Studies of the Mechanism of the Electrical Polyspermy Block Using Voltage Clamp during Cross-species Fertilization

LAURINDA A. JAFFE, MEREDITH GOULD-SOMERO, and LINDA Z. HOLLAND
Department of Biology, University of California, San Diego, La Jolla, California 92093. Dr. Jaffe's present address is Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032.

ABSTRACT Prevention of polyspermic fertilization in sea urchins (Jaffe, 1976, *Nature (Lond.)*. 261:68–71) and the worm *Urechis* (Gould-Somero, Jaffe, and Holland, 1979, *J. Cell Biol.* 82:426–440) involves an electrically mediated fast block. The fertilizing sperm causes a positive shift in the egg's membrane potential; this fertilization potential prevents additional sperm entries. Since in *Urechis* the egg membrane potential required to prevent fertilization is more positive than in the sea urchin, we tested whether in a cross-species fertilization the blocking voltage is determined by the species of the egg or by the species of the sperm. With some sea urchin (*Strongylocentrotus purpuratus*) females, ≥90% of the eggs were fertilized by *Urechis* sperm; a fertilization potential occurred, the fertilization envelope elevated, and sometimes decondensing *Urechis* sperm nuclei were found in the egg cytoplasm. After insemination of sea urchin eggs with *Urechis* sperm during voltage clamp at +50 mV, fertilization (fertilization envelope elevation) occurred in only nine of twenty trials, whereas, at +20 mV, fertilization occurred in ten of ten trials. With the same concentration of sea urchin sperm, fertilization of sea urchin eggs occurred, in only two of ten trials at +20 mV. These results indicate that the blocking voltage for fertilization in these crosses is determined by the sperm species, consistent with the hypothesis that the fertilization potential may block the translocation within the egg membrane of a positively charged component of the sperm.

The prevention of polyspermic fertilization in sea urchins (13), starfish (21), the marine worm *Urechis* (10), and anuran amphibians (3) involves an electrically mediated fast block. The fertilizing sperm causes the egg's membrane potential to shift in a positive direction, and this "fertilization potential" prevents subsequent sperm from fusing with the egg.

Our work is directed at understanding the mechanism of the voltage dependence of fertilization. Previous studies with *Urechis* have established that the fast polyspermy block does not inhibit the binding of sperm to eggs; rather it apparently acts at the level of sperm-egg plasma membrane fusion (10, 23). The effectiveness of the polyspermy block is a graded function of the egg's membrane potential, fertilization becoming less probable as potential becomes more positive (10). The inhibition of fertilization is due to the change in membrane potential per se and not the accompanying ion movements (10).

The molecular mechanism whereby positive membrane potential prevents fertilization is not known. The electric field across the egg plasma membrane could regulate sperm-egg fusion by affecting components in the egg membrane, or in the sperm membrane, or both. There could be potential-sensitive components in the egg membrane, for example, putative "sperm receptors," whose exposure to sperm in the external medium is potential dependent. Alternatively, there may be charged components in the sperm membrane that effect fusion by moving within the bilayer of the egg membrane; such movements would also be influenced by the electrical field in the egg membrane.

To investigate these alternatives we have used fertilization between two species which differ in the voltage sensitivity of fertilization: the sea urchin *Strongylocentrotus purpuratus*, in which egg membrane potentials of 0 mV will block fertilization (13), and the marine worm *Urechis caupo*, in which with comparable sperm concentrations egg membrane potentials of +30 mV are required to block fertilization (10). Thus, with cross-species fertilization, we ask whether the egg membrane potential required to block fertilization is determined by the species of the egg or by the species of the sperm. If the voltage to which the membrane must be clamped to prevent fertilization were determined by the sperm species, the hypothesis that

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there are potential-sensitive elements in the sperm membrane would be supported. Alternatively, if the blocking voltage were determined by the egg species, the hypothesis that the potential-sensitive components reside in the egg membrane would be supported. In our results, we will describe the morphological and electrophysiological characteristics of the cross-fertilization between *Urechis* sperm and sea urchin eggs and show that the voltage dependence is characteristic of the sperm species.

MATERIALS AND METHODS

Procedures for obtaining gametes of *U. caupo* and *S. purpuratus* have been described (7, 12). After being spawned into sea water, sea urchin eggs were washed twice by allowing them to settle. All experiments were made in natural sea water at 14–17°C. Unless otherwise noted, inseminations were performed with eggs and semen diluted 1:1,000 (vol/vol). At this dilution, there are ~700 *Urechis* eggs/ml and 700–1,900 sea urchin eggs/ml (depending on the amount of egg jelly). At a dilution of 1:1,000, there are $2.4 \pm 0.7 \times 10^7$ *Urechis* sperm/ml ($n = 4$) and $4.4 \pm 0.8 \times 10^7$ sea urchin sperm/ml ($n = 5$).

Assay of Incorporation of Sperm Nuclei

The presence of *Urechis* sperm nuclei within the sea urchin egg cytoplasm could be determined in whole-mounts of fixed eggs only after the sperm nuclei had begun to decondense. Egg pronuclei or condensed chromosomes were also visible in these whole-mounts. Externally bound *Urechis* sperm are not dislodged by fertilization envelope elevation and can be so numerous as to obscure the interior of the egg (Fig. 1); therefore, before fixation most of the bound sperm were removed by suspending the eggs for 15 to 30 min with occasional agitation in 1 mg/ml pronase (B grade, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) in sea water. The pronase treatment did not interfere with egg development. Fixation was carried out for a minimum of 15 min each in 4% neutral formaldehyde (in deionized water) and a 3:1 mixture of 100% ethanol and glacial acetic acid (three changes of each fixative). The eggs were then cleared in 60% acetic acid and observed at × 400 under phase contrast.

Electron Microscopy

Eggs for electron microscopy were fixed by the method of Gould-Somero and Holland (8), with the concentration of NaCl reduced to 0.5 M. Contrast of silver sections was enhanced with uranyl acetate and lead citrate.

Electrophysiology

Electrophysiological methods for recording fertilization potentials were described previously (16). For voltage-clamping, we used two intracellular microelectrodes, one for passing current and one for recording potential, and electronics built by Akishige Ono, of Biodyne Electronics Laboratory (Santa Monica, CA). The leakage with two electrodes was more severe than with a single electrode; therefore, recorded membrane potentials were reduced compared with those observed with a single electrode. However, neither the fertilization potential nor fertilization envelope elevation was prevented by the presence of the two electrodes (15).

Several hundred eggs (with jelly coats intact) were placed on the bottom of a plastic petri dish containing ~3 ml of natural sea water. Observations were made with a Wild stereoscope (Wild Heerbrugg Instruments Inc., Farmingdale, NY), at × 100. After inserting the electrodes and establishing a stable baseline, sperm were added from a Pasteur pipet. Approximately 0.1 ml of a 1:100 dilution of semen was added ~1 cm from the egg. The sperm suspension did not mix uniformly in the dish; we estimate that near the egg the sperm dilution was ~1:1,000.

RESULTS

Urechis Sperm Fertilize Sea Urchin Eggs

Urechis sperm added to *S. purpuratus* eggs can undergo the acrosome reaction and bind to the egg surface, with the membranes of the acrosomal processes closely apposed to the egg plasma membrane (Figs. 1 and 2). Among eggs from different females, the number of bound sperm ranged from a scattered few to a densely packed layer (Fig. 1). Between 2 and 5 min

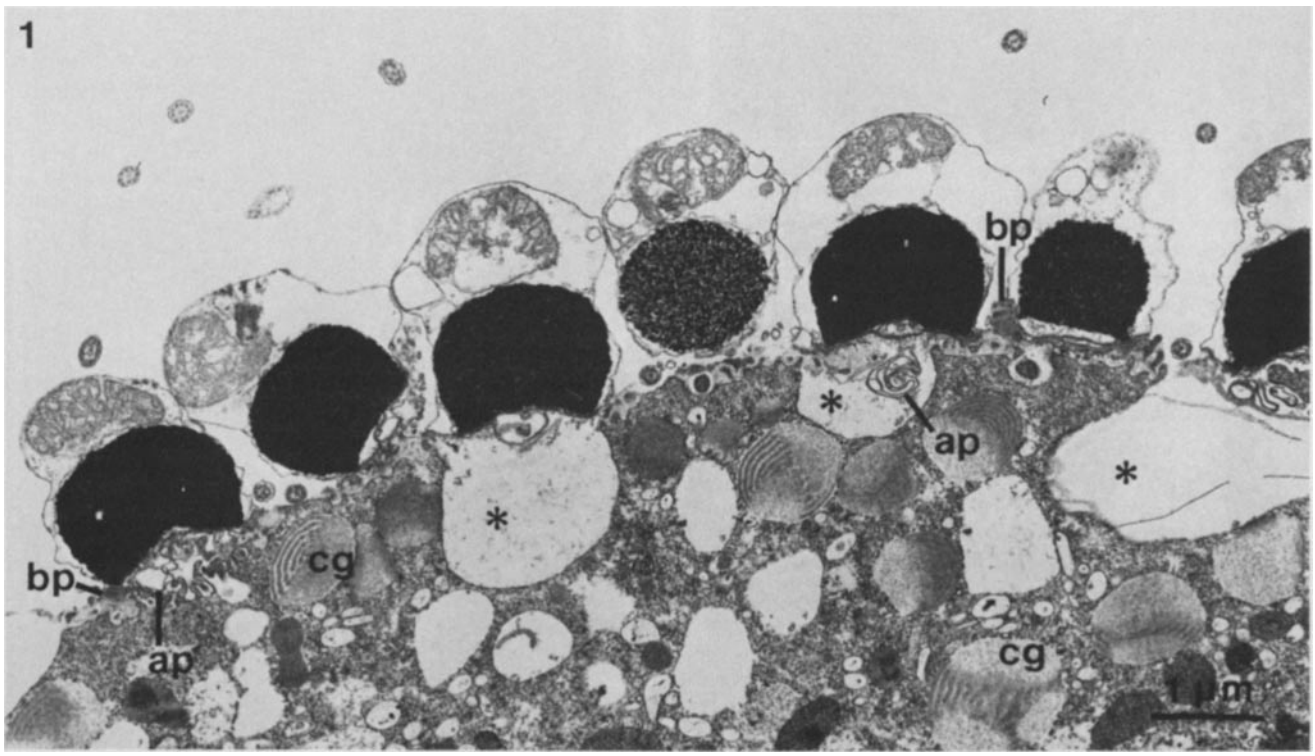

FIGURE 1 Acrosome-reacted *Urechis* sperm bound to a sea urchin egg fixed 16 min after insemination at 15°C. Eggs diluted 1:100 (vol/vol) were inseminated with a 1:500 dilution (vol/vol) of sperm. Fertilization envelopes elevated on 24% of the eggs. No fertilization envelope elevated from the portion of the egg surface in the figure, despite exocytosis of some cortical granules (*). Intact cortical granules (*cg*) are present both in the cortical cytoplasm and several microns beneath the egg surface. In areas of the egg surface lacking bound sperm, cortical granules are typically restricted to the egg cortex. *ap*, acrosomal process. *bp*, binding protein. × 15,000.

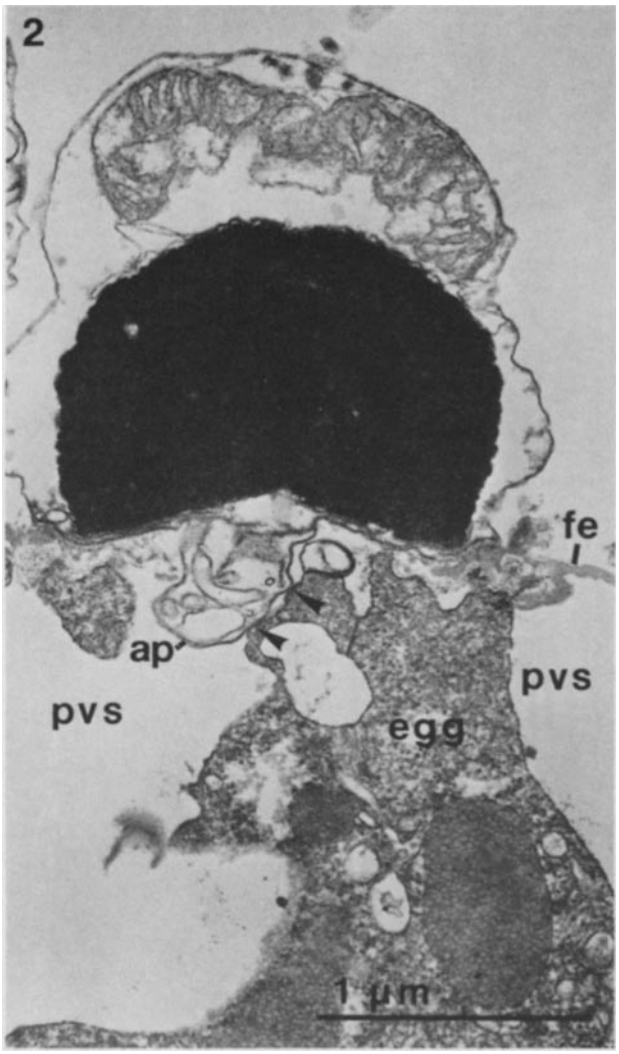

FIGURE 2 *Urechis* sperm bound to a sea urchin egg which has elevated a fertilization envelope (*fe*), showing the close association between the sperm acrosomal process (*ap*), and the egg plasma membrane (arrows). The egg is from the same sample as that in Fig. 1. As is typical in eggs with elevated fertilization envelopes, a tongue of egg cytoplasm extends through the perivitelline space (*pvs*) to the site of sperm attachment, where the fertilization envelope is incomplete. × 36,750.

after insemination, fertilization envelopes elevated on from 0 to 100% of the eggs; the percentage depended on the female but not the male. When insemination was done as described in Materials and Methods, ≥90% of the eggs from each of 41 out of 188 females (22%) elevated fertilization envelopes; unless otherwise stated, experiments were restricted to eggs from these 41. Typically, eggs binding few sperm failed to elevate fertilization envelopes, but those binding many sperm did not always elevate them. Sometimes the fertilization envelopes were less elevated than normal, or formed over only a part of the egg surface, probably due to failure of some cortical granules to undergo exocytosis (Fig. 1). A greater percentage of eggs of *S. purpuratus* than of *Lytechinus pictus* elevated fertilization envelopes in response to *Urechis* sperm.

A jelly coat around the eggs was necessary for a high percentage of sperm binding and fertilization envelope elevation; removal of jelly (either mechanically or with pH 5 treatment) significantly reduced cross-fertilizability (e.g., from 90% to ≤10% fertilization envelope elevation in two experiments). A jelly coat, however, does not guarantee cross-fertilization. If the coat is too thick, sperm may fail to reach the egg surface, but for eggs with thin jelly coats there must be other unknown variables to explain why eggs from some females were not fertilized by the bound *Urechis* sperm. Treatment of eggs with dithiothreitol (5) to partially remove the vitelline layer as well as the jelly coat, thus exposing egg plasma membrane directly to sperm, did not increase the frequency of cross-fertilization, as assayed by cleavage.

A higher sperm:egg ratio was necessary for the cross-species fertilization than for either homologous cross. A sperm:egg ratio in the incubation mixture of 2,800 ± 1,800 (SD, $n = 4$; see Materials and Methods) *Urechis* sperm per sea urchin egg was required for 50% of the eggs to elevate fertilization envelopes, whereas a ratio of 16 ± 11 ($n = 4$) sperm:egg sufficed for the homologous sea urchin fertilization. 50% of *Urechis* eggs fertilized with 50 ± 8 ($n = 2$) *Urechis* sperm per egg. (With a higher egg concentration, 50% fertilization of *Urechis* eggs occurs with a lower sperm:egg ratio.)

Sea urchin eggs which elevated a fertilization envelope in response to *Urechis* sperm also underwent pronuclear breakdown. In eggs from five females the percentages with partial or complete fertilization envelopes ranged from 77 to 100% and were within 3% of the percentages that had undergone pronuclear breakdown in corresponding samples. Although both egg chromosome condensation and aster formation occurred in

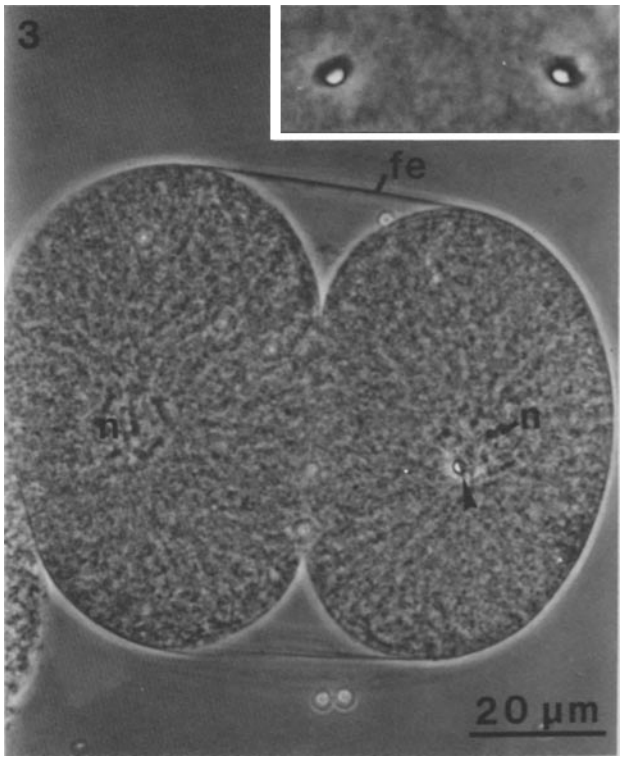

FIGURE 3 Whole-mount of a 2-cell sea urchin embryo fixed 2 h after insemination with *Urechis* sperm. A partially decondensed *Urechis* sperm nucleus (arrow) is associated with one of the interphase nuclei (*n*). *fe*, fertilization envelope. × 925. The *inset* shows a higher magnification of two partially decondensed *Urechis* sperm nuclei in the cytoplasm of another sea urchin egg, with decondensing chromatin (phase dense) extending from a core of condensed chromatin (highly refractile) (cf. reference 8). × 2,950.

most of the activated eggs, fewer cleaved (Fig. 3). Occasionally, however, cross-fertilized eggs developed to swimming plutei (Stephano Hornedo and Gould-Somero, unpublished observations).

Decondensing *Urechis* sperm nuclei were often, but not always, observed in the activated sea urchin eggs. For example, in the five samples described above with 77 to 100% fertilization envelope elevation, the percentages of eggs with decondensing sperm nuclei (see Fig. 3) ranged from 34 to 86%. Whether or not *Urechis* sperm had fused (i.e., established cytoplasmic continuity) with those activated eggs that had no decondensing sperm nucleus could not be determined, since it is not practical to distinguish sperm which have fused with the egg, but have not undergone nuclear decondensation from sperm merely bound to the egg surface (see Materials and Methods in reference 9). In eggs containing decondensing sperm nuclei, the average number per egg was 2.2 ± 1.1 (SD, $n = 4$ of the above five samples).[1] We did not look for decondensing *Urechis* sperm nuclei in the sea urchin eggs from which we made electrical recordings.

Urechis chromosomes, distinguishable from sea urchin chromosomes by their larger size, were only rarely seen in sea urchin eggs. Partially decondensed sperm nuclei frequently persisted through one or more mitotic cycles (see Fig. 3). However, *Urechis* sperm chromosomes apparently do not contribute to subsequent development. There have been several other reports of activation and penetration of sea urchin eggs by sperm from other phyla (annelids and molluscs). In these crosses, as in the *Urechis* × sea urchin cross, the paternal genome also apparently fails to contribute to embryonic development: "defects" range from failure of sperm nuclear decondensation to elimination of male chromatin from the zygote nucleus (6, 17, 18, 20, 27).

Sea Urchin Sperm Did Not Fertilize Urechis Eggs

Attempts to fertilize *Urechis* eggs with *S. purpuratus* sperm were unsuccessful. Sperm binding was poor and the eggs were not activated, even if the pH was raised to 9 (which enhances cross-fertilization between some species, e.g., reference 19). Partial or complete removal of the *Urechis* egg surface coat with 1 M glucose, 10 mM EGTA, pH 7 (9), resulted in no significant improvement, nor did addition of sea urchin egg jelly to induce acrosome reactions. Attempts to fertilize *Urechis* eggs with sperm from *Lytechinus pictus*, *Chaetopterus pergamentaceous*, and *Patiria miniata* were also unsuccessful.

A Fertilization Potential Accompanies Fertilization of S. purpuratus Eggs by Urechis Sperm

In all of the electrophysiological studies, we used only those *S. purpuratus* eggs from females from which ≥90% of the eggs elevated fertilization envelopes when aliquots were exposed to *Urechis* sperm. The electrical response of a sea urchin egg to *Urechis* sperm is illustrated in Fig. 4 *a*. After addition of *Urechis* sperm to the recording chamber (see Materials and Methods), a response was usually seen within 1 min and sometimes as early as 15 s (Fig. 4 *a*). The fertilization potential consisted of a shift from the unfertilized egg potential of about −70 mV (16) to a positive value, followed by a return to −70 mV. The response to *Urechis* sperm of five eggs from five females was recorded with a single microelectrode: in all five, a fertilization potential occurred and the fertilization envelope elevated. The peak amplitudes of fertilization potentials elicited by *Urechis* sperm in sea urchin eggs were similar to those elicited by sea urchin sperm but were significantly less positive than in *Urechis* eggs fertilized by *Urechis* sperm (Fig. 4 and Table I). Thus, the amplitude of the fertilization potential is a characteristic of the species of egg, as expected since the fertilization potential channels are not introduced by the sperm but are in the egg before fertilization (2, 11, 14, 24, 25). On the other hand, the duration of the fertilization potential was longer when sea urchin eggs were fertilized with *Urechis* sperm than when they were fertilized with homologous sperm (Fig. 4 and Table I). The significance of the long duration is not clear, since it is not known for either species how the duration of the fertilization potential is controlled.

Potential Dependence of Cross-fertilization

Fertilization of *S. purpuratus* eggs with *S. purpuratus* sperm and of *Urechis* eggs with *Urechis* sperm does not occur if the egg membrane potential is held at a sufficiently positive level (10, 13). To test whether cross-fertilization is voltage dependent, we voltage-clamped *S. purpuratus* eggs during exposure to *Urechis* sperm. When the *S. purpuratus* egg membrane was clamped at +50 mV, fertilization by *Urechis* sperm occurred in only nine of twenty trials, as determined by elevation of a fertilization envelope. In all of these experiments, ≥90% of the surrounding eggs in the recording chamber elevated fertilization envelopes. In contrast, when the clamp voltage was +20 mV, cross-fertilization occurred in ten of ten trials.

Fertilization is always accompanied by a change in membrane resistance, indicated by a change in the current required

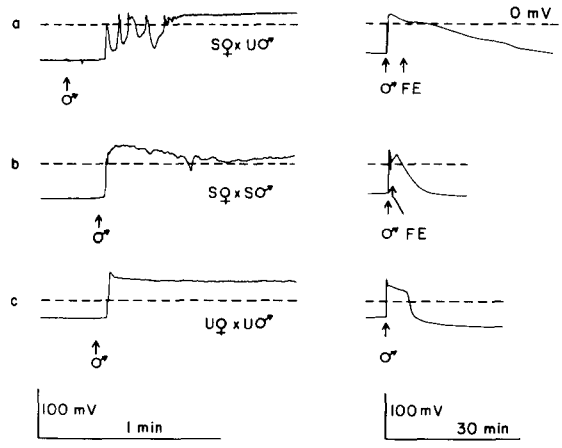

FIGURE 4 Fertilization potentials. Membrane potential as a function of time. On the left are portions of the original chart records. On the right are replots on a contracted time-scale showing the entire fertilization potentials. (*a*) *S. purpuratus* egg × *U. caupo* sperm. (*b*) *S. purpuratus* egg × *S. purpuratus* sperm. (*c*) *Urechis* egg × *Urechis* sperm. ♂ indicates addition of sperm. *FE* indicates the time at which a fertilization envelope was first visible on the egg.

[1] Eggs were scored 2 to 2½ h after insemination, when the percentage of eggs with decondensing *Urechis* sperm nuclei was maximal. Before egg pronuclear breakdown, at 1 to 1½ h, decondensing *Urechis* sperm nuclei were never seen, and only condensed sperm which had not been removed by pronase digestion (see Materials and Methods) were associated with the eggs. Since some sperm may have fused with eggs but failed to undergo nuclear decondensation, the number of decondensing nuclei is a minimal estimate of the number of incorporated sperm. After 2½ h, there were fewer decondensing *Urechis* sperm nuclei, presumably due to dispersion or degradation of the sperm chromatin.

to maintain the voltage clamp. About the time of fertilization envelope elevation, the clamping current begins to increase (Fig. 5 *b* and *c*); this corresponds to the hyperpolarization that occurs in the unclamped egg. One might also expect to see an earlier current associated with the positive-going phase of the fertilization potential; however, probably because the clamp potential is near the equilibrium potential of the response, this current is too small to be detectable. A change in the clamping current was not observed in eggs that did not elevate a fertilization envelope (Fig. 5 *a*).

In seven of the eleven trials in which no fertilization envelope elevated during the +50 mV clamp, we tested to see whether *Urechis* sperm could fertilize the egg after the clamp was turned off. In all seven trials, a fertilization potential occurred. In four of the seven trials, the fertilization envelope elevated as well, e.g., see Fig. 5 *a*. In the other three, we could not determine whether or not a fertilization envelope elevated because of the opaque layer of *Urechis* sperm which bound to the egg surface during the clamp period. In summary, for those trials which could be scored, previous exposure to +50 mV did not prevent subsequent fertilization.

These experiments indicate that cross-fertilization is potential-dependent. The most direct criterion for fertilization would be sperm-egg plasma membrane fusion, but a conclusive assay for the initial events of fusion has not been developed. (With electron microscopy of serial sections, fusion sites have been seen, but smaller initial points of fusion may have gone undetected; for this reason, it is not known exactly when fusion occurs. See discussion in reference 10.) Sperm nuclear penetration is an indicator that fusion has occurred, but we couldn't determine whether a sperm had been incorporated unless it had undergone nuclear decondensation (see above). Therefore, our criterion for fertilization was fertilization envelope elevation, and it was important to establish that fertilization envelope elevation itself is not blocked by clamping the egg membrane potential at +50 mV. To do this, we inseminated *S. purpuratus* eggs with *S. purpuratus* sperm and, at 10 s after the rise of the fertilization potential, clamped the membrane at +50 mV. (We used the homologous fertilization for this control because the rise of the cross-species fertilization potential is often gradual [Fig. 4 *a*] and, therefore, it was not always practical to precisely define the initiation point of the fertilization potential.) The secretion which causes the elevation of the fertilization envelope starts ~30–45 s after the rise of the fertilization potential (at 15°C) (15). In nine of ten trials, fertilization envelope elevation proceeded normally at +50 mV. We conclude that the potential dependence of fertilization results from the potential dependence of a step preceding fertilization envelope elevation, which we assume is sperm-egg plasma membrane fusion.

Next, we investigated how the voltage required to block fertilization in the cross compared to that in self-species fertilization, by voltage clamping *S. purpuratus* eggs at +20 mV and then exposing them to *S. purpuratus* sperm, at the same concentration as the *Urechis* sperm (estimated to be ~3 × 10^7 sperm/ml; see Materials and Methods). In each experiment, the clamp was maintained until ≥90% of neighboring eggs had elevated fertilization envelopes, then the clamped egg was scored for elevation of a fertilization envelope. *S. purpuratus* sperm succeeded in fertilizing the *S. purpuratus* egg in only two of ten trials at +20 mV (see Fig. 6).[2] We did not test fertilization of *Urechis* eggs by *Urechis* sperm under the identical conditions used in our experiments, because voltage-clamping at a positive

TABLE I

Fertilization Potential Characteristics in the S. Purpuratus × Urechis and Homologous Crosses

Cross	Peak amplitude*	*p*‡	Duration*	*p*‡	*n*
	mV		*min*		
S♀ × S♂	+17 ± 11	>0.1	1.4 ± 1.2	<0.01	7
S♀ × U♂	+24 ± 4	<0.01	6.6 ± 2.2	>0.6	5
U♀ × U♂§	+51 ± 6		7.0 ± 1.2		16, 10

* Values given are means ± SD.

‡ Calculated by the Student's *t* test, comparing the means of the two samples. Values <0.01 are significantly different at the 99% confidence level, whereas values >0.1 are not significantly different at the 90% confidence level.

§ Data from reference 14. The two values for *n* refer to peak amplitude and duration, respectively.

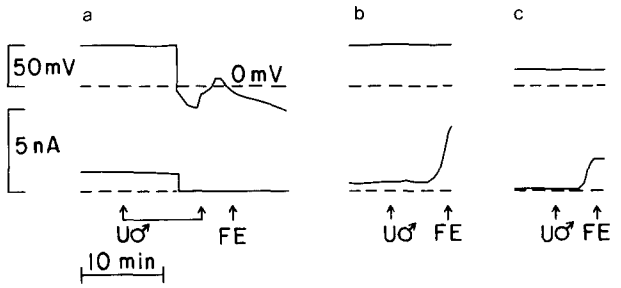

FIGURE 5 Voltage clamp during insemination of *S. purpuratus* eggs with *Urechis* sperm. Upper traces are membrane potential as a function of time; lower traces are current as a function of time. (*a*) Clamp at +50 mV. *U♂* indicates addition of *Urechis* sperm. During the clamp period, fertilization envelopes elevated on 97% of neighboring eggs but not on the experimental egg. When the clamp was turned off and a freshly prepared sample of *Urechis* sperm at the same concentration was added, a fertilization potential occurred and a fertilization envelope elevated on the experimental egg (*FE*). In some trials, when the clamp was turned off, fertilization occurred without reinsemination. (*b*) Clamp at +50 mV. A fertilization current occurred and a *FE* elevated on the experimental egg during the clamp. (*c*) Clamp at +20 mV. A fertilization current occurred and a *FE* elevated on the experimental egg during the clamp.

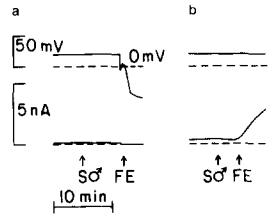

FIGURE 6 Voltage clamp during insemination of *S. purpuratus* eggs with *S. purpuratus* sperm. Upper traces are voltage as a function of time; lower traces are current as a function of time. (*a*) Clamp at +20 mV. *S. purpuratus* sperm were added at S♂. During the clamp, fertilization envelopes elevated on 96% of neighboring eggs but not on the experimental egg. When the clamp was terminated, a fertilization potential occurred and a *FE* elevated on the experimental egg. (*b*) Clamp at +20 mV. A fertilization current occurred and a *FE* elevated on the experimental egg during the clamp.

[2] To achieve cross-fertilization, it was necessary to use a high sperm concentration (see above). Previous studies of the voltage dependence of homologous fertilization used a lower sperm concentration (10, 13). Therefore, because the probability of sperm entry is a function of sperm concentration as well as membrane potential, the absolute voltages required to block fertilization in our present experiments cannot be directly compared with those observed in previous studies.

level for a comparable period of time activates or kills *Urechis* eggs. However, it has been previously established, using a shorter period of positive potential and a lower sperm concentration, that the voltage required to block self-fertilization of *Urechis* is ~30 mV more positive than that required to block self-fertilization of *S. purpuratus* (see Introduction, and references 10, 13). Therefore, all available data are consistent in indicating that a more positive voltage is required to block fertilization by *Urechis* sperm than by *S. purpuratus* sperm. We conclude that the voltage required to prevent fertilization depends on the species of the sperm.

DISCUSSION

When sea urchin eggs are voltage-clamped at +20 mV and inseminated with sea urchin sperm, fertilization only rarely occurs (in two of ten trials). When sea urchin eggs at +20 mV, however, are inseminated with *Urechis* sperm, fertilization always occurs (in ten of ten trials). To prevent the *Urechis* sperm at a comparable concentration from fertilizing the sea urchin eggs, it is necessary to hold the egg membrane potential more positive: at +50 mV, fertilization occurred in nine of twenty trials. Since the potential dependence of fertilization is probabilistic and not an absolute threshold phenomenon (10), it might be argued that a higher potential is required to exclude *Urechis* sperm because these sperm are more efficient at fertilizing sea urchin eggs than are sea urchin sperm. The probability, however, of *Urechis* sperm fertilizing sea urchin eggs is in fact lower. The sperm:egg ratio required for fertilization of sea urchin eggs by *Urechis* sperm is 175 times that required for fertilization of sea urchin eggs by sea urchin sperm.

Our results indicate that, at least for the species studied, the voltage level necessary to block fertilization is characteristic of the sperm species and suggests that the electric field across the egg plasma membrane may regulate fertilization by affecting charged components in the sperm membrane. An alternative hypothesis, that the egg membrane contains a "sperm receptor" whose exposure to sperm depends on the egg membrane's potential, is not ruled out; however, the expectation of such a model in its simplest form would be that the voltage-dependence of fertilization should exhibit the same blocking voltage, independent of the sperm species.

Our findings suggest the possibility that the voltage-dependence of fertilization is a consequence of some positively charged element in the sperm membrane which must insert in the egg membrane and move across the electric field in the egg membrane during the process of membrane fusion in fertilization. Thus, an electric field opposing this translocation could inhibit fertilization. Potential-dependent insertion of proteins and translocation of proteins across lipid bilayers has been demonstrated for diphtheria toxin and for asialoglycoprotein receptor (1, 4). It is then interesting to ask whether the sperm acrosomal process membrane, which fuses with the egg membrane, contains a positively charged protein. Whole sea urchin sperm have a net negative surface charge (22), and a study of the distribution of the surface charge between the acrosomal process and other regions of the starfish sperm did not show any regional specializations (26). A biochemical analysis, however, of acrosomal membrane proteins and their charge remains to be made.

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