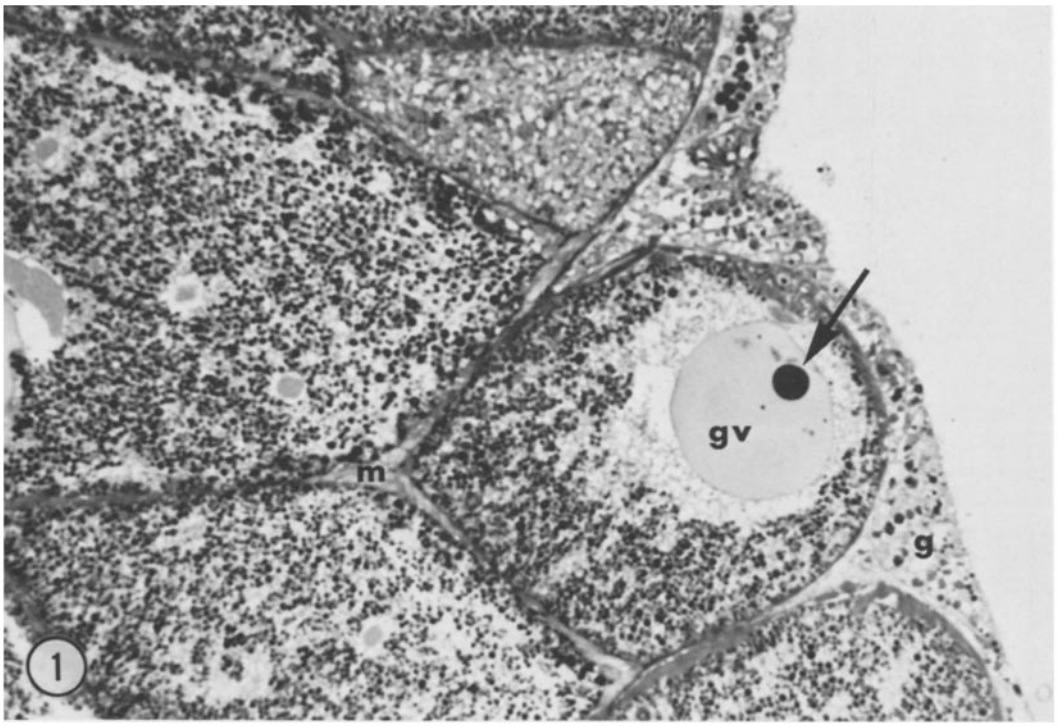


FIGURE 1 A light micrograph showing oocytes lying within the gastrodermis of the incomplete mesenteries of the gastrovascular cavity. Mesoglea separates the oocytes from one another and from the overlying gastrodermis. *g*, gastrodermis; *gv*, germinal vesicle; *m*, mesoglea; arrow, nucleolus. × 1,200.

and recent light microscope studies in reference to their appearance at the magnification level of the light microscope (11, 15, 16, 28).

The germinal vesicle probably breaks down before egg release since it is never present in spawned eggs, whether or not they have undergone a cortical reaction. We have not yet observed polar bodies associated with the surface of the egg. Perhaps the mechanical disturbance incurred during the release of the eggs from the fibrous mesoglea results in the loss of these bodies from the egg surface. With the exception of the nonmembrane-bound fibrous bodies all the organelles and inclusions characteristic of the ovarian oocyte are also found in the spawned egg (compare Figs. 2, 3 with Fig. 8). Notably, however, the cortical granules in the spawned egg have a less spherical contour than those in the ovarian oocytes (compare Fig. 4 with Figs. 8, 11).

Eggs that had undergone a cortical reaction were obtainable only when both sexes spawned simultaneously. For this study the reacted eggs were collected by inserting a Pasteur pipette into the mouth opening of the female and withdrawing the eggs as they were released into the gastrc vascular cavity. Some of the eggs thus obtained were still loosely bound by ovarian tissue (W. C. Dewel, unpublished data). Of these, several showed a cortical reaction over only a portion of their surfaces (Fig. 12). It is important to note that only those eggs which displayed a cortical reaction developed normally. From samples taken before fixation we found that normal cleavage occurred in greater than 90% of those sampled (three replicates, 10 eggs per replicate). Many of those embryos not fixed developed to the planula stage (W. C. Dewel, unpublished data). Eggs which had not undergone a cortical reaction were collected both in the manner described above and from the bottom of aquaria either when females spawned before males or when males failed to spawn. If a female began spawning before any males it was possible to obtain both unreacted (before male's spawning) and reacted (after male's spawning) eggs from the same animal. Of the eggs released at spawning unreacted ones never developed and all attempts at in vitro fertilization were unsuccessful even when they

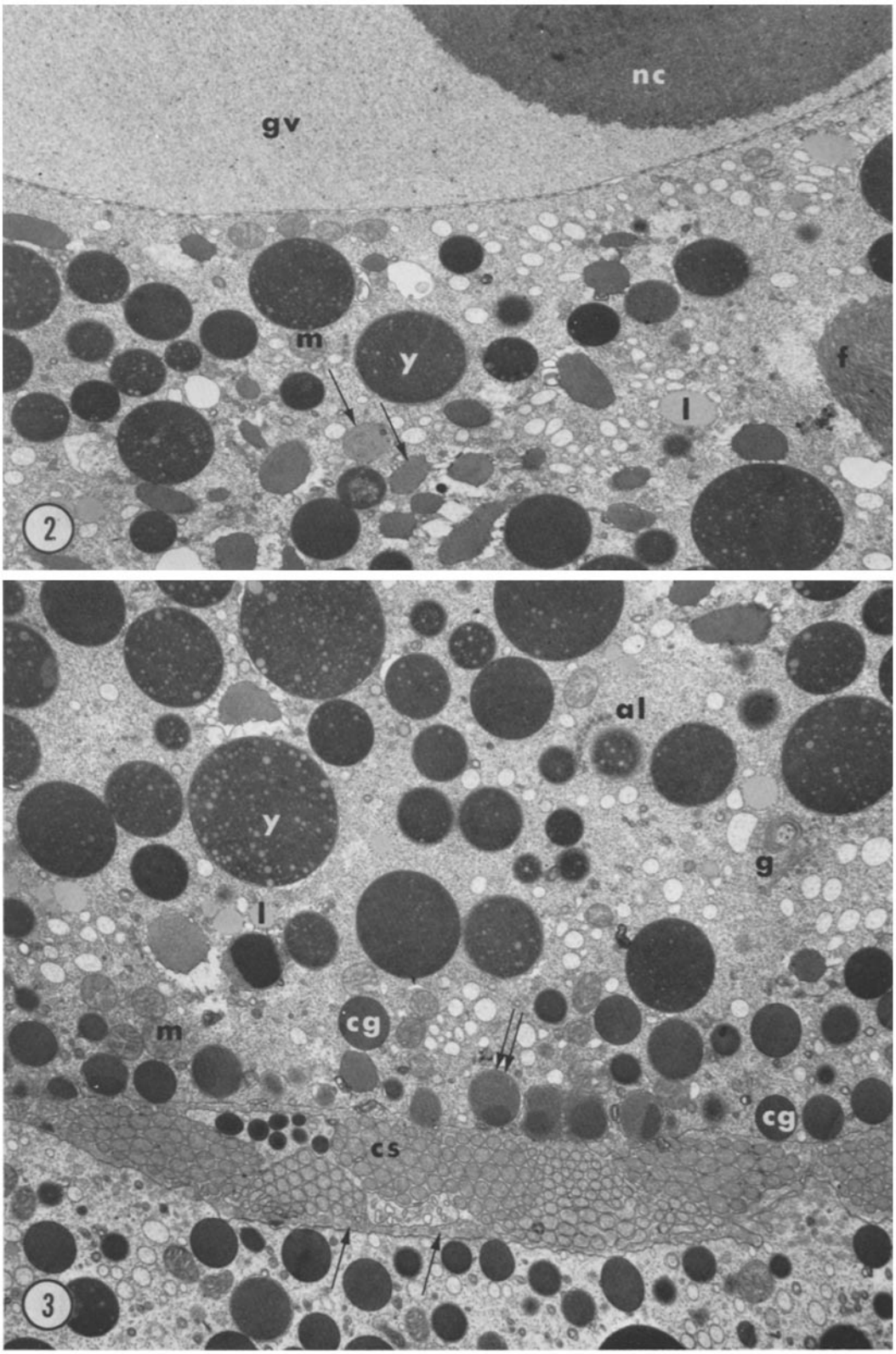


FIGURE 2 An electron micrograph of the nuclear region of an oocyte. *gv*, germinal vesicle; *nc*, nucleolus; *y*, yolk; *l*, lipid-like body; *m*, mitochondrion; *f*, fibrous inclusion; arrows, membrane-bound fibrous bodies. × 9,200.

FIGURE 3 An electron micrograph showing the cortical region of a maturing oocyte. *y*, yolk; *m*, mitochondrion; *g*, Golgi; *l*, lipid-like body; *al*, annulate lamellae; *cs*, cytospines; arrows, mesoglea; *cg*, cortical granule; double arrow, discharging cortical granule. × 9,600.

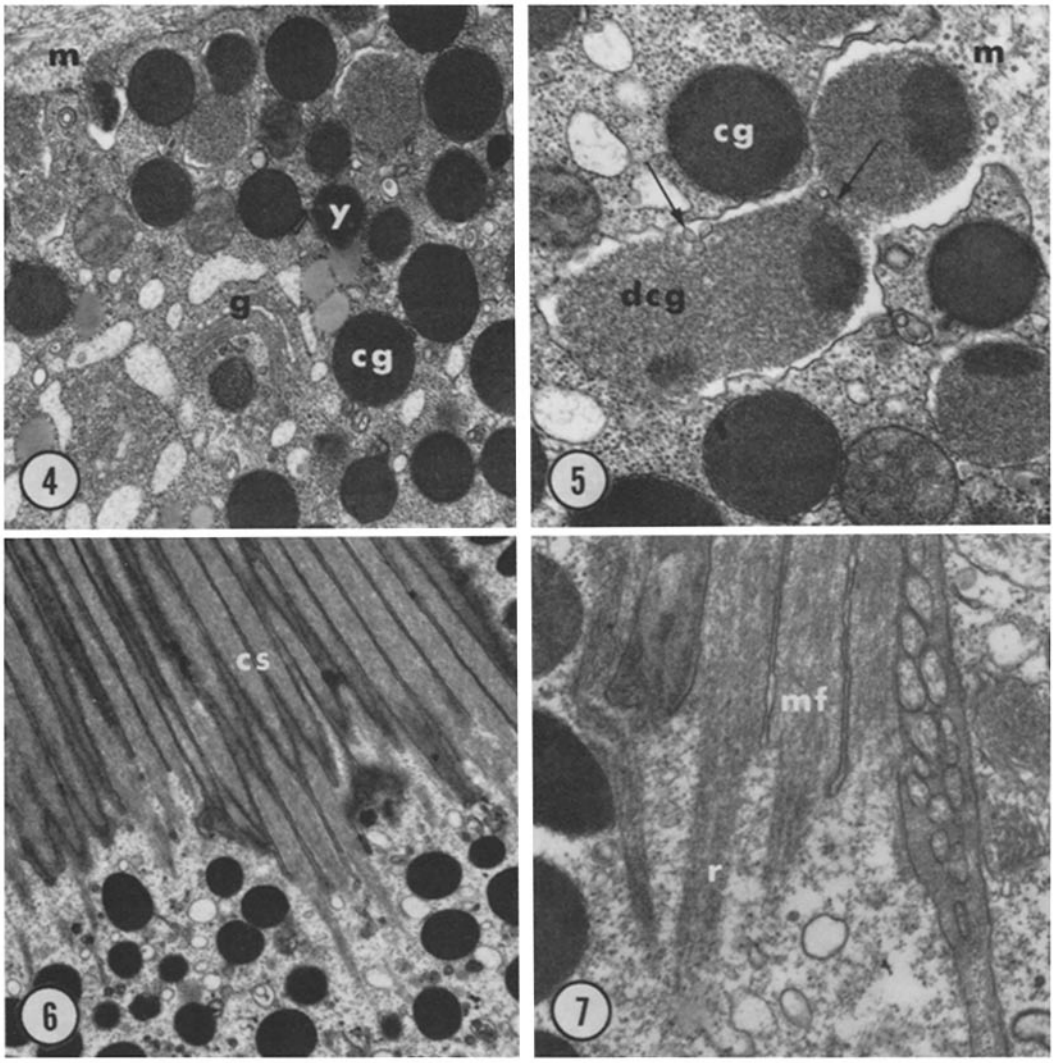


FIGURE 4 A section showing Golgi elements associated with developing yolk and cortical granules. *y*, yolk; *cg*, cortical granule; *g*, Golgi; *m*, mesoglea. × 14,800.

FIGURE 5 The oocyte surface showing cortical granule discharge. The cortical granule at the surface has undergone fusion and vesiculation with oolemma while those subjacent to it have fused and vesiculated with the peripheral cortical granule. *cg*, cortical granule; *dcg*, discharging cortical granule; *m*, mesoglea; arrows, vesicles. × 23,200.

FIGURE 6 A low magnification electron micrograph of the basal region of the cytospines present on the ovarian oocyte. *cs*, cytospines. × 8,800.

FIGURE 7 A higher magnification of the cytospine. Note the core of microfilaments which extend as a rootlet into the ooplasm. *mf*, microfilaments; *r*, rootlet. × 26,200.

were exposed to increasing concentrations of sperm from males responsible for successful fertilization (i.e., sperm from males in adjoining aquaria where eggs underwent cleavage and developed to the planula stage).

Except for the cortical region, the cytoplasm of the reacted egg is similar to that of the unreacted egg with respect to density and presence and disposition of cellular organelles and inclusions (Figs. 12, 19). However, in the cortical region the major

portion of the cortical granules undergoes a marked change as the reaction takes place. The membranes of those granules adjoining the egg surface fuse and vesiculate with the egg plasma membrane (Fig. 12). Concomitantly the membranes of subjacent granules fuse and vesiculate with the membranes of each other and/or those of already discharging granules (Figs. 12–14). As a result several cortical granules may share a common matrix interrupted only by plate-like vesicular areas marking the original membranous boundary (Fig. 13). Fusion between granules does not necessarily result in structural changes in the enclosed contents (Fig. 13). Apparently contact of this cortical granule material with the external milieu results in its morphological alteration. Initially the material loses its homogeneous appearance and exhibits a granularity of two different densities (Figs. 12, 14). Subsequently, this material becomes a dispersed flocculent that moves apically between the cytospines (Figs. 12, 15). This flocculent does not immediately dissipate but forms a layer of material over the egg surface (Figs. 17–19).

Significantly, somewhat comparable events also occur in the unreacted egg, as well as in the ovarian oocyte (Figs. 3, 5, 8, 11), but involve only peripheral granules, usually on an individual basis and seemingly always without previous fusion. Evidently, the discharge is at such a reduced rate that the relative number of granules remains essentially unchanged for several hours (W. C. Dewel, unpublished data).

The extensive fusion and vesiculation of cortical granule membrane with egg plasma membrane as well as with subjacent cortical granule membrane establishes a honeycombed collection of chambers or channels over the cortical region of the egg (Fig. 14). The channels thus formed open to the outside between the cytospines (Figs. 12, 15). Excluding the membrane of the spines, the limiting membrane of the egg is now a highly convoluted mosaic of both egg plasma membrane and cortical granule membrane. Qualitatively, the cortical granule membrane is by far the largest contributor to this mosaic. At first the developing surface appears extremely irregular (Figs. 16, 20) but later it exhibits a smoother profile (Figs. 18, 19). Confined outside this surface are numerous vesicular remnants of the original oolemma and cortical granule membranes (Figs. 12, 16, 19, 20). There is considerable variability in vesicle size. Even membrane-enclosed portions of the cortical cytoplasm with characteristic cellular constituents (e.g. mitochondria, lipid-like inclusions, endoplasmic reticulum, and ribosomes) are occasionally present (Figs. 16, 18, 20).

As a result of the reaction the newly organized oolemma lies centripetal to the former oolemma. In other words, the diameter, when it is measured from the surface of the egg (48 reacted and 50 unreacted) is significantly less ($P < 0.001$, Wilcoxon two sample test) in the reacted egg than in the unreacted egg (Fig. 21; compare also Figs. 8, 9 with Figs. 17, 19).

In addition to the above cortical changes the morphology of the basal region of the cytospines changes as a result of the cortical reaction. In the unreacted egg, the cytospines possess a dense core which consists of a compact bundle of microfilaments extending radially from the cortical region of the egg to the apical tips of the cytospines. During the early stages of the cortical reaction the cytospines seemingly increase their overall length as the cortical granules discharge between them and form channels or spaces which eventually surround their individual rootlets (Fig. 15). In later stages, though, their continuity with the newly organized oolemma appears virtually to disappear (Figs. 17–19). The zone formerly occupied by the cortical granules and cytospine rootlets becomes greatly disorganized as the reaction proceeds. Apical to this zone the microfilaments are somewhat dispersed in that area of cytospine where its membrane joined the oolemma before the reaction (Figs. 17, 19, arrows). Basal to this zone the number of rootlets visible in what is now the cortex of the reacted egg is greatly reduced, perhaps reflecting the absence of all but that portion of the rootlet which penetrated beneath the cortical layer before the reaction (Fig. 18, white arrow, 19).

DISCUSSION

It is well known that marine eggs commonly display surface specializations such as microvilli. However, the surface specializations on the eggs of some anthozoans are exceptional with respect to size and arrangement. In several light microscope studies (10, 11, 15, 16, 28) the term spine is used to describe these exceptional structures. However, the results of this study, as well as two other fine structural examinations (12, 29), reveal certain limitations with this term. For instance, a spine customarily refers to the shape of an aggregate as resolved at the light level and not to the structure

of the individual members. In addition, the term does not reflect the cytoplasmic nature of these specializations. To overcome these limitations we propose adoption of the term "cytospine." The use of microvillus as a possible alternative is less appropriate since these structures are not sufficiently similar to microvilli in respect to size, arrangement, and rich microfilamentous content. However, adopting the term cytospine not only would allow for the continuation of differentiation between spiny anthozoan eggs and those with a smoother microvillous surface but also would effectively designate the unit structure and thus facilitate the description of individual cytospine variability among all spiny eggs in terms of their size, arrangement, and fine structure.

Many investigators have described cortical reaction in the eggs of marine invertebrates (1, 4–6, 22, 24). In the case of *B. cavernata* we have not definitely established what event initiates cortical granule discharge. Nevertheless, we do know that spawning females that are either isolated or with nonspawning males produce eggs that (*a*) do not display a cortical reaction, (*b*) cannot (at least to date) be fertilized in vitro, and (*c*) never undergo development. In contrast, spawning females held in the same aquaria with simultaneously spawning males produce eggs which display a cortical reaction and which undergo normal cleavage. Furthermore, these reacted eggs are obtainable from the gastrovascular cavity of the female and in many cases they are still in clusters held together by layers of mesoglea. These considerations lead us to conclude tentatively that (*a*) the cortical reaction can take place internally on or just subsequent to egg release and (*b*) the cortical reaction is in response to fertilization or as yet unknown events related to the earliest stages of development, but not in response to release or exposure to sea water as in certain other species (25, 26).

The cortical reaction in *B. cavernata* involves the multiple fusion and vesiculation of cortical granule membranes with each other as well as with the egg plasma membrane. The mechanism of this reaction is not unusual; it is apparently similar to that described by Barros et al. (8) in the acrosome reaction of a mammal, namely "the occurrence of multiple unions between two cellular membranes lying in close apposition, with the formation first of a double-walled fenestrated layer and ultimately of an array of separate membrane bounded vesicles." The basic process was also found in the cortical reaction of the egg of the sea urchin *Arbacia punctulata* (5).

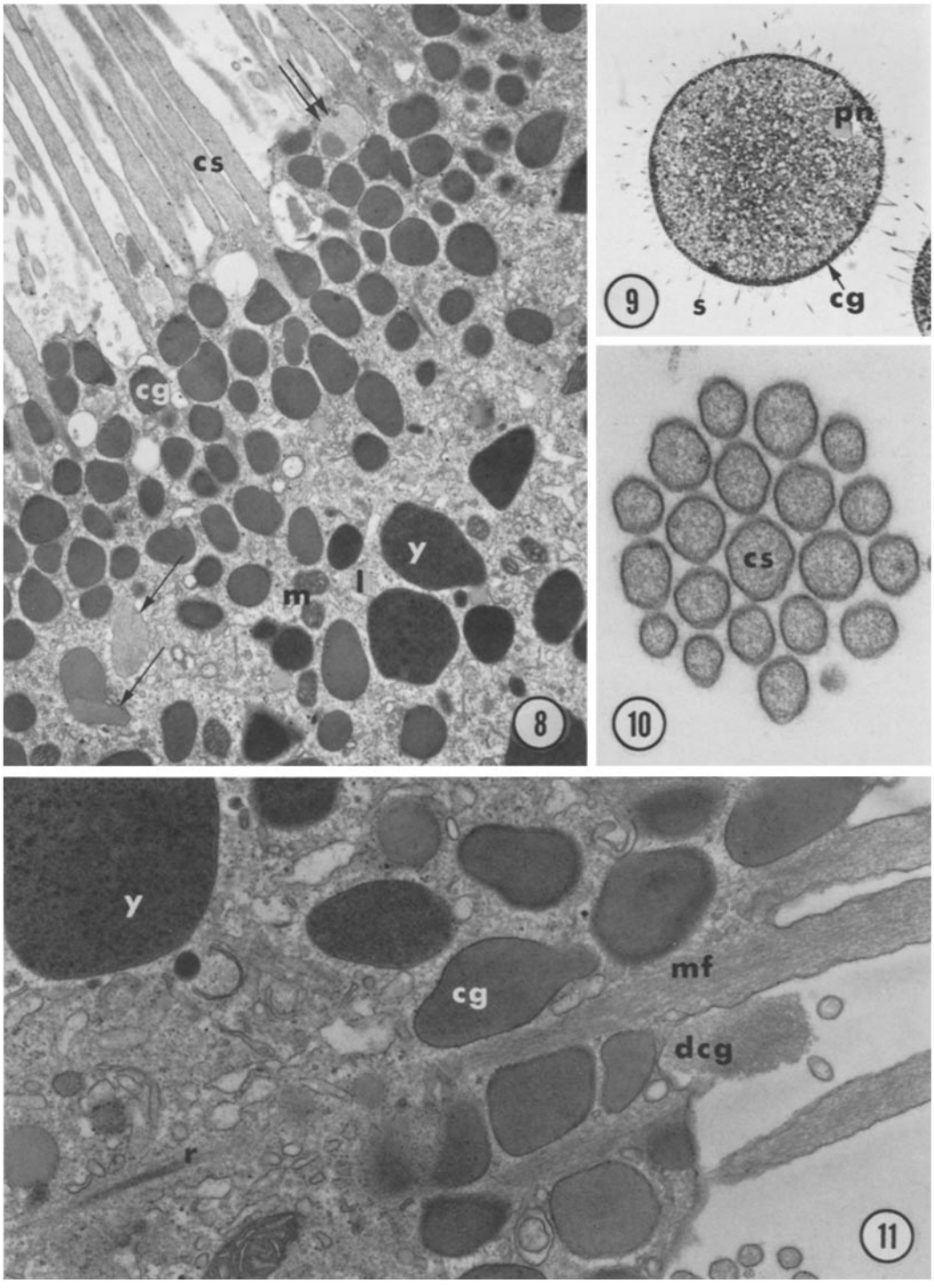
However, in the case of *B. cavernata* a number of unusual characteristics merit discussion. One is the extraordinary massiveness of the reaction. It is perhaps, with the exception of the eggs of *Nereis limbata* (14), unparalleled in terms of the amount of cortex involved. The extensiveness is a result of several factors including the considerable thickness of the cortical layer and the high density of granules within the layer together with the seemingly simultaneous fusion and vesiculation of granule membranes with the oolemma and with each other. Although it is difficult to determine the precise sequence of fusion, there are examples of completely fused granules which do not appear to have established any contact with the outer environment (Fig. 13).

FIGURE 8 The surface of the released but unreacted egg. Note the thick layer of cortical granules. The double arrow points to a cortical granule which is discharging its contents to the outside. Most of the cortical granules, however, remain undischarged. Compare this micrograph with the reacted egg shown in Fig. 19. *y*, yolk: *m*, mitochondria; *l*, lipid-like body; *cg*, cortical granule; *cs*, cytospines; arrows, membrane-bound fibrous bodies. × 7,400.

FIGURE 9 A light micrograph of a released but unreacted egg. Compare this micrograph with Fig. 17 showing the reacted egg. *pn*, pronucleus; *cg*, cortical granules; *s*, spines. × 310.

FIGURE 10 A cross section through a group of cytospines (*cs*) which taken together make up the spines visible at the light microscope level on certain anthozoan eggs. A coat of fibrillar material is visible on the outer surface of the cytospine membrane. × 45,000.

FIGURE 11 A longitudinal section through several cytospines on the surface of the released but unreacted egg. Note the rootlet extending through the cortical layer deep into the ooplasm. *y*, yolk; *cg*, cortical granule; *dcg*, discharging cortical granule; *mf*, microfilament; *r*, rootlet. × 21,300.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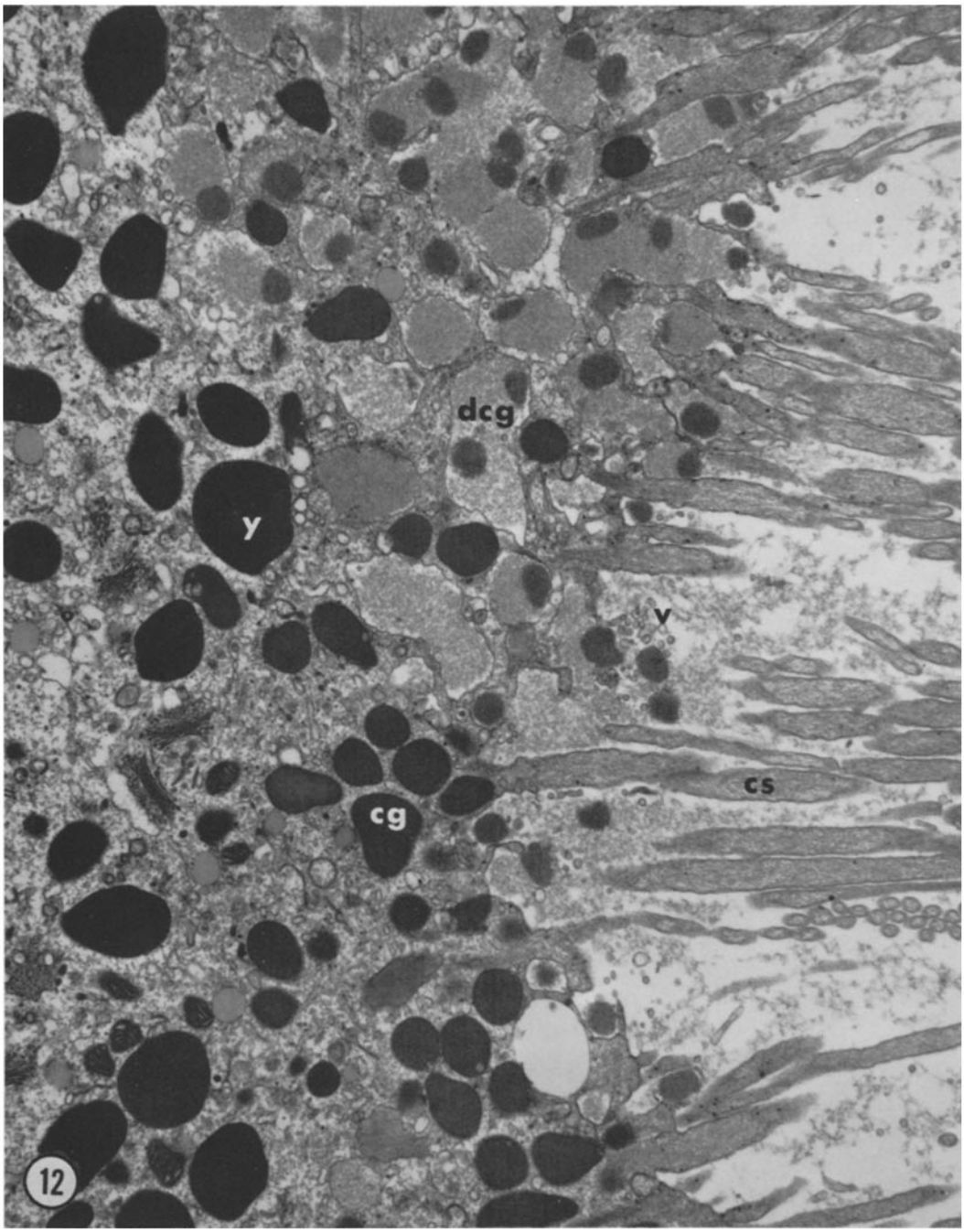

FIGURE 12 A micrograph showing an egg in which the cortical reaction has proceeded over only a portion of its surface. The zone of junction between unreacted surface (lower) and reacted surface (upper) is approximately in the middle of the micrograph. *cg*, cortical granule; *dcg*, discharging cortical granule; *cs*, cytospine; *v*, vesicle; *y*, yolk. × 11,700.

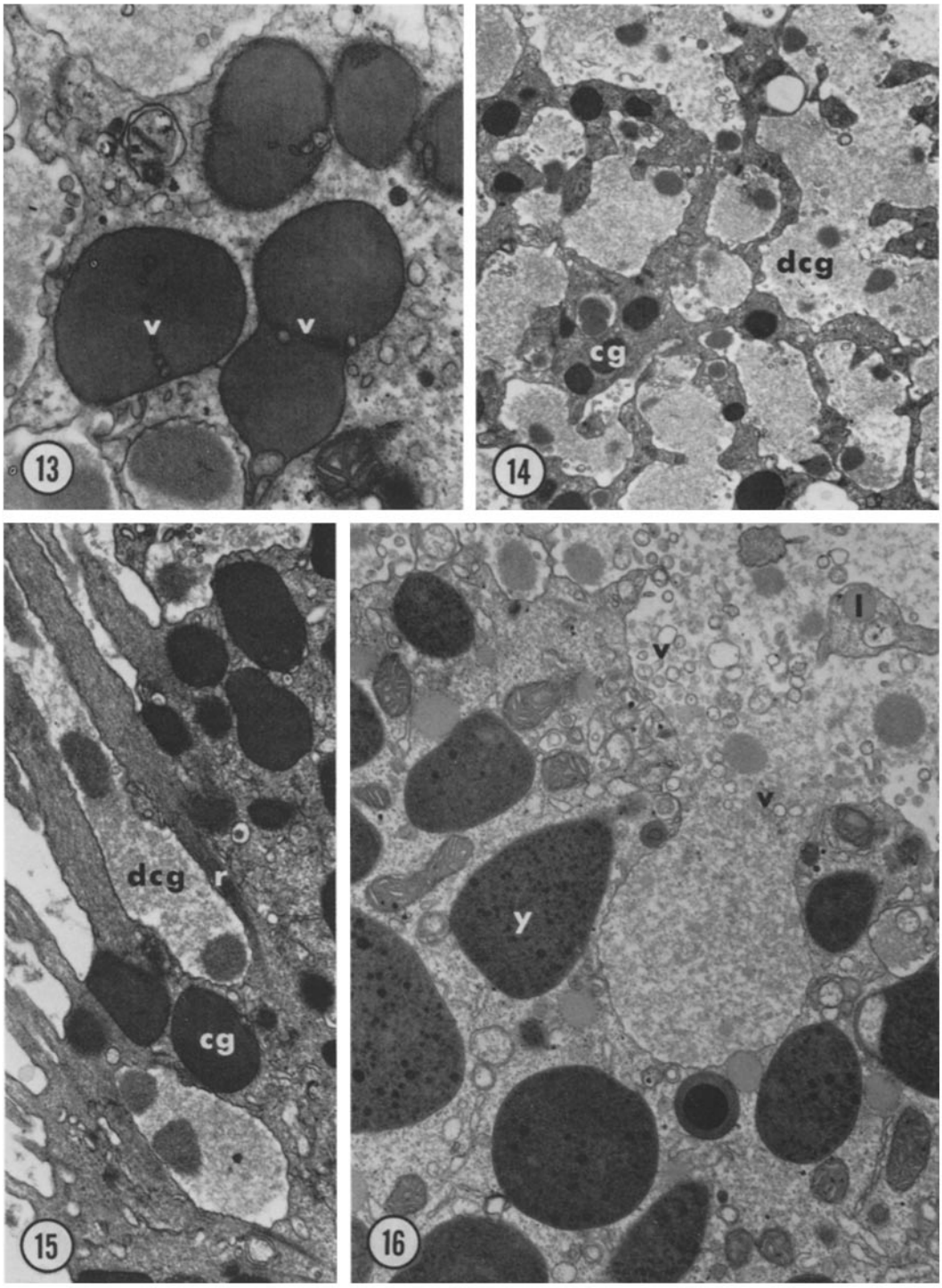

FIGURE 13 A section through a reacting egg showing the vesicular areas between fused but undischarged cortical granules. *v*, vesicles. × 26,100.

FIGURE 14 A tangential section through the egg surface showing its honeycombed appearance after the cortical reaction. *cg*, cortical granule; *dcg*, discharging cortical granules. × 8,900.

FIGURE 15 A micrograph showing the earlier stages of cortical granule discharge which is taking place between the cytospines. *cg*, cortical granule; *dcg*, discharging cortical granules; *r*, rootlet. × 16,300.

FIGURE 16 The surface of the reacted egg. Note the small membrane-enclosed vesicles which are abundant at the opening of a pocket formed by a discharging cortical granule. *y*, yolk; *v*, small vesicles; *l*, lipid-like body. × 15,500.

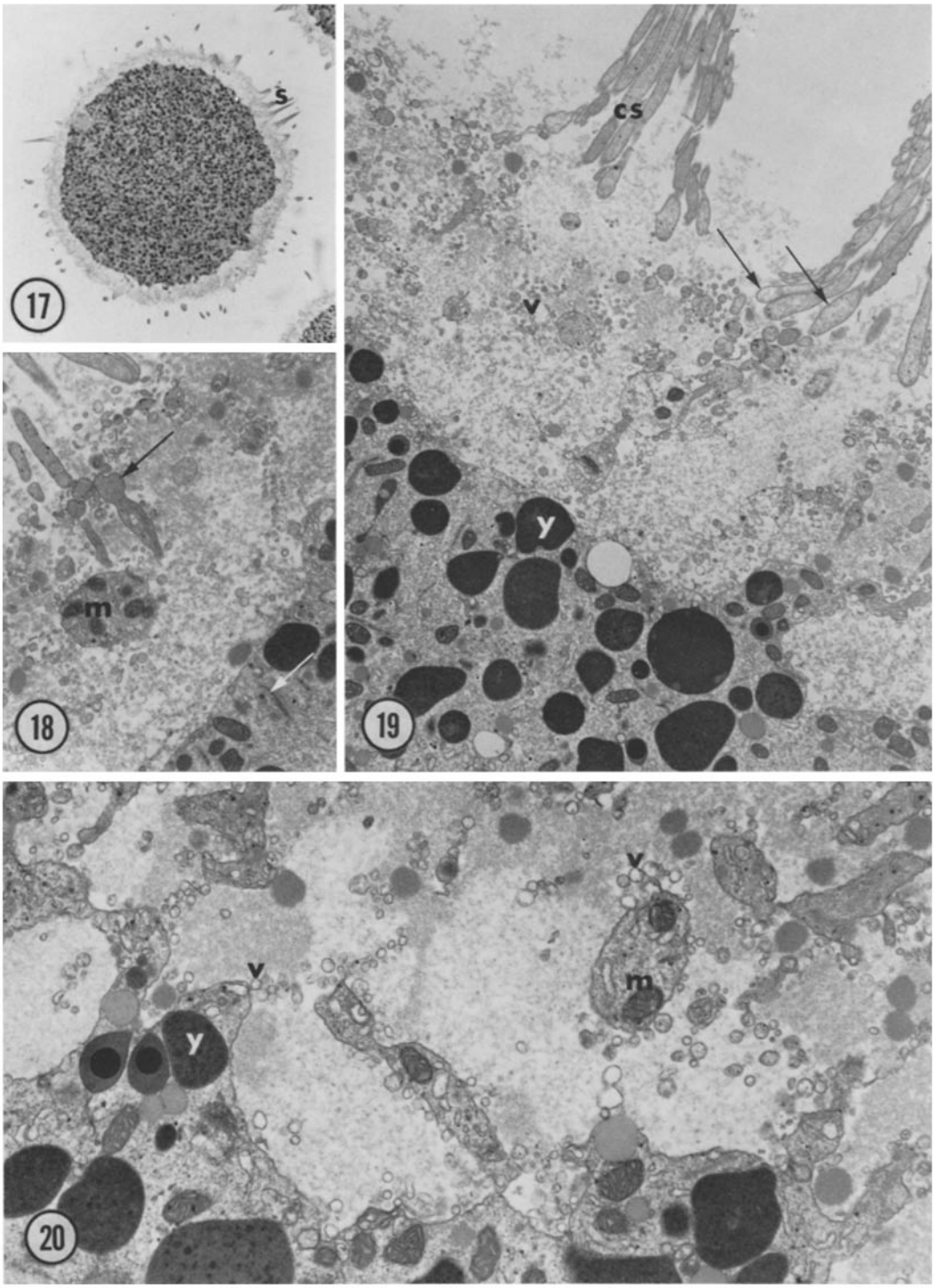

FIGURE 17 A light micrograph of the reacted egg. Compare this micrograph with Fig. 9. *s*, spines. × 310.

FIGURE 18 A section through the surface of the reacted egg. Note the large organelle-containing vesicles and the single rootlet (white arrow) visible in the cortical cytoplasm. At this stage the basal ends of the cytospines are swollen (arrow). See also Fig. 19. *m*, mitochondria. × 6,100.

FIGURE 19 Another section of the egg surface after the cortical reaction. The arrows point to the swollen bases of the cytospines. Their microfilamentous core is disrupted and their former organization has been lost. Compare this micrograph with the unreacted egg shown in Fig. 8. *cs*, cytospines; *y*, yolk, *v*, vesicles. × 4,600.

FIGURE 20 A higher magnification of the egg surface after the cortical reaction. Note the small vesicles (*v*) and the larger vesicles which contain cortical organelles. *y*, yolk; *m*, mitochondrion. × 12,900.

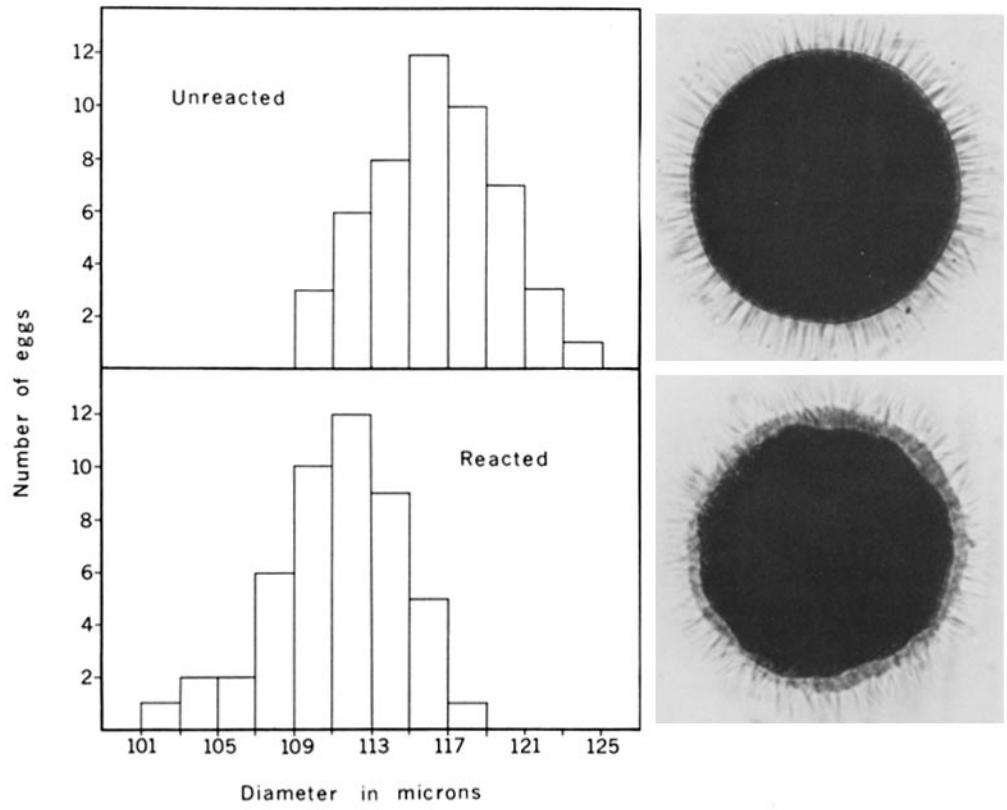

FIGURE 21 A comparison of the diameter of the unreacted and reacted eggs showing the decrease in egg diameter resulting from the cortical reaction (see Results). × 310.

Perhaps the interaction of the granule membranes during the reaction is independent of the development of continuity of the granule contents with the external milieu. Since fused granules exhibiting unaltered contents have rarely been observed in oocytes or in released but unreacted eggs, the process may be largely restricted to the cortical reaction. Evidence of previous fusion is also reported for the eggs of *N. limbata* (14).

Another notable feature is the absence of "all-or-none" behavior in the cortical granules. As previously indicated the granules occasionally discharge their contents to the outside both in developing oocytes and in released but unreacted eggs. The manner in which this occurs is apparently similar to that occurring during the cortical reaction. Perhaps it underlines an inherent lability or reactivity in the cortical granule and egg plasma membranes. Unfortunately, the immediate affects of fixation in possibly causing or augmenting any premature release of cortical material cannot be ruled out entirely. However, there is evidence that released but unreacted eggs held for longer than 18 h show a substantially reduced population of cortical granules (W. C. Dewel, unpublished data) presumably due to this process of gradual discharge. Regardless of whether the discharge is real or artifactual it cannot be overemphasized that it is not at all comparable to the overwhelming disruption of the cortex during the normal cortical reaction.

Another unusual characteristic of the reaction is the formation and subsequent loss of vesicles large enough to contain cellular organelles such as mitochondria and rough endoplasmic reticulum. Presumably these vesicles are derived from areas of cortical cytoplasm interstitial to the cortical granules. If there are multiple sites of fusion and if there is almost simultaneous interaction among adjacent membranes, then the production of these vesicles is easily visualized. Since the packing of granules within the cortex is not optimally close, it is apparent that significant amounts of ooplasm could be lost during the reaction. A quantitatively

similar loss does not occur in the eggs of certain sea urchins (5, 7) nor in the eggs of *N. limbata* (14) although in the latter small vesicular remnants are conspicuous in the perivitelline space. Notably, before the cortical reaction in *N. limbata*, the alveoli seem to be very densely packed with the intervening cytoplasm-lacking organelles such as mitochondria (14). This factor together with the reported preformation of large coalesced alveoli could conceivably reduce the chances of large vesicles becoming isolated during the cortical reaction.

Possibly the most puzzling aspect of the cortical reaction in eggs of *B. cavernata* is the morphological and functional relationship between the cortex and the cytospines. During the earliest stages of the reaction, when the cortical granules just begin to discharge between the cytospines, the integrity of the cytospines is apparently maintained. Nevertheless, there are indications that the microfilamentous rootlets are disrupted and disappear, that the cortical region becomes thoroughly disrupted as the reaction proceeds, and that the continuity of the cytospines with the egg is, at the very least, obscured. It is clear that the high degree of disruption and the unusual changes in cytospine structure, in particular the possibility of active participation by the microfilaments in surface and cortical reorganization, warrant further attention.

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