

Bioelectric Responses at Fertilization: Separation of the Events Associated With Insemination From Those Due to the Cortical Reaction in Sea Urchin, *Lytechinus variegatus*

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The bioelectric responses at fertilization of the sea urchin *Lytechinus variegatus* are a complex series of membrane potential and resistance changes that occur concomitant with gamete fusion, ionic fluxes, and the cortical granule discharge. This work attempts to separate the electrical effects of sperm-egg interactions from those of the cortical reactions. Two approaches were taken to discern the electrical events associated with insemination, distinct from cortical granule discharge: 1) fertilization of eggs treated with 3% urethane, 10 mM procaine, or 10 mM nicotine, to prevent the cortical reaction and 2) refertilization of fertilized eggs (denuded with 1 mM aminotriazole containing 1 mg/ml soybean trypsin inhibitor). Cortical granule discharge in the absence of sperm incorporation was investigated by artificial activation with 5 μ M A23187 or by fertilization in the presence of 10 μ M cytochalasin D, which prevents incorporation.

These results are consistent with a model in which the sperm-egg interaction triggers both a rapid (50–400 msec), but minor (\cong 10 mV), electrical transient that leads to an action potential and then both the Na⁺-dependent fast block to polyspermy and the late block resulting from the secretion of the cortical granules.

Key words: fertilization, sea urchin, bioelectric response, secretion, motility

INTRODUCTION

At fertilization the sea urchin egg undergoes a complex series of electrical changes in membrane potential and resistance. The early changes include an action potential

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[Okamoto et al, 1977; Chambers and de Armendi, 1979] and a Na⁺-dependent reversal of the membrane potential [Steinhardt et al, 1971], which both together may be responsible for the fast block to polyspermy [Jaffe, 1976]. The repolarization of the membrane is accompanied by an increase in K⁺-conductance and by intracellular changes in [Ca²⁺], which trigger the metabolic activation of the egg [Steinhardt et al, 1971; Graininger et al, 1979; Steinhardt and Winkler, 1979; Shen and Steinhardt, 1980; Winkler et al, 1980].

Membrane and surface modifications occur concomitant with these electrical changes at fertilization, including the fusion of the acrosome-reacted sperm with the plasma membrane of the egg [Longo and Anderson, 1968; Schatten and Mazia, 1976] and the secretion of the cortical granules, which result in the elevation of the fertilization coat [Endo, 1961]. In this report, the separation of these two membrane events (sperm-egg membrane fusion and the secretion of the cortical granules) during fertilization was attempted, the aim being the assignment of the causality between the cellular events and the dominant bioelectric conditions of the egg at fertilization.

These results, in conjunction with the accumulated literature, support the following conclusions: 1) The membrane potential and input resistance of the unfertilized egg are in the range of -80 mV and 300 MΩ [Chambers and de Armendi, 1979; for review see Hagiwara and Jaffe, 1979], 2) voltage depolarizations of about 10 mV at the approximate moment of sperm-egg fusion, which shortly follows sperm attachment and is independent of sperm incorporation, 3) an action potential that is fired when this depolarization reaches its threshold level [Okamoto et al, 1977; Chambers and de Armendi, 1979], 4) the Na⁺-dependent membrane potential reversal that precedes the secretion of the cortical granules [Steinhardt et al, 1971] and associated capacitance changes [Jaffe et al, 1978], and 5) the repolarization of the egg membrane associated with increased K⁺ conductance and egg activation [Steinhardt and Winkler, 1979; Shen and Steinhardt, 1980]. A model in which sperm-egg fusion results in a minor electrical transient [DeFelice and Dale, 1979] and triggers the secretion of the cortical granules, which results both in the Na⁺-dependent fast block to polyspermy [Jaffe, 1976] and the late block due to the secreted contents of the cortical granules (Vacquier et al, 1972), is proposed.

MATERIALS AND METHODS

Animal Maintenance and Gamete Preparation

The gulf coast sea urchin *Lytechinus variegatus* was collected locally during the summer and fall 1979 and maintained in running sea water at the Florida State University Marine Laboratory at Turkey Point. The water temperature was raised to 24°C during the winter months to maintain fertility. Eggs and sperm were obtained by intracoelomic injection of 0.5 M KCl. Eggs were collected and gently stirred in Millipore filtered (0.22 μm) sea water at room temperature no longer than 8 hr. Dejellying was accomplished by vigorous pipetting through a fine bore pipette. Sperm were collected "dry" on ice and diluted in Millipore filtered sea water immediately before insemination.

Electrophysiological Measurements

Electrophysiological measurements were performed under a Zeiss phase contrast microscope (16 × objective) and with a Leitz micromanipulator. Eggs to be impaled were freely floating, sticking spontaneously to plastic Petri dishes or immobilized on polylysine-coated glass slides or plastic Petri dishes [Mazia et al, 1975]. Microelectrodes

were pulled with a vertical pipette puller (David Kopf Instruments) using Hilgenberg glass (Hilgenberg, West Germany, outer diameter 1 mm, with an inner filament), and filled with 3 M KCl immediately prior to use. Electrode resistances were usually about 40 M Ω in seawater and were continuously compensated by bridge adjustment with 0.1 or 1 msec pulses when membrane resistances were measured with 1 sec pulses [Brennecke and Lindemann, 1971]. Selected eggs were impaled by momentarily vibrating the microelectrode either electrically by overtuning the negative capacitance compensation or mechanically by a jolt to the micromanipulator. Potential differences and membrane resistance were measured with the Intracellular-Preamp-Clamp Model 8500 (Dagan Corp., Minneapolis, MN) and recorded with a Beckman strip chart recorder, a Tektronix 564 storage oscilloscope, and a Mnemotron tape recorder.

Sperm Incorporation in the Absence of a Cortical Reaction

To study the electrical events associated with sperm-egg fusion and incorporation in the absence of a cortical granule discharge, two different approaches were taken, ie, insemination of eggs that had been treated with inhibitors to prevent secretion and refertilization of previously fertilized eggs. Secretion was inhibited by treating unfertilized eggs with 3% urethane (ethyl carbonate) in sea water for more than 3 min [Longo and Anderson, 1970b], 10 mM procaine HCl in sea water (pH 8.1) for 15 min [Vacquier and Brandriff, 1975], or 10 mM nicotine HCl in sea water (pH 8.1) for 15 min [Hägstrom and Allen, 1956; Longo and Anderson, 1970a]. Refertilization was accomplished by first fertilizing the eggs in the presence of 1 mg/mg soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), which prevents the hydrolytic activity of the cortical granule protease [Vacquier et al, 1972], and in 1 mM 3-amino-1,2,4-triazole to prevent the hardening of the elevating fertilization coat [Showman and Foerder, 1979]. The fragile fertilization coats were stripped from the now-fertilized eggs by gently pipetting through a fine bore pipette, and refertilization was then possible.

Cortical Reaction in the Absence of Sperm Incorporation

The discharge of the cortical granules was separated from sperm incorporation by two different methods, ie, artificial activation with an ionophore for divalent cations, A23187 (5 μ M) [Chambers et al, 1974; Steinhardt and Epel, 1974; Steinhardt et al, 1977] and fertilization of eggs treated with 10 μ M cytochalasin D, which cannot incorporate the spermatozoon [Schatten and Schatten, 1980; 1981; reviewed by Schatten, 1982]. To fully evaluate the relations between the motility necessary for the successful completion of fertilization and the electrical events, 1 μ M colcemid, a microtubule inhibitor that prevents the pronuclear migrations [Zimmerman and Zimmerman, 1967; Schatten and Schatten, 1981], was also employed.

RESULTS

Studies on Control Eggs

Membrane-potential and resistance of the unfertilized egg. Unfertilized eggs of *Lytechinus variegatus* can be clearly separated into two groups on the basis of measurements of their membrane potentials: i) On polylysine-coated dishes or on dishes wiped with paper tissues (Kimwipes) the adhering eggs had potential differences of about -20 mV and membrane input resistances of about 50 M Ω , and ii) a small proportion of the eggs will spontaneously adhere to untreated plastic dishes; in some of these attached

TABLE 1. Potential Differences and Membrane Resistances of "Leaky" Eggs Before, During, and After Fertilization (mean \pm SE, n=35)

Unfertilized egg		Reversed potential peak		Second plateau		Final plateau	
mV	M Ω	mV	M Ω	mV	M Ω	mV	M Ω
-18.33	56.33	9.26	10.47	-25.10	37.00	-66.53	15.20
± 1.19	± 1.27	± 0.79	± 1.16	± 2.02	± 5.28	± 1.97	± 3.22

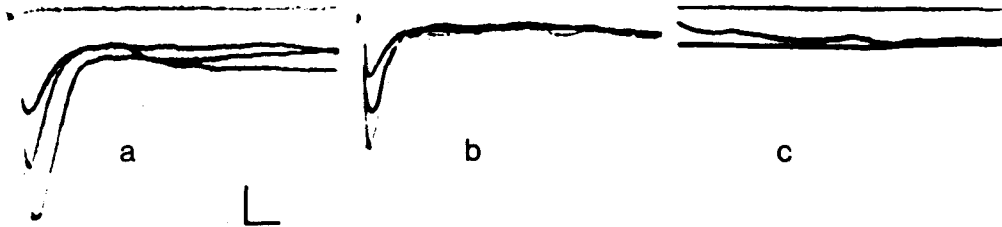


Fig. 1. Registration of potential changes during impalement of unfertilized *Lytechinus variegatus* eggs with glass microelectrodes. (a) Three different eggs, upper trace indicates baseline. (b) Three subsequent insertions of the same electrode into the same egg. The greatest deflection (-35 mV) resulted from the last impalement. Point indicates baseline. (c) One minute after impalement the recorded potential difference was -10 mV (lower trace; upper trace = baseline). Insertion of second electrode (middle trace) led to a minor depolarization, no initial deflection detectable. Horizontal bar: 0.5 msec in (a) and 1 msec in (b) and (c). Vertical bar: 10 mV.

eggs potential differences of -82 ± 2.7 mV (mean \pm SE, $n=14$) with input resistances from 100–500 M Ω were measured, while the remainder of these spontaneously sticking batches displayed potential differences around -20 mV. By injection of hyperpolarizing current, the membrane potentials of some of the -20 mV eggs could be converted into the higher potential difference as described by Chambers and de Armendi [1979].

Several hundred potential difference measurements of the first group have been made, and in Table 1, 35 eggs, where potential and resistance measurements before, during, and after fertilization have been recorded, are tabulated. In these eggs, at higher time resolution, it is found that within <0.5 msec after electrode insertion the registered potential difference changes may be as high as -80 mV and these vary considerably from one impalement to the next. In every case, however, a stable potential difference of -10 to -30 mV is then recorded for hours. During this time the membrane input resistance will often increase to about 100 M Ω . At times changes to higher membrane input resistances (300 M Ω) are often accompanied by an increase in the measured membrane potential to up to -80 mV. Figure 1a shows three impalements of different eggs with initial potential difference recordings between -30 and -70 mV which stabilized between -10 and -20 mV by 3 msec. Three subsequent impalements (within 1 min) of one egg with the same electrode led to different initial potential difference recordings but these stabilized at the same level, eg, -5 mV (Fig. 1b). The last impalement led to the highest initial deflection, indicating a rapid sealing and recovery of the membrane after electrode withdrawal. When a second electrode was inserted into a previously impaled egg (Fig. 1c), no initial deflection in the potential difference was observed; the potential difference of -10 mV was only slightly reduced after the second impalement.

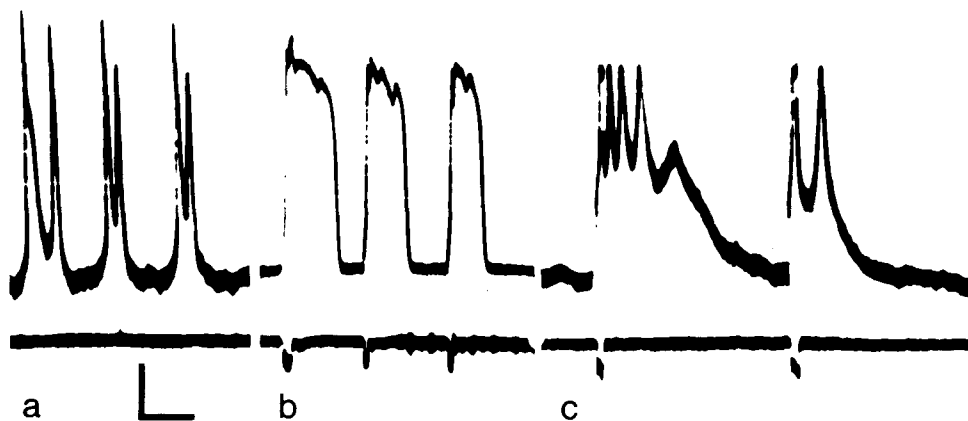


Fig. 2. Action potentials in unfertilized *L variegatus* eggs. (a) Spontaneous firing at threshold level, 40 mV. (b) Electrically stimulated at a potential difference of -72 mV. (c) Electrically stimulated at a potential difference of -55 mV. Lower trace: record of injected current. Horizontal bar: 5 sec in (a) and (b); 2 sec in (c). Vertical bar: 10 mV in (a) and (c); 20 mV in (b); 0.5 nA in (b) and (c) for the lower trace.

Action potentials in unfertilized eggs. Most of the -80 mV eggs, ie, the group with the higher potential difference level, were electrically excitable and produced action potentials that varied in shape and duration for different eggs. Three examples are shown in Figures 2a-c. Action potentials occurred spontaneously at the threshold level (eg, -40 mV, Fig. 2a) or could be electrically induced by applying depolarizing current at higher potential difference levels. The excitability of a low potential difference egg could easily be tested by transiently shifting the potential difference to higher values by injecting hyperpolarizing current. Excitable eggs fired an action potential when the hyperpolarizing current was switched off and the action potential threshold was passed. Furthermore, potential difference recordings from eggs that subsequently were found to have a long-lasting action potential always started with positive values after impalement; the negative capacitance compensation, used for impalement, masked the initial depolarizing part of the action potentials.

Fertilization potentials. Fertilization potentials, ie, the electrical events following sperm-egg attachment and probably triggered by the initial sperm-egg fusion, are clearly influenced by the electrically excitable or nonexcitable state of the unfertilized egg. Following sperm attachment to nonexcitable eggs, rapid potential changes were observed from -80 mV to values between -20 and -5 mV with potential reversal to $+20$ mV. In excitable eggs sperm attachment and the ensuing fusion action potentials were fired when the threshold was attained. In Figures 3a and 3b two different sperm-induced potential changes can be seen. For comparison, the electrically induced action potential of the egg in Figure 3b while still in the unfertilized state is shown in Figure 3c. In Figures 3d and 3e, a long-lasting action potential appears to cover the early electrical events in the egg triggered by the sperm, which can clearly be resolved after short action potentials (Figs. 3f and 3g). Following sperm attachment and apparent fusion in an egg with a potential of -15 mV a step-like depolarization occurs, the amplitude of which is dependent on the input resistance (Fig. 3h). Eggs with measured potential differences of about -10 mV will undergo normal fertilization and cleavages. Figure 3i shows the

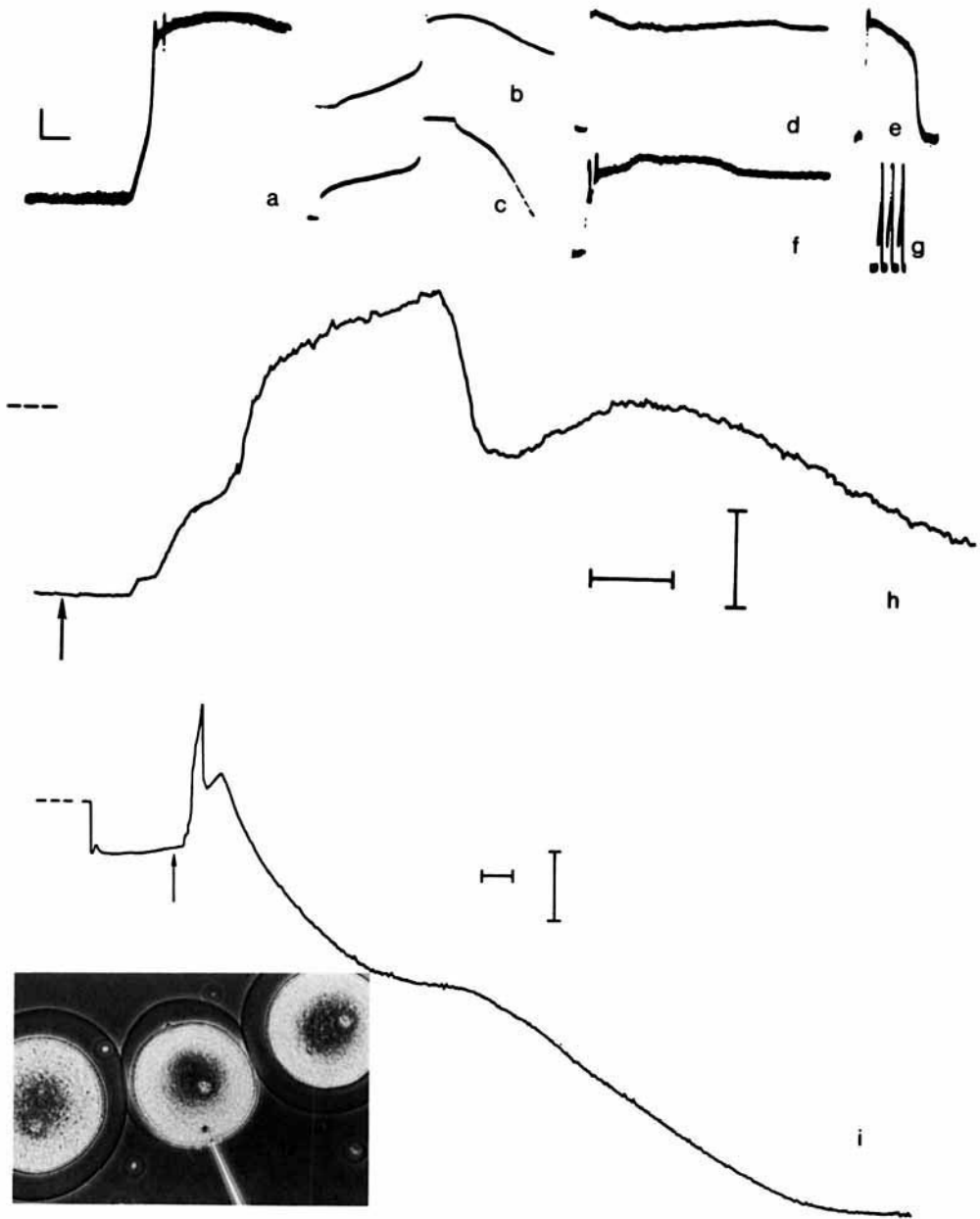


Fig. 3. Fertilization potentials in *L. variegatus* eggs. (a) High time resolution of the initial slope of a sperm-egg fusion and induced-action potential in a -64 mV egg. (b) High time resolution of the initial slope of a sperm-egg fusion and induced action potential in a -84 mV egg. (c) For comparison: electrically induced action potential of egg (b), 30 sec before fertilization. (d) Fertilization at -85 mV. (e) Same egg as (d), electrically induced action potential. (f) Fertilization at -79 mV. (g) Same egg as (f), electrically induced action potential. (h) Fertilization at -19 mV; dashed line = baseline (zero). (i) Fertilization at -8 mV; dashed line = baseline (zero). Inset: developing fertilization coat after sperm-egg fusion. Horizontal bar: 0.1 sec in (a), (b), and (c); 5 sec in (d), (e), (f), and (g); 10 sec in (h); 1 min in (i). Vertical bar: 20 mV in (b), (c), (d), (e), (f), and (g); 10 mV in (a), (h), and (i).

changes in membrane potential after fertilization of an -8 mV egg. After sperm-egg attachment and perhaps fusion, indicated by a 2 mV step [Dale et al, 1978], the potential reverses with a step to positive values and reaches a peak of about $+19$ mV before repolarization. Another plateau is reached at about -25 mV, before the final potential of about -70 mV is measured. These membrane-potential changes are accompanied by changes in membrane resistance. The initial resistance change after sperm-egg fusion appears to depend on the sealing of the electrode. At the positive peak the input resistance is only about 10 M Ω and increases with the second positive peak or the first plateau to about 35 M Ω ; it remains at this level until it is gradually reduced to about 15 M Ω at the final -70 mV plateau (see Table 1).

Sperm Incorporation Without Cortical Granule Discharge

In an attempt to clarify which of the potential changes are associated with cortical granule discharge, the addition of several drugs that inhibit the secretion of the cortical granules was performed.

Effects of urethane. Treatment of the eggs with 3% urethane prevents the cortical granule secretion without activating the egg, as shown by Longo and Anderson [1970a] in *Arbacia* eggs. Depending on the duration of treatment and varying with animals, urethane-treated eggs formed fertilization cones upon addition of sperm (Fig. 4a, inset), which were not accompanied by the formation of a fertilization coat. Addition of urethane to impaled eggs decreases the potential difference by 30 mV associated with the firing of an action potential if the threshold level is exceeded. In urethane an excitable egg loses its excitability. The input resistance is also reduced to about 60 M Ω . Addition of sperm to these eggs results in transient depolarizations each with an amplitude of 25 mV; a second sperm entry could be resolved. Since the block to polyspermy is impaired, supernumerary sperm-induced potential changes can be repeated in the same egg (Fig. 4a). Fifteen minutes after replacement of the medium with fresh sea water, unfertilized eggs could be fertilized and activated with fertilization coat formation and accompanying fertilization potentials indicating a rapid recovery from the urethane effects. It is noteworthy that urethane-treated eggs did not elevate fertilization coats when 5 μ M A23187 was added.

Effects of nicotine and procaine. Similarly, 10 mM nicotine or 10 mM procaine inhibits the discharge of the cortical granules after sperm entry, although unfertilized eggs treated with these substances are artificially activated, perhaps because of a shift in internal pH [Johnson et al, 1976; Vacquier and Brandriff, 1975]. After exposure periods of 30 min, the eggs normally display a membrane potential of -70 mV and a low input resistance of about 10 M Ω . Following insemination of these eggs, fertilization cones could clearly be detected and were always preceded by transient depolarizing steps (Figs. 4b and 4c); hyperpolarizing steps were noted at the time of fluid addition (Figs. 4c and 4d). Fertilization coats failed to elevate (Figs. 4b and 4c, insets), an indication of the absence of the cortical reaction.

Effects of refertilization. Membrane potential measurements were also made on eggs refertilized 15 min after the fertilization coats were removed. As is characteristic of eggs at this stage of development, the membrane potential was -70 mV, the input resistance was low, and the eggs were electrically inexcitable. The transient depolarizations observed following sperm attachment are quite similar to those described for nicotine- and procaine-treated eggs; multiple fertilization cones were also noted (Fig. 4d).

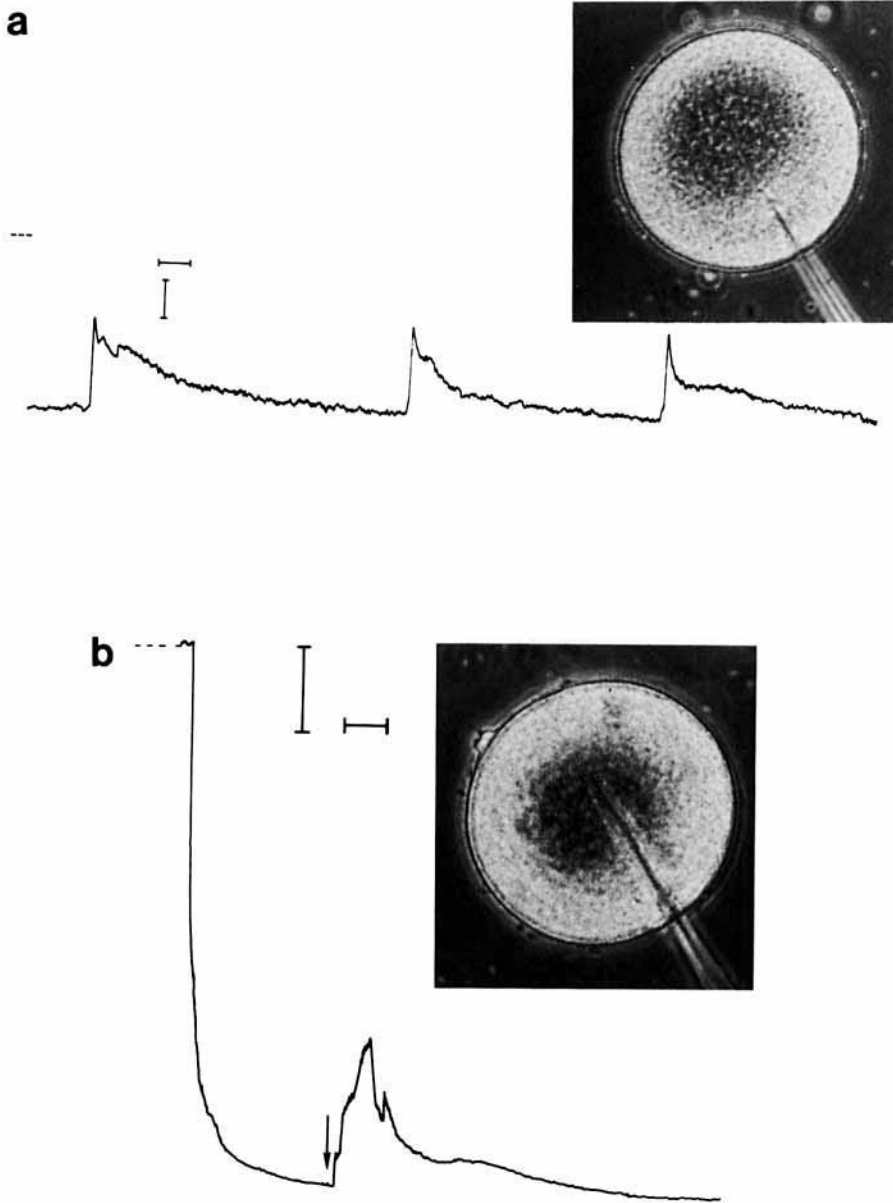


Fig. 4. Changes in potential difference upon sperm-egg fusion in the absence of cortical granule discharge and phase contrast pictures of the observed sperm entry cones. (a) -45 mV egg in 3% urethane. (b) -63 mV egg in 10 mM nicotine. (c) -51 mV egg in 10 mM procaine. (d) -51 mV egg in 1 mM amino-triazole + 1 mg/ml soybean trypsin inhibitor, refertilization. Arrows indicate addition of sperm. Horizontal bar: 10 sec in (a); 1 min in (b), (c), and (d). Vertical bar: 10 mV. Dashed line: Baseline (zero).

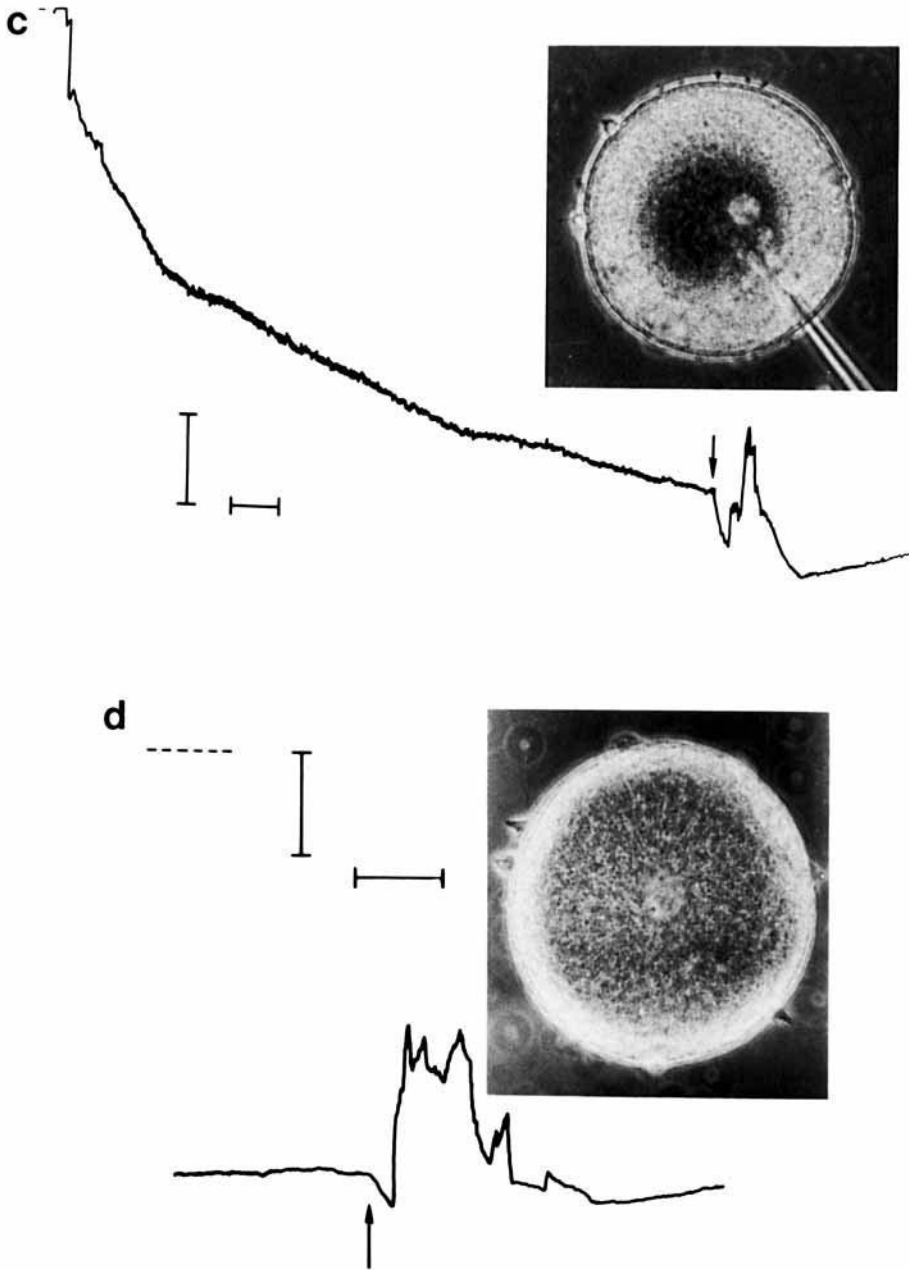


Figure 4.

Cortical Reaction in Absence of Sperm Incorporation

Effects of A23187. In a different approach, to investigate the role of sperm in triggering the fertilization potential, the cortical granule discharge was artificially induced by the release of intracellular Ca^{++} following the addition of $5 \mu\text{M}$ A23187. As can be seen from Figure 5a, the addition of ionophore results in a depolarization of the -55 mV egg. At the threshold level of -45 mV , three short action potentials were fired. These action potentials are similar to those electrically induced in the same eggs prior to fertilization. These are shown at the beginning of the trace of Figure 5a. A potential reversal with a peak at $+6 \text{ mV}$ occurred, a "fertilization" coat developed, and 18 min later a stable plateau of -78 mV was reached.

Effects of cytochalasin and colcemid. In eggs treated with cytochalasin D, a microfilament inhibitor, sperm incorporation cannot occur, even though insemination initiates the cortical granule discharge [Schatten and Schatten, 1980]. The potential reversal and fertilization coat elevation are similar to controls as is demonstrated in an egg with high membrane potential incubated in $10 \mu\text{M}$ cytochalasin D (Fig. 5; Dale and De Santis, 1981). The microtubule inhibitor, colcemid ($1 \mu\text{M}$), does not affect the electrical events at fertilization (Fig. 5c). These observations indicate that the inhibition of motility at fertilization [Schatten and Schatten, 1981] does not have any bioelectric consequences.

DISCUSSION

The cellular events that correlate with the bioelectric responses and the true, as opposed to measured, electrical changes at fertilization are still not fully elucidated. There appears to be a natural variability among different animals, among species, and

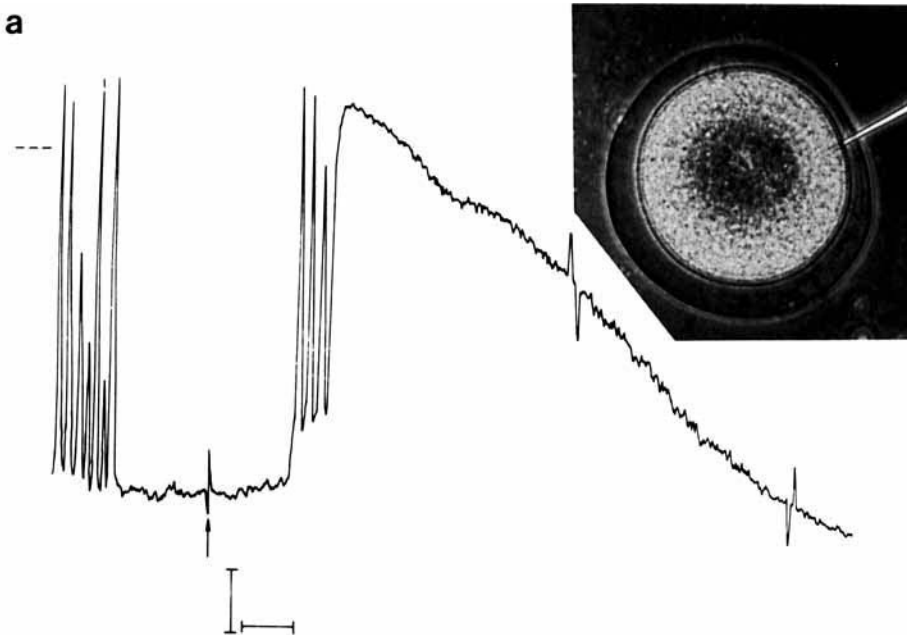


Figure 5a.

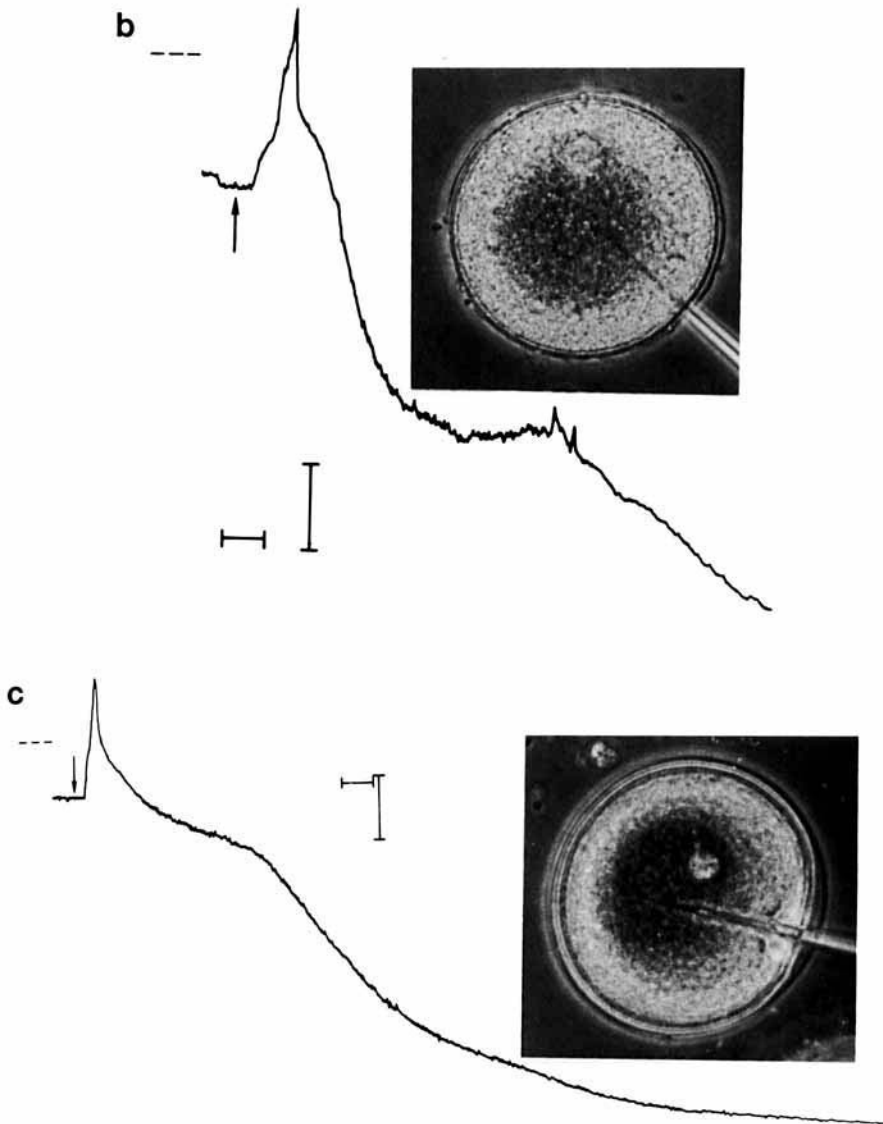


Fig. 5. Changes in potential differences upon cortical granule discharge in the absence of sperm incorporation and phase contrast pictures of the observed "fertilization" coats. (a) An excitable -54 mV egg and addition of $5 \mu\text{M}$ Ca^{++} ionophore A23187. (b) A -15 mV egg in $10 \mu\text{M}$ cytochalasin D and addition of sperm. (c) A -10 mV egg in $1 \mu\text{M}$ colcemid and addition of sperm. Arrows indicate addition of ionophore or sperm. Horizontal bar: 10 sec in (a), 1 min in (b) and (c). Vertical bar: 10 mV. A -15 mV egg in 10 mM cytochalasin D and addition of sperm.

over the gravid season, but this cannot be the reason for the reported large differences in membrane potential measurements [Chambers and de Armendi, 1979; Dale and Monroy, 1981; DeFelice and Dale, 1979; Hagiwara and Jaffe, 1979; Jaffe and Robinson, 1978; Steinhardt et al, 1971]. There is more likely an artificial variability, caused by differences in methods of preparation and cell adhesion, which leads to a selection of

eggs with special properties. The reason for the differences in measured membrane potentials most probably must be accounted for by a nonlinear steady-state current voltage relationship [see Hagiwara and Jaffe, 1979] and the high input resistance of the egg, which makes the recorded value of an electrode impalement strongly dependent on leakage. A leak in an egg with a nonlinear steady-state current voltage relation could either be minor, so that the recorded potential difference remains close to the membrane potential of 170 to 180 mV or with only a slight increase in leakage the recorded potential difference will drop to values around 115 mV. It can be seen that the initial potential difference after insertion of the electrode by a jolt during the first 0.5 msec is considerably higher, ie, 170 to 180 mV, than the "leak-potential" that is recorded after 3 msec, ie, 115 to 120 mV. From the decay curves, membrane potentials have been estimated by Chambers and Armendi [1979] according to the method of Lassen et al [1971], and it appears clear that the true membrane potential is certainly closer to 180 mV than to 110 mV, as previously concluded [Jaffe and Robinson, 1978]. This "leak potential" may vary from egg to egg (see Fig. 1a) but is rather stable in individual eggs.

With the exception of the first 1 to 3 sec, eggs with a "leak potential," ie, -15 to -20 mV eggs, will undergo fertilization with potential and resistance changes similar to those of eggs with a potential close to the expected membrane potential and both impaled groups will develop normally [Steinhardt et al, 1971]. These eggs could be used for the investigation of electrical processes, which start a few seconds after the sperm-egg attachment and fusion; however, they probably are not useful for investigating possible fast blocks to polyspermy [reviewed by Dale and Monroy, 1981]. After sperm-egg fusion, the egg membrane depolarizes to the level of its "leak potential." If this potential is already maintained in the impaled egg, only a minor potential change might be observed. Similar effects have been observed in *Paracentrotus* eggs by Taglietti [1979]. Following sperm-egg attachment and fusion, other later events are triggered. However, the sperm does not trigger development simply by inducing a membrane leak, since the leak set by the electrode is normally not sufficient to initiate the later events. Furthermore, the simple binding of sperm to the eggs clamped to $+5$ mV does not influence the electrical status of the egg [see also Jaffe, 1976]. The ions acquired by the sperm during the acrosome reaction [Tilney et al, 1978; Schackman et al, 1978] are likely candidates for triggering egg activation.

Membrane excitability was found in only a small percentage of the studied eggs. By clamping " -15 mV" eggs to -80 mV and recording the depolarizing off response, we could test whether an egg was excitable and assist the sealing in those eggs with a hyperpolarizing current, enabling them to regain their internally generated -80 mV potentials [Brown and Flaming, 1977; Chambers and de Armendi, 1979]. In most excitable eggs, the potential difference recording quickly decreased to -80 mV indicating a rapid sealing. In eggs with action potentials of a longer duration, the recording always started at positive values, indicating that the leak due to electrode impalement had triggered an action potential. Since action potentials vary in amplitude and duration, sperm-egg fusion processes can only be studied with the knowledge of their individual appearance, and some of the long-lasting action potentials might well be superimposed on effects related to egg activation triggered by the sperm (see Fig. 3).

After fusion of the sperm with the egg, a series of changes in the egg and in its membrane occurs [for review see Epel, 1978]. In an attempt to dissect the contribution of the different early events to the different components of the fertilization potentials, we treated the eggs with several drugs that inhibit the discharge of the cortical granules.

These experiments resulted in eggs with membrane properties that differed from those of the unfertilized egg and that varied with different inhibitors. However, they all revealed a transient depolarization of the membrane potential after the sperm-egg interaction. This depolarization never resulted in a potential reversal and hence is clearly different from the positive shift in potential that occurs simultaneously with the cortical granule discharge. In the interpretation of these experiments, it is essential that the effects of these drugs be considered. Jaffe [1980] has studied the electrical effects of low concentrations of nicotine. However, both nicotine and procaine induce artificial activation of unfertilized sea urchin eggs [Johnson et al, 1976; Vacquier and Brandriff, 1975], with accompanying increase in potassium conductance, and urethane reduced the measured potential difference. In other electrically excitable systems, urethane and procaine have been shown to decrease sodium flux [Pichon, 1978; Weidmann, 1955], and nicotine has been shown to induce membrane depolarizations [Ginsborg and Guerrero, 1964]. The very nature of the effects of these drugs, in blocking the fusion of the cortical granule membranes with the plasma membrane, would, in itself, be expected to have bioelectric correlates, an inherent problem with the pharmacological dissection of the membrane events at fertilization.

The amplitude of these sperm-induced depolarizations depended mainly on the membrane resistance as can be seen from the small depolarization in the refertilized eggs with low resistances (Fig. 4d) and the greater depolarizations in the urethane-treated eggs with high resistances (Fig. 4a). Furthermore these eggs always allowed multiple sperm entry leading only to minor depolarizations, which were superimposed on the depolarization of a preceding sperm-egg interaction (Fig. 4a). The reciprocal experiment, namely the discharge of cortical granules without sperm interaction by the Ca^{++} ionophore A23187 and without sperm incorporation by inhibition with cytochalasin D, clearly showed that the membrane potential reversal is correlated with the cortical granule discharge, as a result of Ca^{++} release.

Lytechinus variegatus eggs clamped to +5 mV cannot be fertilized, a value that is similar to that described for *S. purpuratus* [Jaffe, 1976]. Interestingly, sperm-egg adhesion will occur at this reversed potential. If this voltage-dependent block to fertilization becomes effective immediately upon reaching this level, eggs with action potentials will certainly have a very fast block to polyspermy, depending only on the time for depolarization until the threshold for the action potential is reached (eg, 50 msec, Fig. 3a, and 400 msec, Fig. 3b). If this action potential is long lasting, the block will be effective until the discharge of the cortical granules, which then provides a permanent block to polyspermy due to the resultant elevation of the fertilization coat. In the case of short action potentials, the potential difference repolarizes to negative values at about -10 mV (Fig. 3f) before the discharge of the cortical granules, thus permitting a momentary lapse in this fast block to polyspermy. However, the permanent block to polyspermy will be established, in any case, about 15 sec after sperm-egg fusion. Since we cannot exclude the possibility that action potentials are present only in a small percentage of eggs, this permanent block may well be the most common one. The discharge of the cortical granules was followed by fertilization coat elevations that usually could be observed when the eggs started to repolarize from +10 mV to negative values. At this time the resistance sharply increases to 35 M Ω , but the egg quickly repolarized to -70 mV and the input resistance decreased to about 20 M Ω .

In summary, these results, and the accumulated electrophysiological knowledge, appear to support the following model for sea urchin eggs at fertilization: The unfertil-

ized egg has a resting potential on the order of -80 mV, with a high membrane resistance, and may well be electrically excitable. Sperm-egg attachment and fusion, but not binding or incorporation per se, results in a minor (about 10 mV) and rapid (50–400 msec) transient, which would trigger action potentials in excitable eggs. This transient also triggers both the discharge of the cortical granules and the Na^+ -dependent membrane potential reversal; it is possible that the cortical reaction itself results in the opening of these Na^+ channels, with the later changes in capacitance [Jaffe et al, 1978] being due to a reorganization of this mosaic membrane. The fast block to polyspermy then may have two components—the fast electrical excitability of the egg and the longer Na^+ -dependent membrane potential reversal—while the late block is solely related to the secretion of the cortical granules. These results underscore the importance of the cortical reaction in the establishment of overlapping blocks to polyspermy.

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