ULTRASTRUCTURAL LOCALIZATION OF CALCIUM IN UNFERTILIZED SEA-URCHIN EGGS

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SUMMARY

The pyroantimonate technique was employed to identify the binding sites for calcium in unfertilized Arbacia punctulata and Strongylocentrotus purpuratus eggs. Since antimony is non-specific and binds with a variety of cations, the identification of calcium was established by specific chelation with ethyleneglycol tetra-acetic acid (EGTA) and X-ray microprobe analysis. Antimony deposits were observed on the egg's membranes, i.e. plasma, cortical (secretory) granule, pigment granule, smooth-surfaced vesicle, and yolk platelet. Deposits were also observed in the mitochondria, rod-containing vesicles, and the vitelline layer. Two types of yolk platelets were observed: a more numerous electron-opaque platelet which had precipitate along its limiting membrane as well as within the stored-matrix substance, and a less-frequently seen platelet with lower electron opacity which contained precipitate only along its limiting membrane. Deposits were reduced at all sites following exposure of eggs to EGTA either prior to or after osmium-antimonate fixation. Initial fixation in glutaraldehyde followed by postfixation in osmium-antimonate solutions provided better preservation of structure but less precipitation than direct fixation in osmium-antimonate. The organelle sites of calcium binding identified within unfertilized sea-urchin eggs may participate in stimulus-secretion coupling (exocytosis of the cortical granules) and the activation of embryogenesis at fertilization.

INTRODUCTION

Stimulation of sea-urchin eggs by sperm at fertilization activates cortical granule exocytosis and initiates embryogenesis. These responses in the egg appear to be triggered by a substantial increase in the intracellular concentration of 'free' calcium ions (Steinhardt, Zucker & Schatten, 1977), which is believed to be a universal feature of the stimulation-response coupling mechanism in cells (Heilbrunn, 1956). The secretory product released from the egg's cortical granules at fertilization promotes the elevation of the fertilization membrane to complete the block to polyspermy (Longo & Schuel, 1973; Schuel, Wilson, Chen & Lorand, 1973; Schuel, Kelly, Berger & Wilson, 1974; Schuel, Longo, Wilson & Troll, 1976a).
Most of the calcium in unfertilized sea-urchin eggs is bound (Mazia, 1937; Nakamura & Yasumasu, 1974), although the subcellular location of the binding sites is unknown. While a calcium influx from the external seawater during cortical granule exocytosis at fertilization has been described (Nakazawa et al. 1970), the intracellular stores appear to be a major source of the free calcium. This concept is largely based on experiments with calcium ionophores, a class of antibiotics which selectively transport this cation across biological membranes. These agents, which trigger secretion in adult somatic cells (Foreman, Mongar & Gomperts, 1973), have also been used to stimulate cortical granule discharge and parthenogenetic development of unfertilized sea-urchin eggs (Chambers, Pressman & Rose, 1974; Schuel, Troll & Lorand, 1976; Steinhardt & Epel, 1974). Since the ionophore can elicit these responses in eggs cultured in calcium-free media, it would appear to mimic the stimulating action of sperm, at least in part, by liberating calcium from internal binding sites (Steinhardt et al. 1977). Similar phenomena have been observed with vertebrate eggs (Ridgeway, Gilkey & Jaffe, 1977).

Electron-microscopic cytochemistry has been employed to determine the intracellular location of calcium in a variety of differentiated somatic cells. The present communication describes an attempt to determine the ultrastructural location of calcium in unfertilized sea-urchin eggs by cation precipitation with pyroantimonate (Herman, Sato & Hales, 1973; Spicer, Hardin & Greene, 1968). There are 3 major limitations to the pyroantimonate technique (Herman et al. 1973; Simson & Spicer, 1975): (1) lack of specificity; (2) loss or translocation of cations during fixation; and (3) poor morphologic preservation. These problems have been dealt with, in part, by employing alternative methods of preparation and examination. Preliminary accounts of parts of this study have been presented previously (Cardasis & Schuel, 1976).

MATERIALS AND METHODS

Eggs from mature Strongylocentrotus purpuratus and Arbacia punctulata were collected by means of standard procedures (Schuel et al. 1973) in either artificial or calcium-free seawater at pH 8.1 (Goldstein, 1953). The viability of the eggs was determined by test fertilization.

Several fixation procedures (Herman et al. 1973; Spicer et al. 1968; Simson & Spicer, 1975) were employed in order to improve morphological preservation while retaining antimonate-cation deposits; direct fixation in osmium-pyroantimonate in distilled water, and glutaraldehyde fixation in seawater followed by either a short (15 min) or a long (overnight) seawater wash prior to postfixation in osmium-pyroantimonate. The calcium concentration in seawater is 10 mM. These procedures also served to elucidate cation loss and/or redistribution during processing.

The presence of calcium within the antimony deposits was evaluated by treating the eggs with ethylene glycol tetra-acetic acid (EGTA), a specific calcium-chelating agent (Baker, 1972), either prior to (Legato & Langer, 1969) or after osmium-antimonate fixation. Details of the preparative procedures are shown in Table 1. All groups of eggs were processed for routine electron microscopy using standard procedures; dehydrated in graded ethanol, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and examined with a Philips 300 electron microscope.

In a further effort to characterize the precipitates, X-ray microprobe analysis (Herman et al. 1973) was performed on sections (approximately 0.25 μm in thickness) from 3 blocks of group 2A eggs. The X-ray microanalysis data were obtained using a Philips model 301 electron microscope equipped with a eucentric goniometer stage and an EDAX-711 energy dispersive
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analyser interfaced with a Data General/Nova Computer. The conditions used to obtain the spectra were: 100 s analysing time, 60–80 kV, and 36° specimen tilt. The electron beam was made asymmetrical to probe areas of interest within the sections by means of the condenser stigmators. A control area (yolk platelet) was probed within the sections under the same conditions as were used for the analysis. The controls were run to compensate for any elements that might be introduced during sample preparation. The control spectra were subtracted from the spectra obtained from the areas of interest. The resulting spectra (sample-control) were designated ‘net-counts’ spectra. Additional X-ray microprobe analyses were performed on a Kent Cambridge Mark II Stereoscan microscope (20 kV) equipped with an EDAX analyser system. Eight analyses of deposits on the cortical granule and plasma membrane respectively were made.

Table 1. Details of preparative procedures

<table>
<thead>
<tr>
<th>Group</th>
<th>Collecting solution (1 h)</th>
<th>Primary fixation (1 h)</th>
<th>Wash</th>
<th>Secondary fixation (1 h)</th>
<th>Wash (1 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Seawater*</td>
<td>Osmium-antimonate†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>Seawater</td>
<td>Osmium-antimonate</td>
<td>EGTA§ (1 h)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Seawater</td>
<td>Glutaraldehyde‡</td>
<td>Seawater (overnight)</td>
<td>Osmium-antimonate†</td>
<td>EGTA§</td>
</tr>
<tr>
<td>B</td>
<td>Seawater</td>
<td>Glutaraldehyde</td>
<td>Seawater (overnight)</td>
<td>Osmium-antimonate</td>
<td>EGTA§</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Seawater</td>
<td>Glutaraldehyde‡</td>
<td>Seawater (15 min)</td>
<td>Osmium-antimonate†</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>Seawater</td>
<td>Glutaraldehyde</td>
<td>Seawater (15 min)</td>
<td>Osmium-antimonate†</td>
<td>—</td>
</tr>
<tr>
<td>Group 4</td>
<td>Calcium-free seawater + 10⁻³ M EGTA</td>
<td>Glutaraldehyde in calcium-free seawater + 10⁻³ M EGTA</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* In all instances the seawater is artificially prepared.
† 1% osmium – 2% pyroantimonate (Fisher Lot no. 730312) in distilled water (pH 8.1) (Spicer et al. 1968): This particular lot provided satisfactory results (Simson & Spicer, 1975).
‡ 2.5% glutaraldehyde in artificial seawater (pH 8.0).
§ 10⁻³ M EGTA in distilled water (pH 8.0).

The EDAX analyser was used to detect X-rays from 0 to 20 keV. This ensured that the X-rays detected in the vicinity of 3.6 keV characteristic of the calcium K lines, were not sum peaks, escape peaks, or interfering elements. The spectra were then expanded and photographed over selected ranges. However, other elements in the sections (uranium, antimony and iodine) also emit X-rays in the energy range of 3–4 keV. These peaks could not be completely resolved by the EDAX detector, and computer peak stripping was employed to separate them. The computer statistically generates a peak with a normal gaussian distribution for the interfering element, antimony, and then subtracts it from the original non-gaussian peak of the specimen. Thus calcium which is present, but masked by the overlapped antimony signal, is revealed.

RESULTS

Ultrastructural localization of antimony deposits in unfertilized Strongylocentrotus (Fig. 1) and Arbacia eggs are shown in Figs. 2–4. These deposits were observed on the plasma, cortical granule, small smooth-surfaced vesicles, pigment granule, and yolk platelet membranes. Although the internal structure of cortical granules differ in the
2 species (Figs. 1 and 2) (Anderson, 1968), antimony deposits were located on the membranes in both cases. Precipitate was also observed within the vitelline layer, mitochondria, Golgi apparatus, rod-containing vesicles, some yolk platelets, nucleus, cytoplasmic ground substance, and on the surface of lipid droplets. Two types of yolk platelets were observed (Fig. 3): a more numerous electron-opaque platelet which had precipitate along its limiting membrane as well as within the stored-matrix substance, and a less frequently seen platelet with lower electron opacity which contained precipitate only along its limiting membrane. Rod-containing vesicles had a large precipitated particle within their lumen, in addition to the usual membrane deposit. These structures are secretory organelles which release their product into the perivitelline space, beginning approximately 6 min after fertilization (Anderson, 1968). Some vesicles may represent smooth endoplasmic reticulum rather than rod-containing vesicles. The rough endoplasmic reticulum is not highly developed in unfertilized sea-urchin eggs (Anderson, 1968; Gross, Philpott & Nass, 1960). A reduction in the wash time between the glutaraldehyde and osmium-antimonate fixations (Group 3A) resulted in retention of more deposit at all sites (Fig. 3). Direct fixation in osmium-antimonate (Group 1A, Fig. 4) compared to glutaraldehyde followed by postfixation in osmium-antimonate (Groups 2A and 3A) (Figs. 2, 3) resulted in a heavier deposit on all cellular membranes and a clear localization of deposit in the mitochondrial cristae and between the outer and inner membranes, rather than in the matrix. There also appeared to be a reduction in random deposits within the cytoplasmic ground substance. The remaining deposit was associated with small smooth-surfaced vesicles. All the other sites of deposits were seen with all fixation methods employed. These observations suggest a loss of cations takes place during the glutaraldehyde fixation and subsequent washing steps in the procedure. However, the preservation of normal morphology, particularly with respect to the cortical granules,

Fig. 1. Periphery of *Strongylocentrotus purpuratus* egg fixed in glutaraldehyde and postfixed in osmium-pyroantimonate (Group 2A). Small, dense, rounded particles representing precipitated antimony may be seen on the following cell membranes: plasma (p), vitelline (v), cortical granule (eg) and yolk platelet (y). Deposit is also associated with smooth-surfaced vesicles (sv) in the cytoplasm. × 18300. Bar = 2.5 μm.

Figs. 2, 3. Periphery of *Arbacia punctulata* eggs fixed in glutaraldehyde and postfixed in osmium-antimonate. Comparison of a short post-glutaraldehyde wash (Fig. 2) with a long post-glutaraldehyde wash (Fig. 3).

Fig. 2. Egg fixed in glutaraldehyde; long buffer wash; postfixed in OsO₄-antimony (Group 2). Note sites of deposition similar to Fig. 1. In addition, antimony precipitate is seen within the Golgi apparatus (g). eg, cortical granule. × 20000. Bar = 1 μm.

Fig. 3. Egg fixed as in Fig. 2 except that a short buffer wash intervened between the glutaraldehyde and osmium-antimonate fixation steps (Group 3A). The short buffer wash provided enhanced antimony deposition at similar sites. Two types of yolk platelets are present: one with deposition on the matrix as well as the platelet limiting membrane (y); and the other with deposit only on the platelet membrane (y). In addition, abundant antimony precipitate may be seen within the mitochondria (m). × 47000. Bar = 1 μm.
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was better in the glutaraldehyde-fixed eggs as compared to direct fixation in osmium-antimonate mixtures.

Incubation of the eggs in calcium-free seawater containing EGTA prior to fixation (Group 4) resulted in a general reduction of deposit at all sites (Fig. 5) in Strongylocentrotus eggs. This approach was not as effective in Arbacia eggs. However, exposure of Arbacia eggs to EGTA following fixation in osmium-antimonate (Groups 1B and 2B) resulted in a far more complete reduction in deposits (Fig. 6). The residual deposit in these eggs had an altered appearance, suggestive of a partial extraction of material. This residual substance may represent antimony complexed with other cations.

X-ray microprobe analysis revealed the presence of calcium in antimony deposits located on the plasma membrane (Fig. 7) and cortical granule membranes of group 2A eggs. The 'net counts' spectra obtained by subtraction of probe data from a control region (yolk platelet) contained signals for calcium, antimony and possibly iodine (Fig. 7A). This subtraction step served to cancel out most of the counts derived from uranium in the sections, and also provided an internal control for the artifactual addition of any element during specimen preparation. Subsequent computer-generated sequential subtraction of signals for antimony (Fig. 7B), as well as iodine and background resulted in the retention of residual peaks for the calcium Kα and Kβ emissions (Fig. 7C). Signals for calcium and antimony could not be detected in adjacent cytoplasmic regions that did not contain deposits. X-ray signals for magnesium were not detected in the sections. The presence of several other elements was also detected by virtue of their emitted X-rays. These elements may be accounted for by substances in the eggs, seawater, preparatory materials (plastic), or metallic sites within the microscope.

Similar X-ray microprobe analyses were performed on deposits remaining in the eggs following extraction with EGTA (Fig. 8). The counting rate of X-rays emitted from these residual deposits located on the plasma membrane (Fig. 8A) was much lower than that obtained from eggs that were not treated with EGTA after osmium-pyroantimonate fixation (3628 integral counts vs. 21576 over 100 s recorded in Fig. 7, above). Signals generating peaks which were statistically significant above background were not detected in the energy range of the calcium K lines, even when the counts were collected over a period of 400 s (Fig. 8B). These data suggest that...
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The results described above identify at the ultrastructural level the locations of calcium-binding sites in unfertilized sea-urchin eggs. The bulk of the calcium-antimonate deposits appeared to be associated with the membranes of the egg's cytoplasmic organelles; plasma membrane, cortical granules, yolk platelets, pigment granules, smooth-surfaced endoplasmic reticulum vesicles, rod-containing vesicles, mitochondria, etc. Furthermore, our findings also clarify the results obtained by Steinhardt & Epel (1974) using $^{40}$Ca, which demonstrated that this cation in unfertilized eggs is bound to the large particle fraction isolated by differential centrifugation, which would include the bulk of the cytoplasmic organelles. However, identification of specific granule types, or organelle sites of calcium binding was not possible with their fractionation procedure.

The data on calcium localization obtained in this study should be viewed in the light of the known limitations of the pyroantimonate technique (Herman et al. 1973; Simson & Spicer, 1975) which must now be considered.

Antimony can bind and be precipitated with a variety of cations in addition to calcium. The deposits within sea-urchin eggs produced by antimony were characterized in 2 ways: chelation of calcium with EGTA, either prior to or following antimonate fixation, and X-ray microprobe analysis. The marked reduction in deposits observed in EGTA-treated eggs would suggest that a major portion of the precipitate represents a calcium-bound antimonate salt, since EGTA is known to have a very high affinity for calcium in comparison to any other cation (Baker, 1972). The residual deposit seen in the EGTA-treated eggs may reflect the presence of other cations.

Fig. 7. X-ray spectra and electron micrographs of deposits along surface of unfertilized Arbacia eggs, fixed according to Group 2A.

A, shaded area represents X-ray emission spectra recorded from a portion of the egg's plasma membrane, containing peaks for uranium (U), antimony (Sb), calcium (Ca), and iodine (I). White area represents 'net-counts' spectra (Sb, Ca, I) obtained by subtraction of control probe data from sample spectra. The control was obtained by probing an adjacent yolk platelet until the height of the U peak was approximately the same as that in the sample. Vertical scale: 1000 counts. Horizontal scale: 20 eV/channel. In addition, note that some loss of the Sb and Ca peak occurs following stripping of U as illustrated by the difference in Sb and Ca peaks between shaded and white areas. This may reflect, in part, the presence of deposits within the platelet.

B, white area represents emission spectra for Ca and I, following computer stripping of Sb from 'net-counts' spectra (shaded area).

C, emission peaks for Ca (K-lines) following subtraction of signals for I and background, enlarged on a vertical scale of 100 counts. These peaks represent signals more than 2 standard deviations above background.

D, portion of Arbacia egg surface prior to microprobe analysis.

E, electron micrograph of similar region of egg after microprobe analysis. Dark elliptical zone represents contamination area following 100-s probe and illustrates region of plasma membrane probed.
Fig. 8. X-ray microprobe analysis of the surface of an unfertilized Arbacia egg treated with EGTA following osmium-pyroantimonate fixation (Group 2B).

A, shaded area represents X-ray emission spectra from sample at egg surface, containing signals for U, Sb, and I. White area represents ‘net-counts’ spectra, minus U, obtained by subtraction of control probe through yolk platelet as in Fig. 7, above. Vertical scale: 250 counts.

B, subtraction of Sb and I from net-counts spectra failed to reveal any residual signal for Ca above background. Data from analysis of 400-s counting period shown, although similar results were obtained in 100-s count.

C, electron-micrograph of the surface of an EGTA-extracted egg from which X-ray emission spectra were obtained. Residual deposits on plasma membrane were probed.
Furthermore, X-ray microprobe analysis provides evidence that sufficient calcium was present in the deposits to provide a detectable emission signal for calcium after subtraction of the overlapping antimony peak. This conclusion is confirmed by the absence of detectable emission signals for calcium in residual deposits remaining in EGTA-extracted eggs. X-ray signals for magnesium were not seen. The latter is consistent with previous findings that magnesium is free and highly diffusible within the unfertilized sea-urchin egg (Steinhardt & Epel, 1974), and precludes the possibility that EGTA might be extracting magnesium as well as calcium from the antimonate deposits (Simson & Spicer, 1975).

The loss or translocation of cations during fixation and processing may be a source of potential artifacts in any cytochemical or cell fractionation procedure (Clemente & Meldolesi, 1975). From a comparative standpoint, it would appear that the fixation conditions employed in this study should minimize, but not entirely eliminate, the possibilities for cation displacement. In comparison with blocks of solid tissue several millimetres thick, sea-urchin eggs are only 75–80 μm in diameter, thus ensuring rapid and simultaneous penetration into all the cells while they are suspended in their natural seawater environment which normally contains 10 mM calcium. Nevertheless, evidence suggestive of some cation loss was obtained in the present study (Figs. 2–4). Optimal cation capture, as evidenced by maximum precipitation, was obtained when the eggs were fixed directly in osmium-antimonate. Since the osmium-antimonate was dissolved in distilled water, these data suggest that the deposits were probably primarily derived from cations present within the eggs rather than in the extracellular space. Furthermore, the reduced deposits observed in the eggs prefixed in glutaraldehyde probably reflect some cation loss during this step of the processing. Cation loss also appears to take place during the wash between glutaraldehyde and post-fixation in the osmium-antimonate, since a reduction in wash time yields more deposit (Yarom et al. 1975). Results obtained by other investigators (Mazia, 1937; Nakamura & Yasumasu, 1974) suggest that only 0.6–1.4% of the total calcium in the unfertilized sea-urchin egg is ‘free or ionized’. This small quantity of ‘free’ calcium would probably be washed out of the eggs during fixation, or may represent a minor proportion of artifactually translocated antimony-calcium complex on membranes. In addition, it is possible that some of the bound but potentially diffusible calcium (Nakamura & Yasumasu, 1974) may undergo translocation prior to capture by antimony. Cryofixation and microtomy of fresh or fixed specimens might further reduce but would not entirely eliminate these problems. These alternate procedures designed to minimize loss or translocation of cations also bring additional problems associated with morphological interpretation (Bacaner, Broadhurst, Hutchinson & Lilley, 1973).

The application of the pyroantimonate technique to eggs as well as somatic cells appears to involve a conflict between ideal fine-structural preservation and maximal cation retention (Herman et al. 1973; Simson & Spicer, 1975). Prefixation with glutaraldehyde appeared to offer an acceptable compromise in the case of sea-urchin eggs, since organelle morphology was reasonably well preserved with retention of appreciable deposition. Furthermore, the amount of deposit lost appeared to be equal
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at all cell sites, suggesting that cation localization in eggs prefixed in glutaraldehyde was representative of elemental retention.

The calcium-binding sites in unfertilized sea-urchin eggs probably participate in several essential physiological functions related to the activation of embryogenesis at fertilization. As has been found in the case of nerve cells (Baker, 1972), 2 major pools of bound calcium (Nakamura & Yasumasu, 1974) appear to be present in these eggs: (1) bound and non-diffusible, and (2) bound but potentially diffusible. The application of the pyroantimonate technique to a resting cell such as the unfertilized egg cannot distinguish between these 2 types of binding sites. The first may represent structural sites involved in maintaining the integrity of biological membranes (Poste & Allison, 1973). The increased fragility of cortical granules isolated in the presence of chelating agents (Schuel et al. 1972) may reflect extraction of calcium from these structural sites. The second may represent sequestered pool(s) of calcium that could be mobilized at fertilization to initiate embryogenesis. Our results are consistent with both of these concepts, since calcium was found on membranes of the egg’s organelles as well as at locations which have been identified as physiologically active sequestration sites of diffusible calcium in other cells, i.e. plasma membrane (Baker, 1972; Oschman, Hall, Peters & Wall, 1974), mitochondria (Carafoli et al. 1974), cytoplasmic storage granules (Sato et al. 1975), etc. Furthermore, the unfertilized egg remains in a quiescent metabolic state for an extended period between the completion of oogenesis and fertilization. Hence, some of the bound calcium may participate in maintaining this state of metabolic and functional repression characteristic of the unfertilized egg. Conversely, the liberation of calcium from one or more of these sites may be part of the trigger mechanism that activates the egg at fertilization or upon ionophore stimulation (Steinhardt et al. 1977).

The calcium identified in association with the yolk platelets may exemplify these phenomena. It now appears to be well established that storage macromolecules as well as lysosomal hydrolases capable of degrading them are simultaneously packaged in the yolk platelets during oogenesis (Schuel, Wilson, Wilson & Bressler, 1975), although appreciable mobilization of metabolites from the yolk does not begin until after fertilization (Kavanau, 1954). Calcium in the yolk may regulate these processes. Furthermore, since the platelet compartment contains approximately one third of the total volume and a similar portion of the total protein in unfertilized sea-urchin eggs (Schuel et al. 1973), it may also contain a massive pool of potentially diffusible calcium required for other developmental processes after fertilization.

The possible existence of 2 morphologically different yolk platelets in sea-urchin eggs has been alluded to by other investigators (Gross et al. 1960; Krischner & Chambers, 1970). Further evidence for the existence of heterogeneity has been obtained biochemically (Schuel et al. 1975). Our present study confirms the existence of 2 morphological types of yolk platelets: a more numerous platelet of high electron opacity and another of lower electron opacity. A further characterization of these platelet types may now be made cytochemically: the platelet with high electron opacity exhibits a fine antimony deposit within its stored material as well as on its limiting membrane, while the platelet of low electron opacity contains deposit only
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on its limiting membrane, with very little or no deposit within its stored material.

Calcium is present on the plasma and cortical granule membranes of sea-urchin eggs which, by fusion, play a critical role in exocytosis at fertilization. Sites demonstrated in this study are consistent with similar localization on the plasma membrane and granules in a variety of somatic secretory cells, including beta cells of endocrine pancreas (Herman et al. 1973), acinar cells of exocrine pancreas (Clemente & Meldolesi, 1975), blood platelets (Sato et al. 1975), anterior pituitary (Cramer, Cardasis, Milks & Pereira, 1975) and presynaptic terminals (Politoff, Rose & Pappas, 1974). Such focal deposits may represent calcium-binding sites which function in suppressing membrane fusion necessary to initiate exocytosis. Conversely, perturbations in the binding of calcium to these membranes may be an important aspect of the stimulus-secretion coupling mechanism, as suggested by Poste & Allison (1973). The synchronous and rapid discharge of the cortical granules in sea-urchin eggs at fertilization makes these cells an ideal model system to study the functional role(s) of calcium in exocytosis.

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