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Ionic and Permeability Requirements for Exocytosis In Vitro in Sea Urchin Eggs

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Summary. We study exocytosis in the planar isolated cortex of the egg of the sea urchin *Lytechinus pictus*. Solutions bathing the exocytotic apparatus need not contain appreciable amounts of ions: fusion follows addition of submicromolar calcium to solutions containing only nonelectrolyte. We examine the effects of altering the granule membrane permeability to small molecules with ionophores and digitonin. Introducing holes in the secretory granule membrane to the extent of allowing free passage of small molecules does not cause secretion in vitro. We add the amphipathic compound digitonin at 12 to 15 μM concentrations and demonstrate that the granule membrane can become permeable to lucifer yellow, yet that granules remain intact. Granules still undergo exocytosis after digitonin treatment at such concentrations upon subsequent addition of calcium. Higher concentrations of digitonin lead to granule content swelling and vesicle bursting. We conclude that cortical granule hydration during exocytosis is not mediated by small ionic channels.

Key Words exocytosis · membrane fusion · secretion · calcium · sea urchin egg · cortical granule

Introduction

The release of acetylcholine from nerve terminals, the release of insulin from pancreatic islet cells, the secretion of serotonin by platelets, and cell infection by enveloped virus all share a common event: membrane fusion. Two stable surfaces suddenly become one. Fusion is the strictly controlled process by which membrane proteins are inserted and secretory proteins are extruded. It interests physiologists, cell biologists, and clinicians alike. Membrane fusion is also a challenging biophysical problem. Despite active research in chemically defined model membrane systems, cell membrane preparations in vitro, and single-cell and organ studies in vivo, little is certain regarding the mechanism of fusion in biological systems. One set of hypotheses regarding membrane fusion have centered upon a crucial role for ions, entering membrane vesicles and secretory granules to cause an osmotic swelling of the vesicle which then leads to fusion [6, 11, 17, 28]. In this report we examine the role of ions and secretory granule membrane permeability in biological membrane fusion.

We previously studied the role of osmotic swelling in biological exocytosis, the process by which the bilayer membrane of a secretory vesicle fuses to the plasma membrane of a cell resulting in the discharge of secretory product. We used the cortical granule secretion of sea urchin eggs because (1) individual secretory events can be simply visualized with light microscopy, (2) exocytosis can be triggered with calcium, the proximal physiological stimulus, and (3) individual granules and their calcium-induced exocytosis can be clearly seen both in vivo and in vitro. We found that calcium causes a swelling of secretory vesicles in vitro [36, 37]. This swelling is of interest, either as a driving force in membrane fusion [1, 9–11] or as a means of secretory product dispersal following membrane fusion and exposure of secretory granule contents to the external milieu [5, 18, 19].

When granules swell, water must move into the vesicle to increase intravesicular volume. There must be a decrease in the chemical potential of water within the granule which causes net water influx and swelling. One proposed mechanism for this decrease in potential is by the diffusion-driven entry of small molecules or salts through changes in membrane permeability (such as calcium-activated channels) [1, 6, 11, 17]. As noted, [1, 28], channels exist which open at calcium concentrations known to cause exocytosis in vitro [26, 34]. An alternate hypothesis suggests an activation and hydration of the contents of the vesicle [5, 11, 13, 15, 19, 32, 35], perhaps involving a phase change of granular contents [30]. In this second hypothesis we can further

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differentiate between dissolution of small electrolytes [15] and hydration of macromolecules [5, 13, 19, 32]. Is exocytosis dependent upon the cytoplasmic and extracellular molecules exogenous to the secretory granule and plasma membrane, or is it independent of the composition of the solution bathing these membranes? If calcium causes swelling by opening ionic channels in the secretory vesicle membrane, one then would predict (1) a requirement for concentrations of ions sufficient to drive net flux and (2) that altering the membrane permeability will mimic calcium and cause swelling.

We performed experiments to test these hypotheses. We varied the composition of the solution bathing a planar isolated cortex of the sea urchin egg, a preparation consisting mostly of plasma membrane and secretory granules which undergo exocytosis in vitro [29]. This preparation has been shown to undergo membrane fusion and granule content discharge in response to micromolar free calcium [4, 31, 36]. A single solution bathes both the cytoplasmic and extracellular surfaces, and we can alter the composition of that solution at will. We reduced the ionic constituents to submillimolar levels and added ionophores to determine if ionic fluxes or membrane permeability changes are either required or sufficient for fusion. We removed external ions (other than submicromolar calcium) without inhibiting exocytosis, and increased granule membrane permeability to small molecules (<500 daltons) without causing exocytosis. This restricts the list of possible mechanisms.

Materials and Methods

Lytechinus pictus were purchased from Alacrity Marine Biological, Redondo Beach, Calif. Animals were maintained at 15°C in running seawater. Eggs were obtained by intracoelomic injection of 0.25 ml of 0.5 M KCl, and dejellied mechanically by passage through nylon mesh and hand agitation. After washing three times in a large volume of filtered seawater (0.22 micron, Millipore, Bedford, Mass.), eggs were allowed to settle in a large beaker until used.

Isolated planar cortices of echinoderm eggs were prepared by the method of Vacquier [29] with a shearing buffer which has been devised by Baker and Whitaker [4] to approximate cytoplasm (buffer): 0.5 M glycine, 0.22 M potassium glutamate, 10 mM EGTA, 2.5 mM ATP, 5 mM $MgCl_2$, 0.1 mM DTT. Glass cover slips were treated with 0.005% poly-L-lysine (>300,000 mol wt, Sigma, St. Louis, Mo.) for 15 sec, then rinsed extensively with distilled water for a minute. Dejelled eggs were then applied to the cover slip. Eggs settled and adhered within a minute. To remove traces of seawater and calcium, the cover slip and attached eggs were gently rinsed with buffer. Eggs were then lysed with a forceful stream of buffer through a 5¾" pasteur pipette. After an additional wash with 2 ml of buffer, cortices were treated with a variety of experimental solutions, then observed by light microscopy with dark-field, phase-contrast fluorescence, and/or differential interference contrast microscopy.

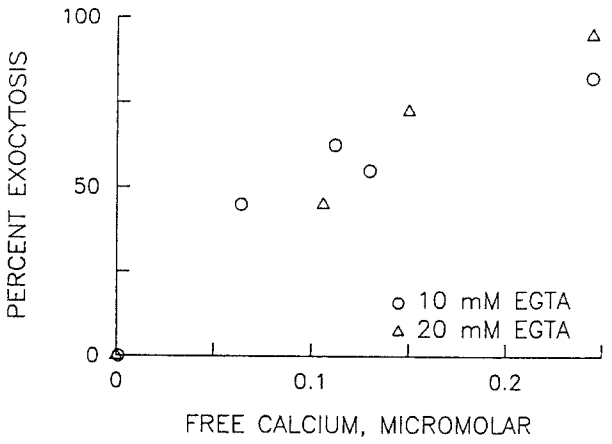


Fig. 1. Calcium causes exocytosis in raffinose solutions. *L. pictus* cortices were prepared by shearing with buffer, then immediately treated with 2 ml of a solution containing 0.5 M raffinose, 10 mM EGTA, 1 mM PIPES, pH 6.7. Next cortices were treated with a solution containing 0.5 M raffinose, 10 mM EGTA, 1 mM PIPES, and various amounts of calcium. After 1½ min, cortices were observed with 1000× phase-contrast microscopy (Reichert). Percent exocytosis was estimated by inspection of many cortices

The extent of exocytosis was estimated with 1000X phase-contrast microscopy by examining a large number of cortices and averaging the estimated percentage of exocytosis in each one. True exocytosis was distinguished from mere bursting of granules by seeing the domes which result from fusion of cortical granules with the plasma membrane and the subsequent interaction of cortical granule contents with the vitelline [36].

Ionophore solutions were prepared as the stock solution in methanol, then diluted into buffer immediately before application to freshly sheared cortices.

Osmotic pressure was measured with a vapor pressure osmometer (Wescor, Salt Lake City, Utah). Raffinose was obtained from Eastman Chemicals, Rochester, N.Y.: digitonin from Fluka, Hauppauqe, N.Y.; sucrose (gold label) from Aldrich, Milwaukee, Wis.; and ATP and ionophores from Sigma, St. Louis, Mo. Water was first deionized, then passed through activated charcoal and two additional mixed bed ion-exchange columns (Millipore).

Free calcium concentrations were determined from $CaCl_2$/EGTA ratios, pH, Mg^{2+}, and ionic strength [20]. Dissociation constants for calcium, magnesium, and protons to EGTA were obtained from data of Martel and Smith [25].

Results

Ions are Not Required for Exocytosis

We treated isolated planar cortices of sea urchin eggs with isotonic sucrose or raffinose solutions containing 5 mM PIPES and 10 mM EGTA, pH 6.7 (with KOH). Granules were stable for over 5 min. After washing fresh cortices with 2 ml of this solution to remove ionic traces, we immediately added a similar solution containing added calcium. Exocytosis of cortical granules followed (Fig. 1). The

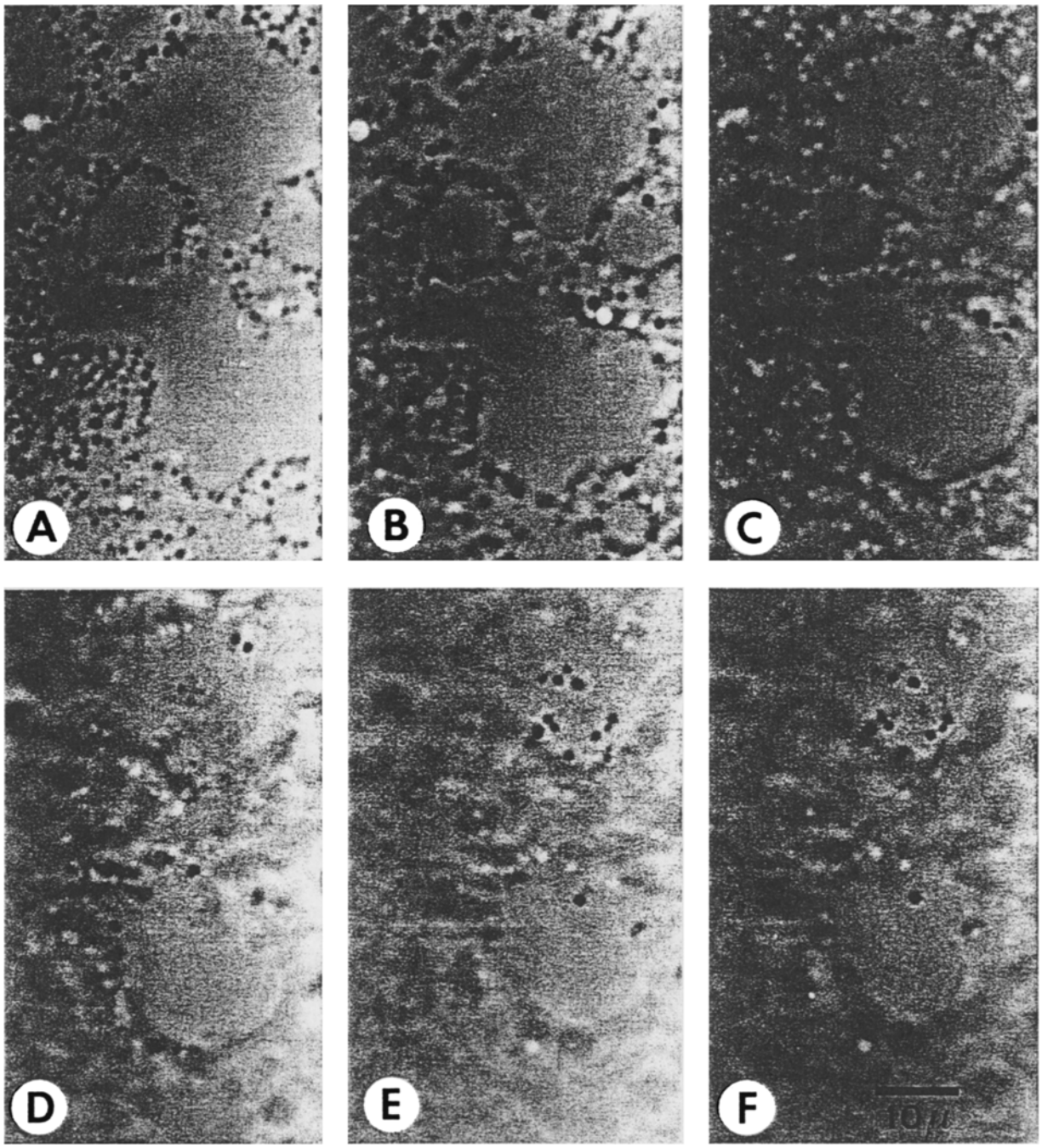


Fig. 2. Dome formation in raffinose solutions with submicromolar calcium. *L. pictus* cortices were prepared by shearing in buffer, then washed with 2 ml of 0.61 M raffinose, 10 mM EGTA, 5 mM PIPES, pH 6.96. Next, cortices were treated with 2 ml of 0.61 M raffinose, 10 mM EGTA, 5 mM PIPES, 1.58 mM $CaCl_2$, pH 6.96 (free Ca^{2+} = 0.07 μM). Two min later the specimen was optically "serially sectioned" by taking a series of photomicrographs at successive focus (phase-contrast optics, Reichert). (*A*) The surface of the cover slip, showing unreacted granule cortices and large clear areas. (*B*) The next focal plane, away from the cover slip, and into the solution (higher). The rim of the exocytotic dome is seen in each of the clear zones, composed of sharply focused, unreacted granules attached to the plasma membrane. (*C–E*) The rim of the dome is smaller in successively higher sections. (*F*) The apex of the upper dome. Granules are seen at the top

domes formed by the plasma membrane plus cortical granule membrane, together lifting off the vitelline coat, demonstrated the vectorial discharge of secretory product (Fig. 2) [36].

Exocytosis also occurred when we added isotonic solutions of raffinose or sucrose (without EGTA or PIPES) to isolated planar cortices. Addition of EGTA to these solutions resulted in a pro-

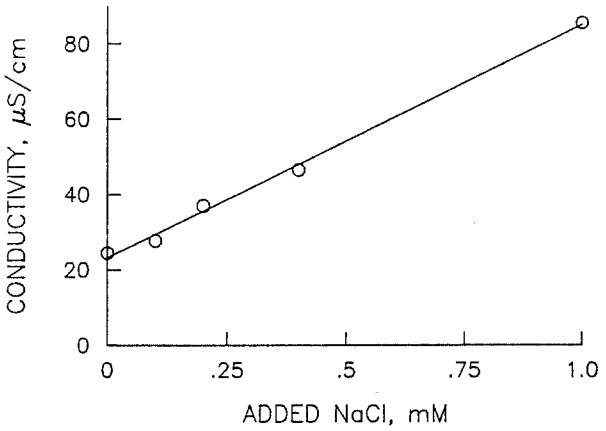


Fig. 3. Approximation of contaminant ion levels in raffinose solutions. The conductivity of 0.5 M raffinose (adjusted to pH 6.7 with 81.1 μM KOH) was measured on a conductivity meter (Markson Electromark Analyzer). Next, small concentrations of NaCl were added to this raffinose solution, and the conductivity was remeasured. Conductivity of the water used for solutions was 1 μS/cm

gressive decrease in exocytosis. We were thus able to demonstrate exocytosis in a solution of raffinose or sucrose, inhibit exocytosis with the addition of EGTA, and reverse that inhibition with subsequent addition of calcium to the EGTA buffer. Exocytosis in nonelectrolyte solutions was presumably due to the presence of contaminant multivalent cations. To exclude the possibility that contaminant calcium was interfering with our estimate of free calcium concentration, we doubled the EGTA and total calcium, yet found the same extent of exocytosis (Fig. 1).

The aggregate contamination level of ions in the raffinose solution was estimated by comparing conductivity measurements of the raffinose solution to raffinose solutions to which NaCl had been added. The total conductivity of a raffinose solution supporting fusion (7.6 to 25 μS/cm for different batches of raffinose) is equivalent to the change in conductivity of a raffinose solution to which 0.1 to 0.5 mM NaCl was added (Fig. 3).

Altering Granule Membrane Permeability Does Not Cause Exocytosis

The occurrence of exocytosis in nonelectrolyte solutions rules out the idea that transmembrane ionic flux into the cortical vesicle is the immediate cause of the water influx and vesicle swelling associated with exocytosis. Granule swelling may still be a result of altering the permeability of the vesicular membrane. This could then lead either to an influx of saccharide and then water, and swelling, or to an activation of vesicle contents by loss of critical molecules (e.g., crosslinkers), changes in pH, ionic strength, etc. If this were the case, altering the permeability of the secretory vesicle should mimic the effect of calcium: treating the preparation in buffer with compounds which make the granule membrane permeable to those hypothetical species should lead to granule swelling.

Table.[a]

Ionophore (units)	Concentration of ionophore	% Granule loss with ionophore	% Exocytosis in 10 μM Ca^{2+} after ionophore
Nystatin	89		
(units/ml)	111	1	93
	333	1	
	1665	3,3,2	90
	6020	5	90
Amphotericin B	150	1	
(μM)	200	1	
	300	2,2,1	98,99
	650	8	90
CCCP[b]	100		95
(μM)	300	1,1,1	95
Valinomycin	100	2	95
(μM)	218	2,5	

[a] Freshly sheared planar isolated cortices were exposed to solutions of ionophore in buffer at the indicated concentration. Percentage granule loss of ionophore after 3 min was estimated by light microscopy. Different experiments at the same concentration are separated by commas. In another set of experiments cortices were exposed to ionophore solutions for minutes, then exposed to 10 μM free calcium in buffer. Percentage exocytosis was then estimated by light microscopy.
[b] Carbonylcyanide *m*-chlorophenyl hydrazone (CCCP).

We therefore treated isolated planar cortices of sea urchin eggs with buffer containing very high concentrations of various substances known to alter the permeability of phospholipid bilayers and biological membranes: nystatin, amphotericin B, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) and valinomycin (*see* Table). Although we had no way of measuring the permeability change caused by these agents, we used concentrations 100 to 1000 times higher than those known to produce a four to five order of magnitude change in the permeability of planar phospholipid bilayers [2, 3, 16]. The concentration of nystatin used was 10 to 100 times higher than the concentration needed to make a red blood cell membrane leaky enough to dialyze the cell [7]. We found that neither carrier nor channel type of ionophore altered the microscopic appearance of the cortices after 1-, 3- and 10-min incubation. With each ionophore, after 2-min treatment, calcium addition in buffer (10 μM free Ca^{2+}) led to complete exocytosis.

We could not measure the presumed membrane

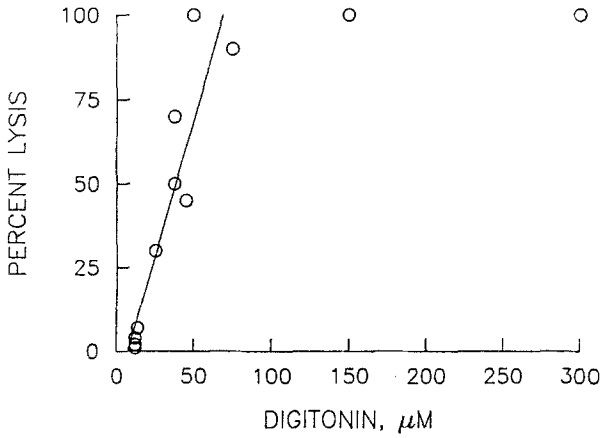


Fig. 4. Effect of varying concentrations of digitonin on vesicle lysis. Cortices were sheared in buffer then washed for 3 min with buffer containing various concentrations of digitonin. Percentage of lysis was then estimated

changes provoked by the addition of ionophores. Ionophore activity is dependent upon lipid composition of the membrane [3, 16]. We do not know the lipid concentration of cortical granule membranes. Therefore we tested the effects of digitonin, known to alter the permeability of cell membranes to somewhat larger molecular weight substances [14, 21, 33]. We could then test the effect of digitonin on permeability directly. As seen in Fig. 4, treatment of cortices with less than 12 μM digitonin in buffer for 3 min had no detectable effect. Increasing concentrations above 12 μM digitonin led to an increasing lysis of cortical granules, maximal at 50 μM. No exocytotic domes were detected, and only granule lysis ("bursting") was observed when the effect of digitonin was monitored in a chamber that allowed constant observation by high resolution light microscopy. Although these concentrations and incubation times are similar to those used to make cell membranes leaky, we could not distinguish experimentally whether high digitonin concentrations caused vesicle bursting by (a) complete solubility of the membrane and contents or (b) large pore formation.

To test digitonin's action, we measured cortical granule permeability to a dye marker. We found that digitonin made membranes permeable to lucifer yellow, a fluorescent marker which we found normally to be impermeant to cortical granules. Whereas a nonbinding marker would quickly diffuse out of the granules during a wash (because of the high surface-to-volume ratio of the granules), a binding dye entering as the result of increased membrane permeability would lead to a permanent staining of cortical granules. Lucifer yellow was demonstrated to be a binding dye in the following experiment. Whole eggs were activated by the calcium ionophore A23187 in seawater containing 1% lucifer yellow. Staining of cortical granule contents with lucifer yellow was seen despite vigorous washing with seawater without dye.

We saw lucifer yellow entry into cortical granules with 12, 14, and 15 μM digitonin using fluorescence microscopy. Under these conditions only limited granule lysis is seen. One experiment is shown in Fig. 5. Isolated planar cortices were incubated with 15 μM digitonin for 30 sec, then with buffers containing 1% lucifer yellow and 20 mM EGTA for 7 min. After a brief irrigation with 6 ml of buffer, cortices were examined with fluorescence microscopy (Fig. 5). These digitonin-treated cortices had a substantially greater number of fluorescent cortical granules than did control cortices treated with lucifer yellow but not digitonin (Figs. 5, 6), demonstrating that granules were initially impermeant to dye. The identity of the observed fluorescent particles as cortical granules was ascertained by (a) comparing phase-contrast and fluorescent images and by (b) observing that calcium-discharged cortices did not fluorescence after digitonin and lucifer yellow treatment. One small set of very bright vesicles which were not phase dense most likely correspond to the acidic vesicles observed by others [8, 24, 27].

Addition of 10 μM free calcium in buffer after 12, 14, and 15 μM digitonin treatment for 2 min resulted in complete cortical granule exocytosis with concomitant formation of domes. Since this digitonin treatment would have led to lucifer yellow entry into half of these granules, perhaps leaky granules can still fuse. Lucifer yellow staining of granules resulted in their being refractory to calcium, an effect presumably secondary to lucifer yellow binding to the granule core, as had been seen in whole eggs. Thus we were unable to directly show that permeable granules undergo exocytosis by detecting dye release with calcium. This experiment will be done with a nonbinding dye.

Discussion

Earlier observations of membrane fusion and exocytosis in the isolated planar cortex of the sea urchin egg show that exocytosis occurs in simple buffer solutions [4, 31]. Vacquier showed cortical granule discharge in glucose solutions [29]. The present study systematically examines exocytosis in nonelectrolyte solutions. We find that large ionic fluxes do not matter. The exocytosis of cortical vesicles with the plasma membrane in nonelectrolyte is essentially identical to exocytosis in vivo. Cyto-

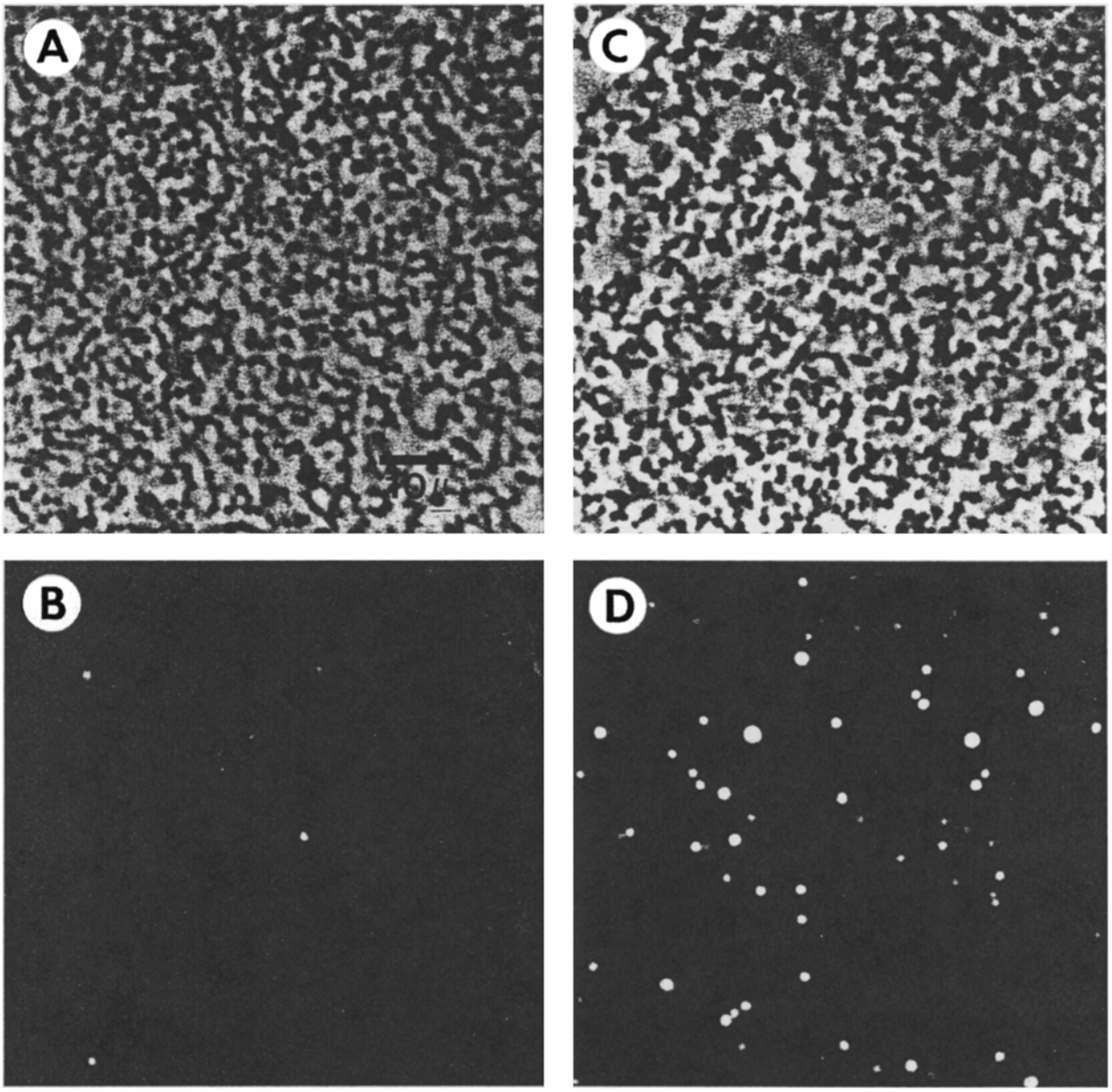
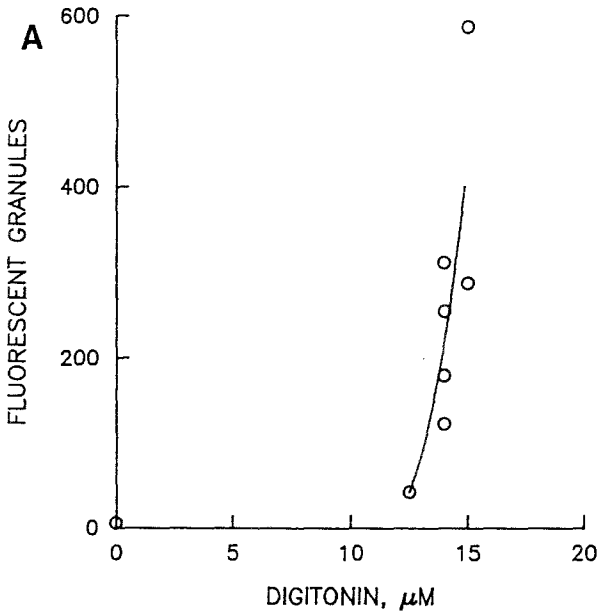


Fig. 5. Digitonin renders cortical granules permeable to lucifer yellow. Cortices were prepared by shearing in buffer, treating for 30 sec in either buffer or buffer containing 14 μM digitonin, then for 7 min in buffer containing 1% lucifer yellow (lithium salt). After irrigation with 6 ml of buffer, cortices were examined by phase-contrast and fluorescence microscopy. The same piece of cortex is seen in (*A*) and (*B*), (*C*) and (*D*). (*A*) No digitonin phase-contrast 1000×; (*B*) no digitonin fluorescence 1000×; (*C*) 14 μM digitonin phase-contrast 1000×; (*D*) 14 μM digitonin fluorescence 1000×

plasm is not needed. The essential molecular components needed in this exocytosis appear to be contained on, in, or enclosed by the fusing membranes: the isolated planar cortex has self-contained exocytotic capability.

Are these results similar to those in other exocytotic systems? In permeabilized chromaffin cell preparations, exocytosis of catecholamine-packed granules can be shown to occur in sucrose solutions in the presence of approximately 10 mM of ions other than calcium [21, 22, 33] and in sucrose solutions with minimal ionic constituents (D. Knight, *personal communication*).

While these experiments rule out hypotheses involving ion fluxes they support hypotheses involving a swelling of gels. It is unlikely that the unreacted cortical granule contents exert an appreciable colloid osmotic pressure, otherwise the digi-



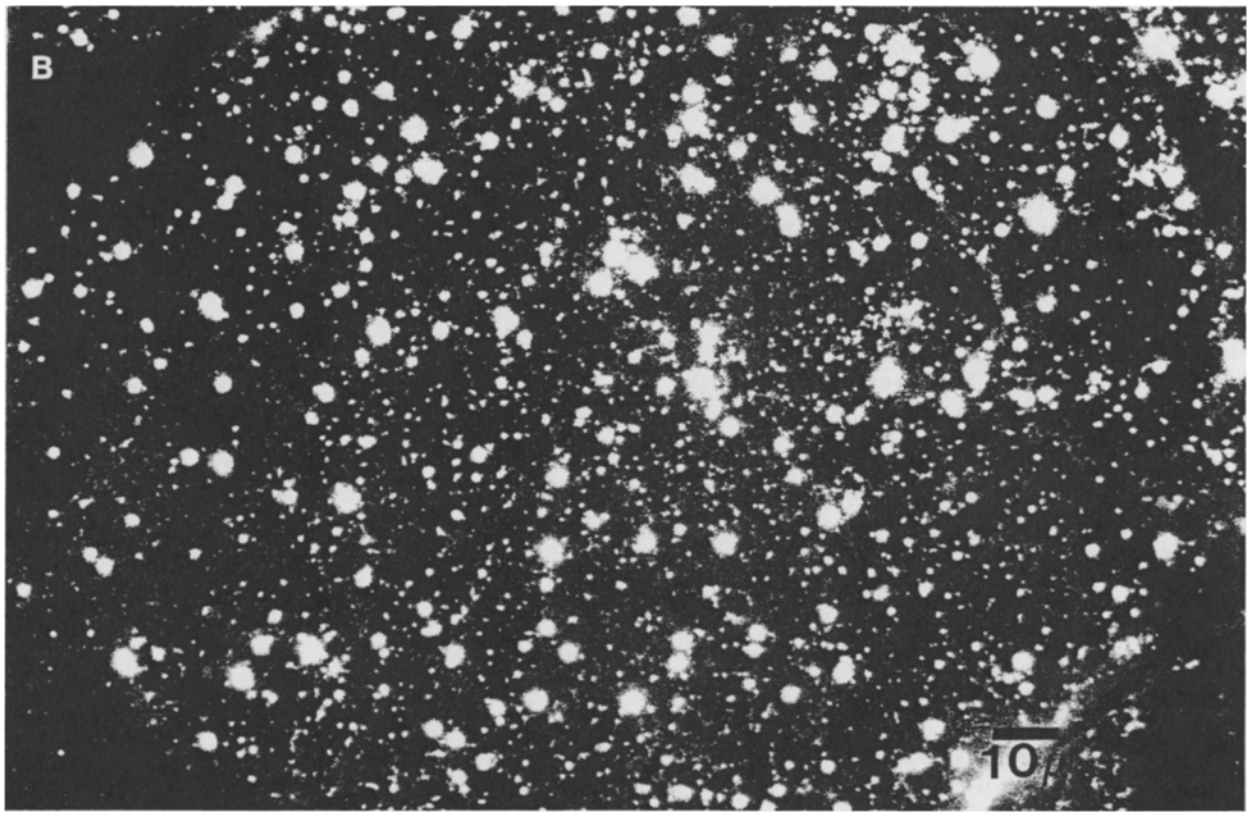


Fig. 6. (*A*) Freshly sheared, planar isolated cortices were treated with various digitonin concentrations in buffer for 30 sec, then incubated with various digitonin concentrations in buffer for another 30 sec, then incubated with 1% lucifer yellow in buffer containing 20 mM EGTA. After 7 min, cortices were washed with 6 ml of buffer, then viewed and filmed under phase-contrast microscopy. The microscope light was switched to excitation and a fluorescent image was filmed. The number of fluorescent granules in each identical cortex area was counted. To correct for granule lysis (*see* Fig. 4 and text), these raw counts were normalized by dividing each experimental count by the fractional percentage lysis at that digitonin concentration. (*B*) A fluorescence photomicrograph of one cortex treated as above with 15 μM digitonin

tonin-permeabilized granules should have burst. On the other hand, the cortical granule contents exert a large colloid osmotic pressure after being secreted, raising the fertilized envelope. Applying colloid osmotic pressures of 300 mOsm in seawater prevents this cortical granule content dispersal [32]. Thus at some point in exocytosis the granule contents become osmotically active. This activation of granule contents is the simplest explanation for the granule swelling associated with exocytosis, and could be seen as a hydration of those macromolecular contents.

The fact that high digitonin concentrations lead to granule bursting rather than detachment suggests that digitonin, too, can trigger the hydration of the cortical granule contents. Is digitonin acting to dis-

solve the granule membrane *and* swelling the contents, or is it making large channels in the granule membrane to allow some critical large molecular species to leave, thereby causing swelling? Recent studies of digitonin on chromaffin cells suggest that channels are formed [21]. If digitonin merely dissolved the attachment of the granule core to the plasma membrane (be it membrane, protein or both) then we would have seen the intact granule core start to undergo Brownian motion and lift off the cortex. Indeed this can be observed with digitonin in the presence of sufficient dextran to prevent granule core hydration [32], but is never seen with digitonin alone.

We suggest that when calcium stimulates exocytosis in the sea urchin egg, one event is an activation of the cortical granule contents. The fact that *isolated* granules do not swell and burst [23] in the presence of a calcium concentration sufficient for fusion unless contact is made with the plasma membrane [12] suggests that this swelling activation requires the adherent plasma membrane. Perhaps calcium leads to a large permeability pathway between the inside of the granule and the extracellular space (such as would be formed by membrane fusion) which then leads to the swelling needed to finalize exocytosis with content dispersal, as has been suggested for other systems [5, 18, 35]. Though we show that alteration of membrane permeability to small molecules is unimportant, it is possible that granule content hydration is achieved through alteration of membrane permeability to larger macromolecules. Alternatively, a calcium-activated enzymatic activation or a direct action of intragranular calcium may be involved.

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References

1. Akabas, M.H., Cohen, F.S., Finkelstein, A. 1984. Separation of the osmotically driven fusion event from vesicle-planar membrane attachment in a model system for exocytosis. *J. Cell Biol.* **98:**1063–1071
2. Andersen, O.S., Finkelstein, A., Katz, I., Cass, A. 1976. Effect of phloretin on the permeability of thin lipid membranes. *J. Gen. Physiol.* **67:**749–771
3. Andreoli, T.E., Tieffenberg, M., Tosteson, D.C. 1967. The effect of valinomycin on the ionic permeability of thin lipid membranes. *J. Gen. Physiol.* **50:**2527–2545
4. Baker, P.F., Whitaker, M.J. 1978. Influence of ATP and calcium on the cortical reaction in sea urchin eggs. *Nature (London)* **276:**513–515
5. Bilinski, M., Plattner, H., Matt, H. 1981. Secretory protein decondensation as a distinct, Ca^{2+}-mediated event during the final steps of exocytosis in *Paramecium* cells. *J. Cell Biol.* **88:**179–188
6. Brown, E.M., Pazoles, C.J., Creutz, C.E., Aurbach, G.D., Pollard, H.B. 1978. Role of anions in parathyroid hormone release from dispersed bovine parathyroid cells. *Proc. Natl. Acad. Sci. USA* **75:**876–880
7. Cass, A., Dalmark, M. 1973. Equilibrium dialysis of ions in nystatin-treated red cells. *Nature, New Biol.* **244:**47–49
8. Christen, R. 1983. Acidic vesicles and the uptake of amines by sea urchin eggs. *Exp. Cell Res.* **143:**319–325
9. Cohen, F.S., Akabas, M.H., Finkelstein, A. 1982. Osmotic swelling of phospholipid vesicles causes them to fuse with a planar phospholipid bilayer membrane. *Science* **217:**458–460
10. Cohen, F.S., Akabas, M.H., Zimmerberg, J., Finkelstein, A. 1984. Parameters affecting the fusion of unilamellar phospholipid vesicles with planar bilayer membrane. *J. Cell Biol.* **98:**1054–1062
11. Cohen, F.S., Zimmerberg, J., Finkelstein, A. 1980. Fusion of phospholipid vesicles with planar phospholipid bilayer membranes. II. Incorporation of a vesicular membrane marker into the planar membrane. *J. Gen. Physiol.* **75:**251–270
12. Crabb, J.H., Jackson, R.C. 1985. In vitro reconstitution of exocytosis from plasma membrane and isolated secretory vesicles. *J. Cell Biol.* **101:**2263–2273
13. Curran, M., Brodwick, M.S. 1985. Mast cell exocytosis and the gel-swell of granules. *Biophys. J.* **47:**172a
14. Dunn, L.A., Holz, R.W. 1983. Catecholamine secretion from digitonin-treated adrenal medullary chromaffin cells. *J. Biol. Chem.* **258:**4989–4993
15. Ferris, R.M., Viveros, O.H., Kirshner, N. 1970. Effects of various agents on the Mg^{2+}-ATP stimulated incorporation and release of catecholamines by isolated bovine adrenomedullary storage vesicles and on secretion from the adrenal medulla. *Biochem. Pharmacol.* **19:**505–514
16. Finkelstein, A., Mauro, A. 1977. Physical principles and formalisms of electrical excitability. *In:* Handbook of Physiology—The Nervous System I. pp. 161–213
17. Finkelstein, A., Zimmerberg, J., Cohen, F.S. 1986. Osmotic swelling of vesicles: Its role in the fusion of vesicles with planar phospholipid bilayer membranes and its possible role in exocytosis. *Annu. Rev. Physiol.* **48:**163–174
18. Green, D.P.L. 1978. The induction of the acrosome reaction in guinea-pig sperm by the divalent metal cation ionophore A23187. *J. Cell Sci.* **32:**137–151
19. Green, D.P.L. 1982. The course of the acrosome reaction in guinea-pig sperm. *J. Cell Sci.* **54:**161–171
20. Harafuji, H., Ogawa, Y. 1980. Re-examination of the apparent binding constant of EGTA with calcium around neutral pH. *J. Biochem.* **87:**1305–1312
21. Holz, R.W., Senter, R.A. 1986. The effects of osmolality and ionic strength on secretion from adrenal chromaffin cells permeabilized with digitonin. *J. Neurochem.* **46:**1835–1842
22. Knight, D.E., Baker, P.F. 1982. Calcium-dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. *J. Membrane Biol.* **68:**107–140
23. Kopf, G.S., Moy, G.W., Vacquier, V.D. 1982. Isolation and characterization of sea urchin egg cortical granules. *J. Cell Biol.* **95:**924–932
24. Lee, H.C., Epel, D. 1983. Changes in intracellular acidic compartments in sea urchin eggs after activation. *Dev. Biol.* **98:**446–454
25. Martel, A.E., Smith, R.M. 1974. Critical Stability Constants. Plenum, New York
26. Marty, A. 1981. Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. *Nature (London)* **291:**497–500

27. Sardet, C., Chang, P. 1985. A marker of animal-vegetal polarity in the egg of the sea urchin *Paracentrotus lividus:* The pigment band. *Exp. Cell Res.* **160:**73–82
28. Stanley, E.F., Ehrenstein, G. 1985. A model for exocytosis based on the opening of calcium-activated potassium channels in vesicles. *Life Sci.* **37:**1986–1995
29. Vacquier, V.D. 1975. The isolation of intact cortical granules from sea urchin eggs: Calcium ions trigger granule discharge. *Dev. Biol.* **43:**62–74
30. Verdugo, P. 1986. Polymer phase transition: A novel mechanism of product release in secretion. *Biophys. J.* **49a:**231a
31. Whitaker, M.J., Baker, P.F. 1983. Calcium-dependent exocytosis in an in-vitro secretory granule plasma membrane preparation from sea urchin eggs and the effects of some inhibitors of cytoskeletal function. *Proc. R. Soc. London B* **218:**397–413
32. Whitaker, M., Zimmerberg, J. 1987. Inhibition of exocytosis in sea urchin eggs by polymer solutions. *J. Physiol. (London)* **389:**527–539
33. Wilson, S.P., Kirshner, N. 1983. Calcium-evoked secretion from digitonin-permeabilized adrenal medullary chromaffin cells. *J. Biol. Chem.* **258:**4994–5000
34. Yellen, G. 1982. Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. *Nature (London)* **296:**357–359
35. Zimmerberg, J., Curran, M., Cohen, F.S., Brodwick, M. 1987. Simultaneous electrical and optical measurements show that membrane fusion precedes secretory granule swelling during exocytosis of beige mouse mast cells. *Proc. Natl. Acad. Sci. USA* **84:**1585–1589
36. Zimmerberg, J., Sardet, C., Epel, D. 1985. Exocytosis of sea urchin egg cortical vesicles in vitro is retarded by hyperosmotic sucrose: Kinetics of fusion monitored by quantitative light-scattering microscopy. *J. Cell Biol.* **101:**2398–2410
37. Zimmerberg, J., Whitaker, M. 1985. Irreversible swelling of secretory granules during exocytosis caused by calcium. *Nature (London)* **315:**581–584

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