Ionic Mechanism of the Fertilization Potential of the Marine Worm, *Urechis caupo* (Echiura)

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Abstract Microelectrode and tracer flux studies of the *Urechis* egg during fertilization have shown: (a) insemination causes a fertilization potential; the membrane potential rises from an initial level of $-33 \pm 6$ mV to a peak at $+51 \pm 6$ mV ($n = 16$), falls to a plateau of about $+30$ mV, then returns to the original resting potential $9 \pm 1$ min ($n = 10$) later; (b) the fertilization potential results from an increase in Na$^+$ permeability, which is amplified during the first 15 s by a Ca$^{++}$ action potential; (c) the maximum amplitude of the fertilization potential, excluding the first 15 s, changes by 51 mV for a 10-fold change in external [Na$^+$]; (d) in the 10 min period after insemination, both Na$^+$ and Ca$^{++}$ influxes increase relative to unfertilized egg values by factors of $17 \pm 7$ ($n = 6$) and $34 \pm 14$ ($n = 4$), respectively; the absolute magnitude of the Na$^+$ influx is 16 $\pm$ 6 times larger than that of Ca$^{++}$; (e) in the absence of sperm these same electrical and ionic events are elicited by trypsin; thus, the ion channels responsible must preexist in the unfertilized egg membrane; (f) increased Na$^+$ influx under conditions of experimentally induced polyspermy indicates that during normal monospermic fertilization, only a fraction of available Na$^+$ channels are opened; we conclude that these channels are sperm-gated; (g) Ca$^{++}$ influx at fertilization is primarily via the membrane potential-gated channel, because kinetics are appropriate, and influx depends on potential in solutions of varying [Na$^+$], but is independent of number of sperm incorporations in normal sea water.

Introduction

A change in membrane potential at fertilization was first observed with an intracellular microelectrode by Tyler et al. (1956) in the egg of a starfish. Fertilization initiated a transient positive shift in potential lasting for $\sim 1$ min. Subsequently, similar changes were observed in the eggs of sea urchins (Hiramoto, 1959; Higashi and Kaneko, 1971; Steinhardt et al., 1971; Ito and Yoshioka, 1972; Chambers et al., 1974; Jaffe, 1976; MacKenzie and Chambers, 1977; Jaffe and Robinson, 1978), amphibians (Maeno, 1959; Morrill and Watson, 1966; Ito, 1972), and fish (Ito and Maeno, 1960; Ito, 1962, 1963; Kiyohara and Ito, 1968; Nuccitelli, 1977). Because the electrical response could be elicited by
artificial activation as well as by fertilization, Maéno (1959) named it the activation potential. In this paper we use the term fertilization potential for the response to fertilization, and activation potential for a response to artificial activating agents. In the amphibian egg the activation potential, and perhaps the fertilization potential as well, is due to a selective increase in chloride permeability (Maéno, 1959; Ito, 1972). In the sea urchin egg, however, the fertilization potential is Na⁺-dependent, at least in part (Steinhardt et al., 1971; Chambers and de Armendi, 1978). For a review of the electrical properties of egg cell membranes, see Hagiwara and Jaffe (1979).

In the present paper, we describe the fertilization potential in eggs of the marine worm Urechis caupo. Using both intracellular microelectrode and tracer flux techniques, we have analyzed the ion channels which exist in the unfertilized egg membrane and which open and close in the 20-min period after fertilization. Our analysis has been facilitated by experimentally induced polyspermy. By varying the number of sperm admitted per egg, we could alter the egg membrane's permeability to different ions and distinguish sperm-gated from voltage-gated pathways. A separate report (Gould-Somero and Jaffe, 1977; Gould-Somero et al., 1979) describes evidence that one function of the Urechis fertilization potential is to provide an electrical polyspermy block, similar to that in the sea urchin (Jaffe, 1976).

MATERIALS AND METHODS

Specimens of Urechis caupo were collected year round from Bodega Bay (University of California Marine Station) or Elkhorn Slough (Sea Life Supply, Sand City, Calif.). Methods for maintaining the adult worms and obtaining gametes from the storage sacs have been described by Gould (1967). The unfertilized Urechis egg has a diameter of ~120 μm, and a shape "similar to that which would be produced in a spherical rubber ball by indenting the surface at one point" (Tyler, 1932). The egg is fertilized externally in seawater, at the germinal vesicle stage. A schedule of developmental events at 17°C is shown in Table I (see also Gould-Somero, 1975). Within the first 4 min after insemination, the dent disappears as the egg "rounds out." Between 4 and 20 min, a surface coat elevates, and at 6 min, the germinal vesicle begins to break down. These events are collectively referred to as "activation."

Microelectrode Measurements

All experiments were done at 17°C; temperature was controlled by working in a constant temperature room or by using a water-cooled stage. Eggs were immobilized in small scratches on the bottom of a plastic petri dish. In some cases, a glass micropipette with a tip diameter of ~5 μm was used to hold the egg in position while penetrating it with the microelectrode. The dish containing the eggs was supported in a Plexiglas frame and was observed under a Wild stereomicroscope at ×50 (Wild Heerbrugg Instruments Inc., Farmingdale, N.Y.). Two types of dishes were used: (a) when solution exchange was not to be done and when the egg was to be recovered from the microelectrode for fixation, we used a dish without partitions. After recording, we lifted the electrode with its attached egg from the bottom of the dish, rotated the dish in the frame to isolate the egg from others in the dish, and removed the electrode by lowering the egg to the bottom of the dish and drawing the electrode back along the plastic surface. The egg was picked up with a mouth-controlled pipette and transferred to fixative. (a) For solution exchange with the microelectrode in the cell, the dish had plastic partitions which made a narrow
channel containing a total volume of 3 ml. Plastic baffles at each end of the channel restricted continuity of inflow and outflow pools with the recording chamber to a narrow slit at the bottom of the dish. This minimized mechanical disturbance and insured that less dense solutions such as those containing choline+ were forced to the bottom of the dish where the egg was located. Solutions were exchanged with a hand-controlled push-pull syringe system connected to the inflow and outflow pools. To ensure complete exchange was occurring, we (a) exchanged solutions with added dye; (b) compared recordings after exchange with those obtained by starting in the test solution; and (c) observed no further change in membrane response when a second volume of new solution was exchanged. 20 ml of new solution sufficed to exchange completely the 3-ml volume.

| Time | Event |
|------|-------|
| 0 | Insemination |
| 0-4 | Egg “rounds out” from concave to spherical shape.† |
| 4-20 | Surface coat elevates.§ |
| 6 | Germinal vesicle begins to “break down” (cytoplasm and nucleoplasm mix).|| |
| 25 | Sperm chromatin maximally decondensed |
| 35 | First polar body |
| 45 | Second polar body |
| 90 | First cleavage |

* From review by Gould-Somero (1975) except as noted. Only morphological changes are listed. † Tyler (1932); Paul (1975 b). Diameter changes from 118 to 108 μm. Volume changes <5%. § Paul (1975 b); Gould-Somero and Holland (1975). Unlike the corresponding event in the sea urchin egg, the elevation of the surface coat in the Urechis egg proceeds from several points simultaneously, without an explosive secretion of cortical vesicles; some may discharge asynchronously, but most remain intact in the early embryo. || Paul (1970).

Test solutions are listed in Table II. The same results were obtained with natural or artificial seawater. Ion-substituted seawaters were prepared as follows: equimolar substitution of choline+ (Sigma Chemical Co., St. Louis, Mo.) for Na+, methanesulfonate- (Aldrich Chemical Co., Inc., Milwaukee, Wis., reagent grade) for Cl-, and Mg++ for Ca++. For high K+, K+ was substituted for Na+. All were at pH 8.0, buffered with 2.4 mM HCO3-. For 10 Ca++ + 20 Co++, 1 part 400 mM CoCl2 was added to 20 parts seawater; final pH was 7.6 to prevent precipitation.

Microelectrodes with resistances of 30–60 MΩ were prepared by back-filling micropipettes with 3 M KCl; a fine glass fiber in the pipette facilitated filling. Penetration was effected by first positioning the electrode to slightly depress the cell surface, and then applying an oscillating current through the microelectrode by transiently turning up the “negative capacitance.” One electrode was used for both recording voltage and passing current; the IR drop across the electrode was electronically balanced by means of a bridge circuit (Bio-Dyne Electronics Laboratory, Santa Monica, Calif., model AM-2).
Maximum applied currents were \(3 \times 10^{-10}\) A, small enough that electrode resistances remained constant. The bath was grounded through a seawater agar bridge, to prevent contact of eggs with Ag\(^+\) ions, which even at \(2 \times 10^{-10}\)M activate *Urechis* eggs. In order to avoid junction potential changes at the agar interface during solution exchange, we recorded differentially with respect to a 3 M KCl-filled micropipette (1-10 MΩ), for all experiments with methanesulfonate\(^-\) and some with choline\(^+\). In most of the other experiments, we recorded nondifferentially, since the estimated junction potential does not exceed 4 mV in the solutions used.\(^1\) Membrane potential and applied current were recorded on a chart recorder (Gould Inc., Cleveland, Ohio, model 220) with a frequency response up to 40 cycles/s; this is fast enough to give true potential changes in the present experiments.

Eggs were inseminated in the recording chamber by adding drops of sperm suspension near the egg. Except as noted, the final sperm concentrations were about \(10^6-10^7\) sperm per ml (≈ 1:30,000 to 1:3,000 dilution of “dry” sperm).

### Table II

| Solution, mM | Na\(^+\) | K\(^+\) | Ca\(^++\) | Mg\(^++\) | Cl\(^-\) | Choline\(^+\) | Methane sulfonate\(^-\) | Co\(^++\) |
|--------------|---------|---------|---------|--------|--------|---------|----------------|--------|
| Natural SW   | 470     | 10      | 10      | 54     | 548    | -       | -              | -      |
| Artificial SW| 490     | 10      | 10      | 56     | 570    | -       | -              | -      |
| 100 K\(^+\)  | 400     | 100     | 10      | 56     | 570    | -       | -              | 484    |
| 86 Cl\(^-\)  | 490     | 10      | 10      | 56     | 86     | 485     | -              | -      |
| 5 Na\(^+\)   | 5       | 10      | 10      | 56     | 570    | 440     | -              | -      |
| 50 Na\(^+\)  | 50      | 10      | 10      | 56     | 570    | 370     | -              | -      |
| 120 Na\(^+\) | 120     | 10      | 10      | 56     | 570    | 370     | -              | -      |
| 360 Na\(^+\) | 360     | 10      | 10      | 56     | 570    | 130     | -              | -      |
| 1 Ca\(^++\)  | 490     | 10      | 1.0     | 65     | 570    | -       | -              | -      |
| 10 Ca + 20 Co| 470     | 9.5     | 9.5     | 53     | 570    | -       | 20             |        |

In addition, all solutions contained 29 mM SO\(_4\)\(^-\) and 2.4 mM HCO\(_3\)\(^-\). pH was adjusted to 8.0, except for Co\(^++\) solutions and appropriate controls, which were at pH 7.6.

**Ion Content Determination**

For analysis of K\(^+\), Na\(^+\), and Mg\(^++\), egg samples were washed for 10-15 min (five washes) in “ion-free seawater,” i.e., artificial seawater in which the ion to be measured had been replaced with an appropriate substitute. Egg pellets were dissolved in 70% nitric acid and analyzed on a Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.). Samples were also assayed for K\(^+\) and Na\(^+\) by Environmental Engineering Laboratory, San Diego; their results were similar to ours; all data are pooled to give the values in Table III. For analysis of Cl\(^-\), egg samples were washed in Cl\(^-\) and SO\(_4\)\(^-\)-free seawater, and Cl\(^-\) was extracted by the method for red blood cells in Cotlove (1964, p. 372). Cl\(^-\) content was assayed colorimetrically by Environmental Engineering Laboratory. Standards contained the same reagents as the

\(^1\) Control experiments showed that for all of our solutions, liquid junction potential changes were <4 mV. Calculations using the Goldman-Hodgkin-Katz equation (Hodgkin and Katz, 1949), substituting self-diffusion coefficients for permeabilities (permeability = mobilities x diffusion coefficient) also indicated that such junction potentials should be <4 mV. \(D_{Na} = 1.3, D_{K} = 1.9, D_{Ca} = 2.0, D_{choline} = 1.0, D_{methanesulfonate} = 1.5.\) (The latter two values were estimated from self-diffusion coefficients of similar molecules [see Hille, 1975].)
samples, plus amounts of K\(^+\), Na\(^+\), Mg\(^{++}\), Ca\(^{++}\), and Cl\(^-\) to approximate the ionic composition of the sample.

Control experiments showed that egg ion contents did not decrease significantly during washing in ion-free seawaters. Eggs (two batches for each ion) were preincubated for 24 h in seawater containing \(^{42}\)K, \(^{24}\)Na, or \(^{36}\)Cl. External tracer was removed by washing eggs in normal seawater; then the amount of tracer remaining in one aliquot was compared to that remaining in a second aliquot washed five additional times in ion-free seawater. The ion-free washes did not significantly lower tracer content of samples; the possible error in these wash controls was \(\sim 20\%\) for Na\(^+\) and Cl\(^-\), and \(\sim 10\%\) for K\(^+\).

We estimated molar concentrations of each ion, assuming uniform distribution of the ion in the cell water, from ion content data and the volume of cell water per egg, as follows: from wet and freeze-dried weights of packed egg samples, and the volume of extracellular space determined with \(^{[3H]}\)methoxy-inulin (New England Nuclear, Boston, Mass.), we calculated that 70\% of the total egg volume is water. For egg volume, we used \(6.3 \times 10^{-10}\) liter, determined by geometrical analysis of photomicrographs (Tyler, 1932), which is similar to that which we estimated from inulin displacement (\(7.7 \times 10^{-10}\) liter). Cell water volume was then calculated to be \(6.3 \times 10^{-10}\) liter (\(\times 0.70\)) = \(4.4 \times 10^{-10}\) liter.

### TABLE III

| Ion     | Estimated internal concentration | External concentration |
|---------|----------------------------------|------------------------|
|         | pmole/egg \(\pm SD\) (n)*        | mM                    |
| K\(^+\) | 104 \(\pm 16\) (11)              | 240                   |
| Na\(^+\) | 5.9 \(\pm 0.9\) (5)              | 13                    |
| Cl\(^-\) | 48 \(\pm 5\) (3)                 | 110                   |
| Mg\(^{++}\) | 7.9 \(\pm 0.6\) (4)     | 18                    |
| Ca\(^{++}\) | 0.56 \(\pm 0.01\) (2) | 56                    |

* Contents of K\(^+\), Na\(^+\), and Cl\(^-\) were the same in eggs freshly removed from animals (above values) and eggs incubated in seawater for 6 h.

\(\)$ Assuming that the ion is uniformly distributed in the cell water. Cell water volume = \(4.4 \times 10^{-10}\) liter (see Methods).

\(\)$ Data from Johnston and Paul (1977).

### Tracer Flux Measurements

Eggs (diluted 1:50 or 1:100 vol/vol) were incubated at 17\(^\circ\)C, in a shallow layer on the bottom of a beaker. Egg batches were not used if \(> 5\%\) of the eggs were artificially activated, or if fertilization in normal sea water resulted in \(< 95\%\) activation. Packed egg volumes were measured after centrifugation at 300 g for 20 s; 1 ml of 1:100 egg suspension contains \(\sim 7,000\) eggs as determined by counting eggs in 50-\(\mu\)l aliquots. (The jelly coat of *Urechis* eggs does not dissolve in seawater.) Sperm were used at final dilutions of 1:2,500-1:100,000, as indicated. These proportions of eggs and sperm ensure that at least 90\% “fertilization” occurred within 10 s after insemination (Paul, 1975 a). Paul determined the time of fertilization by measuring (a) the time at which eggs had established a polyspermy block, or (b) the time at which sperm could be killed with detergent without blocking development.

Radioactive tracers were obtained from New England Nuclear. The usual tracer concentrations (\(\mu\)Ci/ml) in egg suspensions were as follows: \(^{32}\)Na, 10; \(^{42}\)K, 5; \(^{40}\)Ca, 1-2. \(^{24}\)Na and \(^{42}\)K samples (0.5-2 ml) were washed four times (10 ml each wash) by centrifuging and resuspending with chilled seawater (4-10\(^\circ\)C), for a total of 10-15 min. \(^{40}\)Ca samples...
were washed the same way, five to seven times, for 10–20 min. Eggs washed in chilled seawater retained the same amount of radioactivity as eggs washed in 17°C seawater. Washed egg samples labeled with 24Na or 42K were dissolved in 2 ml 0.5% SDS (sodium dodecyl sulfate, in deionized water), and radioactivity was measured in a Nuclear Chicago gamma radiation counter (Nuclear Chicago Corp., Des Plaines, Ill.). Washed egg samples labeled with 46Ca were collected by suction onto Whatman GFA glass fiber filters (Whatman, Inc., Clifton, N.J.) which were placed in scintillation vials with 0.4 ml Protosol:water (9:1) to dissolve the eggs. 10 ml scintillation fluid (4 g PPO, 0.05 g POPOP, per liter toluene) was added to each vial, and radioactivity was determined in a Beckman liquid scintillation counter (Beckman Instruments, Fullerton, Calif.). For specific activity determinations, samples (10–40 µl) of supernatant seawater were counted. Since unlabeled eggs added to known amounts of 24Na, 42K, and 46Ca produced no significant quenching, eggs were omitted from the seawater samples.

Assay for Sperm Penetration

The number of sperm entering eggs was determined after fixation according to Paul (1975 a) and Gould-Somero et al. (1977; 1979). For microelectrode experiments, the egg was fixed individually; for tracer flux experiments large numbers of eggs were fixed simultaneously. If sperm enter but fail to activate eggs, as, for example, with light insemination in 5 or 50 mM Na+, the penetrated sperm nuclei are not distinguished from externally bound sperm (see Gould-Somero et al., 1977). Therefore, before fixation, such eggs were activated by incubation in seawater with 370 mM K+ substituted for Na+.

RESULTS

The Unfertilized Egg

Before investigating fertilization, we need to understand the membrane properties of the unfertilized egg, the background upon which the fertilization events are superimposed. The unfertilized egg has a negative resting potential which usually reaches a steady maximum amplitude a few minutes after electrode penetration. Eggs with steady resting potentials less negative than −25 mV were discarded; we considered these eggs to be damaged because they had small input resistances and often died or activated spontaneously. Of those eggs which attained a steady resting potential more negative than −25 mV, the average was −33 ± 6 mV (SD, n = 133). 2 eggs out of 133 had resting potentials more negative than −50 mV.

In Table III, we list ion content data for the unfertilized egg, and calculations of internal concentrations assuming uniform distribution of the ions in the cell water. Although these data can provide only an estimate of ionic activity near the membrane, they do tell us that the negative resting potential of the unfertilized eggs could be due to a major selectivity for either K+ or Cl−. Varying [Cl−] while measuring potential gave a surprising result—the potential sometimes shifted slightly in a direction opposite that expected (Table IV). This effect is not understood, but it is not a junction potential artifact (see Methods) and it has also been reported in Ascaris muscle (Del Castillo and Morales, 1967; Weisblat et al., 1976) and in certain mammalian skeletal muscles (Dulhunty, 1978). In any case, the result indicates that the membrane is not responding as a Cl− electrode. When [K+] was varied, the potential shifted in the expected direction, but the magnitude of the shift was ~ 20 mV/10 times Δ[K+], as...
opposed to 57 mV expected for a K\(^+\) electrode (Table IV). Varying [Na\(^+\)] (choline\(^+\) substituted) also caused the potential to shift \(\sim 10\) mV/10 times \(\Delta[Na^+]\) (Table IV, Fig. 5). We conclude from these data that the negative resting potential is due largely to K\(^+\) permeability with some Na\(^+\) component. Possibly there may also be a component due to leak around the microelectrode. Since the measured input resistance of the membrane is very high, \(\sim 500\) M\(\Omega\), even a small leak could be significant. However, the measurement of a \(-60\)-mV resting potential in 5 mM Na\(^+\) (Table IV, Fig. 5) indicates that the leak component, if it exists, is small.\(^2\) The possibility of an electrogenic pump contribution to the resting potential was not investigated.

When outward current was applied to the membrane of the unfertilized egg, two distinct inflections in the rise of the voltage response were observed (arrows in Fig. 1). The first occurred at about \(-30\) mV, the second at about \(+10\) mV. We refer to these two responses as action potentials Nos. 1 and 2, respectively.

| Ion   | Concentration | \(\Delta V\)  |
|-------|---------------|---------------|
|       | mM            | mV ± SD (n)   |
| K\(^+\) | 10–100 (×10) | +20 ± 3 (2)   |
| Na\(^+\) | 490-50 (×1/10) | -10 ± 5 (17) |
|       | 490-5 (×1/100) | -27 ± 6 (5)   |
| Cl\(^-\) | 570-86 (×1/7) | -7 ± 7 (4)    |

Both action potentials can be observed as either on or off responses (Fig. 1a and b). Action potentials in eggs were first reported by Miyazaki et al. (1972) in tunicates, and have subsequently been observed in starfish, sea urchin, mouse, annelid, and coelenterate (reviewed by Hagiwara and Miyazaki, 1977; Okamoto et al., 1977; Hagiwara and Jaffe, 1979). The two-threshold response of the Urechis egg is seen in most of these other eggs as well.

In the Urechis egg, the peak voltage of the first action potential varies, depending on stimulus intensity, resting potential, and the particular batch of eggs; the peak voltage of the second action potential is, however, largely independent of these factors and is \(+52 \pm 7\) mV (SD, \(n = 101\)). The duration of the first action potential is \(< 1\) s; however, the second action potential does not reach steady state in response to applied current until \(10 \pm 3\) s (SD, \(n = 17\), Fig. 1c).

Fig. 2 shows the ionic dependence of the action potentials. When [Na\(^+\)] was reduced from 490 to 5 mM, neither action potential was significantly changed (\(n = 6\), Fig. 2a). The Na\(^+\) independence of the second action potential is also shown in Fig. 5, where peak voltage is plotted as a function of [Na\(^+\)]. However,

\(^2\) The hyperpolarization of the membrane when external Na\(^+\) is replaced with choline\(^+\) is too large to be attributed to a liquid junction potential at a leak site, because the self-diffusion coefficient of Na\(^+\) is only \(\sim 1.3\) times that of choline\(^+\). (The calculated change in liquid junction potential for a cell in 500 mM NaCl or 500 mM choline Cl, with cytoplasm containing 200 mM K\(^+\) and essentially immotile anions, is about 6 mV [see footnote 1].)
a precise comparison of the first action potential in varying \([Na^+]\) could not be made, because lowering \([Na^+]\) significantly shifted the resting potential in the threshold range. When \([Ca^{++}]\) was reduced from 10 to 1 mM, the first action potential was slightly reduced and the second action potential was eliminated \((n = 7, \text{Fig. 2} \ b)\). 20 mM cobalt (a Ca++ channel-blocking agent; Hagiwara and Takahashi, 1967) added to seawater reduced the first action potential, and eliminated the second \((n = 6, \text{Fig. 2} \ c)\). CI− substitution \((86 \text{mM CI}^-, \text{substituted})\) had almost no effect on action potential amplitude. We conclude that the second action potential is Ca++-dependent, although the ionic basis of the first action potential is uncertain.

**Electrical Responses to Fertilization and Artificial Activation**

The voltage response of the egg membrane to fertilization, referred to as the fertilization potential is shown in Fig. 3a. On the left is a copy of the original chart record, showing the first 1-min period after insemination; on the right, data from the same egg are replotted to show the membrane potential during a 20-min period. Within 2 s (in the fastest cases) of sperm addition, the membrane potential of the egg shifts to a positive level. Presumably, the duration of the
interval between insemination and the potential shift is related to the time required for the sperm to swim to and contact the egg. An inflection in the rise is often seen at about +10 mV, where the second action potential is initiated (arrow in Fig. 3a). The potential reaches a maximum amplitude at +51 ± 6 mV, 0.8 ± 0.2 s (SD, n = 16) after the start of the rise. If the first 15 s of the fertilization potential is excluded, to minimize the contribution of the action
potential, the maximum amplitude reached during the fertilization potential is +38 ± 5 mV (SD, n = 16). After reaching the maximum, the potential falls off to a plateau phase; to characterize the average plateau potential, we calculated the average potential during the 5-min period after insemination, excluding the first 15 s (see Fig. 4 legend): +27 ± 4 mV (SD, n = 12). Finally, the potential becomes negative again, reaching 0 mV at 7 ± 1 min (SD, n = 10) after the initial rise and the level of the unfertilized egg resting potential at 9 ± 1 min (SD, n = 10). We refer to this latter time as the fertilization potential “duration.” The membrane continues to hyperpolarize, reaching a level more negative than that measured in the unfertilized egg. In eight eggs whose average membrane potential before fertilization was −37 ± 8 mV, the average membrane potential

![Figure 3](image)

**Figure 3.** Fertilization and activation potentials. (a) Voltage response to insemination. On the left is a copy of the original chart record showing the first 1-min period after insemination. Sperm were added at the vertical arrow. The horizontal arrow marks the action potential inflection. On the right is a replot of data for 20 min. (b) Voltage response to trypsin (1.0 mg/ml in seawater); data presented as in (a). Trypsin was perfused into the recording chamber during the bar and was not removed during the experiment.

20 min after fertilization was −62 ± 6 mV. Both action potentials can be elicited at 20 min after insemination, but the current required to reach the threshold of the second action potential is two to four times larger (n = 7). The membrane potential stays at approximately this level until at least 40 min after insemination.

Of the 16 eggs for which we recorded fertilization potentials after insemination in normal seawater, 11 were inseminated with a moderate concentration of sperm (approximately 10⁶ sperm/ml), and 5 were inseminated with a heavy concentration (approximately 10⁷/ml). After recording, we fixed 10 of these 16 eggs for counting of incorporated sperm nuclei; 4 eggs were monospermic, and 6 were dispermic. The fertilization potential characteristics described above—maximum amplitude excluding first 15 s, average plateau potential and dura-
tion — did not differ significantly between eggs receiving a heavy vs. moderate sperm concentration, or between mono- and dispermic eggs. However, the fertilization potentials of three of the six dispermic eggs had a second step in the initial rise (similar to what is shown in Fig. 4b), and in a fourth dispermic egg the potential fell briefly below 0 mV during the plateau phase.

Paul (1970, 1975b) showed that trypsin initiates the morphological changes which normally occur at fertilization: rounding out, surface coat elevation, and germinal vesicle breakdown (see Table I). Fig. 3b shows that 1.0 mg/ml trypsin in sea water also elicits an activation potential similar to the fertilization potential. On the left is a copy of the chart record to show the first 1-min period; on the right is a replot of the data for a 20-min period from the same egg. The maximum amplitude of the responses to 1.0 mg/ml trypsin was similar to that of fertilization potentials initiated by sperm: +52 ± 4 mV (SD, n = 3), or +40 ± 3 mV (SD, n = 3) excluding the first 15 s after the potential went more positive than 0 mV. Durations of the trypsin responses were also similar to sperm initiated responses: 9 ± 3 min (SD, n = 3).

Occasionally, activation potentials occurred spontaneously, soon after electrode penetration. In three cases, spontaneous activation potentials were accompanied by morphological activation; in other cases, the eggs died. The activation potentials of all three activated eggs were similar to fertilization potentials, although the shapes were more variable.

Fertilization Potentials in Low Calcium and Low Sodium Seawater

In order to determine which ions carry the current during the fertilization potential, we recorded fertilization potentials in solutions in which the concentrations of Ca++ or Na+ had been reduced. Fig. 4 shows data obtained in 1 mM Ca++ and the normal 10 mM Ca++. The maximum amplitudes of the responses recorded in 1 mM Ca++ were +38 ± 6 mV (SD, n = 12), compared to +51 ± 6 mV (SD, n = 16) in 10 mM Ca++. The maximum amplitude in 1 mM Ca++ is slightly smaller than that in 10 mM Ca++, but if we measure the maximum amplitude excluding the first 15 s, to minimize the action potential contribution, there is no significant difference: +38 ± 6 mV (SD, n = 12) in 1 mM Ca++, compared to +38 ± 5 mV (SD, n = 16) in 10 mM Ca++. Also the average plateau potential (defined above) is similar in 1 and 10 mM Ca++: +24 ± 8 mV (SD, n = 9) and +27 ± 4 mV (SD, n = 12), respectively. These results indicate that Ca++ is not the major current carrier of the fertilization potential, but carries a minor portion of the current during the early phase.

Examples of fertilization potential records in 1 mM Ca++ are shown in Fig. 4b. Typically, the rise of the fertilization potential has multiple steps. These probably correspond to multiple sperm entries, since eggs in 1 mM Ca++ are somewhat more susceptible to polyspermy than eggs in 10 mM Ca++ (Gould-Somero et al., 1979). Under insemination conditions comparable to those used for the microelectrode recording, an average of two to five sperm per egg was found in 1 mM Ca++, compared to one to two sperm in 10 mM Ca++ (determined from experiments with four-egg batches). However, we were not able to count sperm in individual eggs from which we had made electrical recordings in 1 mM Ca++, because the eggs almost always died after recording.
In contrast to the small effects of Ca ++ replacement, Fig. 5 shows that the amplitude of the fertilization potential is greatly reduced when external Na + is replaced with choline +. The maximum voltage, excluding the first 15 s after the rise of the fertilization potential, to minimize the action potential contribution, has a 51-mV slope for a 10-times concentration change between 490 and 50 mM Na +. Calculated from the average plateau potentials, the slope is 46 mV for a 10-times concentration change. These slopes are close to the expected 57 mV for a Na + selective channel. Fig. 5a also shows data for the maximum amplitude of the fertilization potential, including the first 15 s after its rise; in this case, the slope of voltage as a function of [Na +] is 62 mV for a 10-times change in [Na +]. A slope greater than 57 mV probably results from the opening of voltage-
FIGURE 5. Fertilization potentials in varying Na⁺. (a) Voltage ± SD is plotted as a function of Na⁺ concentration in the external solution. (O) Resting potentials of the unfertilized egg; (□) peak of the action potential (No. 2) in the unfertilized egg. (▲) Fertilization potential maxima; (●) maxima excluding the first 15 s; (■) average plateau potential (see legend for Fig. 4). The number of eggs for each point for a particular Na⁺ concentration is shown in parentheses; an exception is the 5-min average for 490 mM Na⁺, n = 12. (b) Examples of data used for (a); each example is shown on two time scales. Arrows mark the time of sperm addition. The second example with 50 mM Na⁺ shows a light followed by a heavy insemination (see text). All recordings were obtained from eggs in which the recording was started in the test solution (no solution exchange). (c) Fertilization potential amplitude (maximum excluding first 15 s) and morphological activation as a function of number of sperm entries in 50 mM Na⁺. (O) Eggs did not activate (observed for 30 min after insemination). (●) Eggs activated. Time-course was slightly slower than in normal seawater, but by 30 min sperm chromatin decondensation had occurred. (▲) Partial activation, incomplete surface coat elevation, and germinal vesicle breakdown, at 30 min. In each case, development of surrounding eggs in the dish was similar to that of the penetrated egg.
sensitive Ca++ channels: in 50 mM Na+, the potential shift caused by the Na+
permeability increase is insufficient to open Ca++ channels; in 490 mM Na+, however, the Ca++ channels are opened, and amplify the Na+ response. The
amplitude of the trypsin-induced activation potential is also Na+-dependent;
maximum amplitudes excluding the first 15 s were $-28 \pm 4$ mV (SD, $n = 2$) in
50 mM Na+, compared to $+40 \pm 3$ mV (SD, $n = 3$) in 490 mM Na+ (1.0 mg/ml
trypsin).

Fig. 5 b shows examples of fertilization potentials in various Na+ concentra-
tions. In 5 and 50 mM Na+, the fertilization potential sometimes has an initial
brief action potential component (action potential No. 1), usually seen only if
the resting potential of the unfertilized egg is more negative than the threshold
potential; after 1 s, however, the potential falls back to a negative level. Two
examples are shown for 50 mM Na+. The first is representative of our usual
procedure in which eggs were inseminated with a final sperm dilution of $\sim 1:
30,000$ to $1:3,000$. The single insemination brought the potential to its maximum
level. In the second example, representative of five cases, eggs were first
inseminated with a sperm dilution of $\sim 1:300,000$ at the first arrow; then with a
more concentrated sperm suspension ($\sim 1:3,000$) at the second arrow. The first
insemination produced some shift in potential, but the maximum was not
reached until after the second insemination. These different responses can be
correlated with the number of sperm which enter the eggs. In low Na+ solutions,
Urechis eggs are highly susceptible to polyspermy, and the number of sperm
which enter is dependent on the concentration of sperm to which the egg is
exposed (Gould-Somero et al., 1979). As the number of sperm entries increases,
the fertilization potential amplitude increases; in 50 mM Na+, the voltage
response saturates beyond $\sim 10$–20 sperm per egg (Fig. 5 c). All points in Fig. 5
a were determined from inseminations such that the maximum voltage response
was obtained for a given Na+ concentration. (Note that, in the above results, in
normal 490 mM Na+ seawater, the sperm concentration or number of sperm
entries does not significantly affect any of the characteristics plotted in Fig. 5 a.)
In low Na+ seawater, morphological activation (see Table I) does not occur
unless a certain minimum number of sperm, $\sim 40$ in 50 mM Na+, enter the egg
(Fig. 5 c).

The durations of fertilization potentials in both low Ca++ and low Na+
seawater were greater than in normal seawater: $34 \pm 9$ min (SD, $n = 2$) in 1 mM
Ca++ and $18 \pm 7$ min (SD, $n = 13$) in 50 mM Na+, compared to $9 \pm 1$ min (SD,
$n = 10$) in normal seawater (see Figs. 4 b and 5 b). One possible reason for the
long duration fertilization potentials in these solutions is that in both solutions
there is polyspermy. Three experiments in 1 mM Ca++, in which sperm were
killed at various times after insemination, showed that additional sperm enter at
least as late as 6 min after the initial insemination. Therefore, successive sperm
entries may contribute to the long duration of the 1 mM Ca++ fertilization
potential. As noted above, eggs in 50 mM Na+ become even more polyspermic
than those in 1 mM Ca++. However, two experiments in which sperm were
killed at various times after insemination showed that $\geq 95\%$ of sperm entries in
50 mM Na+ occur within the first 1-min period after insemination, probably
because most sperm are immotile after 1 min in 50 mM Na+. In 120 mM Na+,
80% of sperm entered within 1 min after insemination. Therefore, in low Na⁺, the long fertilization potential duration cannot be accounted for by continued sperm entries. However, we cannot exclude the possibility that the long duration is a consequence of multiple sperm entries occurring in the first 1-min period after insemination.

**Sodium and Calcium Influx at Fertilization**

By measuring radioactive tracer uptake, Johnston and Paul (1977) showed that Ca²⁺ influx in *Urechis* eggs increases at fertilization. Inasmuch as our electrophysiological experiments indicated that Na⁺ permeability increases at fertilization, we looked for a Na⁺ influx change using a Na⁺ tracer. This section describes a large increase in Na⁺ influx, and also further characterizes the Ca²⁺ influx described by Johnston and Paul.

First, we measured ion influxes in the unfertilized egg, summarized in Table V. Na⁺ and K⁺ influxes are similar: about 0.02 and 0.03 pmol/egg·min, respectively, while Ca²⁺ influx is much smaller, about 0.0006 pmol/egg·min. These values were determined by taking three samples at successive 10-min times after isotope addition, and measuring the slope of uptake vs. time.

**TABLE V**

| Ion   | Influx pmol/egg·min ± SD (n) |
|-------|------------------------------|
| Na⁺   | 0.019 ± 0.005 (7)            |
| K⁺    | 0.028 ± 0.008 (5)            |
| Ca²⁺  | 0.00058 ± 0.00018 (2)        |

During the 10-min period after insemination, Na⁺ uptake is 17 ± 7 (n = 6) times that during a 10-min period in the unfertilized egg (Fig. 6 a; unfertilized egg data used to calculate ratio are from Table V). Fig. 6 b shows 2-min uptake pulses in the 0–10-min period; most of the uptake occurs during the first 6 min after insemination, indicating that Na⁺ channels close as the fertilization potential terminates. Fig. 6 a also shows that Na⁺ uptake during the 10–20- and 20–30-min intervals after insemination is much smaller than during the first 10 min. The Na⁺ uptake from 10 to 30 min is somewhat larger than that in the unfertilized egg suggesting that a slightly elevated level of Na⁺ influx may continue after the termination of the fertilization potential; however, the significance of these results should be confirmed with additional experiments. Also Na⁺ uptake during activation by 0.5 mg/ml trypsin is comparable to that during fertilization (Fig. 6 c).

The Ca²⁺ uptake determined by Johnston and Paul (1977) in the 10-min period after insemination is 22 ± 10 (n = 8) times that of the unfertilized egg.

³ Flux data are presented in terms of pmol per egg·min rather than per cm²·s because the true surface area of the microvillus-covered egg is not known. From an analysis of electron micrographs, Paul (1975 b) estimated the surface area of the unfertilized egg to be ~2–4 times larger than the value (4.54 × 10⁻⁴ cm²) calculated by assuming the egg is a sphere of 60 μm radius. Whether there are significant changes in egg surface area after fertilization has not been investigated.
Similarly, we measured the 0-10-min uptake to be $34 \pm 14$ (SD, $n = 4$) times the 10-min uptake in the unfertilized egg (Fig. 6d; unfertilized egg data used to calculate this ratio are from Table V). The absolute magnitude of the Ca$^{++}$ uptake in the 10-min period after insemination is $16 \pm 6$ times smaller than the absolute magnitude of the Na$^{+}$ uptake. (Note that the Ca$^{++}$ uptake scale in Fig. 6d is expanded 10 times compared to the Na$^{+}$ uptake scale in Fig. 6a.) Relative to the 0-10-min period, Ca$^{++}$ uptake is reduced during the 10-20- and 20-30-

![Figure 6](image)

**Figure 6.** Na$^{+}$ and Ca$^{++}$ uptake at fertilization. (a) $^{24}$Na uptake ± SD during 10-min pulses. The bars indicate the average rate of Na$^{+}$ influx for 10 min in the unfertilized egg (U) and for three 10-min periods after insemination (arrow). Averaged data from two experiments. (b) $^{24}$Na uptake during 2-min pulses during the first 10 min after insemination. Dotted line indicates uptake during a 10-min pulse measured simultaneously with the 2-min pulses. Averaged data from two experiments. (c) $^{24}$Na uptake during 20-min pulses after introduction of 0.5 mg/ml trypsin (- - -) or after insemination (---). Averaged data from two experiments. (d) $^{45}$Ca uptake during 10-min pulses. Averaged data from four experiments. (e) $^{45}$Ca uptake during 2-min pulses during the first 10 min after insemination. Dotted line indicates uptake during a 10-min pulse measured simultaneously with the 2-min pulses. Averaged data from two experiments. Sperm dilutions for all experiments were 1:15,000 or 1:30,000.

In interpreting tracer uptake data, it is important to be certain that uptake measurements represent transmembrane influx, and not extracellular binding. To minimize this problem, we used 10-15-min wash periods (see Methods). The following arguments indicate that the amount of extracellular tracer remaining after this procedure is small compared to the transmembrane fluxes of Na$^{+}$ and Ca$^{++}$.

Most of the Ca$^{++}$ uptake occurs in the first 6 min after insemination (Fig. 6e). These results are consistent with those of Johnston and Paul (1977); their paper also shows that Ca$^{++}$ uptake during trypsin activation is the same as that during fertilization.

In interpreting tracer uptake data, it is important to be certain that uptake measurements represent transmembrane influx, and not extracellular binding.
Ca++ during the 0-10-min period after insemination: (a) we can estimate the amount of extracellular tracer associated with the unfertilized egg by subtracting the 10-min uptake determined from the slope of the uptake vs. time curve (Table V) from the uptake in a 10-min pulse (Fig. 6d). For Ca++, these figures are 0.006 ± 0.002 pmol/egg (SD, n = 2) and 0.013 ± 0.004 pmol/egg (SD, n = 4), respectively, indicating that up to 0.01 pmol/egg may remain bound extracellularly after washing. This amount is small compared to the 0.20 pmol/egg uptake during the first 10 min after insemination. A similar argument can be made for Na+. (b) A post-fertilization increase in extracellular isotope binding due to surface coat elevation can be ruled out. Uptake of both Na+ and Ca++ increases before surface coat elevation, which begins at 4 min after insemination (Table I). (c) Finally, we compared the summed uptake from five 2-min uptake pulses with the uptake during a single 10-min pulse in the 0-10-min period after insemination. For both Na+ and Ca++, the summed 2-min uptakes exceeded the 10-min uptake by < 10% (Fig. 6b, e). Therefore, extracellular binding is negligible during the 0-10-min period, since an extracellular fraction would be sampled five times by the five pulses, but only once by the single 10-min pulse (assuming extracellular binding is essentially complete after 2 min). This experiment also eliminates a possible “isotope saturation” artifact, i.e., an increase in intracellular specific activity to the point that significant isotope is lost by efflux. If this occurred, the sum of the 2-min uptakes would exceed the 10-min uptake. In summary, these controls show that the increased uptake of Na+ and Ca++ in the 10-min period after insemination is due to transmembrane influx. For the 10-30-min period, we have not done corresponding controls, so we cannot unambiguously interpret the small increases in uptake relative to the unfertilized egg.

**Sodium Uptake As a Function of the Number of Sperm Entering per Egg and As a Function of External Sodium Concentration**

Results from previous sections have shown that sperm cause an increase in Na+ permeability of the egg membrane. That the increased permeability results from the opening of channels already in the egg membrane, rather than the addition of new channels from the sperm membrane, was indicated by the finding that trypsin could substitute for sperm in opening the channels. These results led us to ask whether normal monospermic fertilization opened all of the Na+ channels available in the egg membrane. We investigated this by determining whether, if many sperm fertilized a single egg, additional Na+ channels would be opened. Fig. 7a shows two such experiments. Eggs in normal seawater were inseminated with either a moderate sperm concentration, in which case almost all eggs were monospermic, or with a high sperm concentration, in which case many eggs admitted two or more sperm. Na+ uptake was measured under each of these conditions, and plotted as a function of the average number of sperm entering per egg. A linear relationship was obtained, indicating that each sperm opens additional Na+ channels.

In normal seawater, even with the highest sperm concentrations, not more than a few sperm can enter each egg. However, by lowering the external Na+ concentration in seawater, it is possible to obtain up to 150 sperm per egg
(Gould-Somero et al., 1979). This procedure allowed us to ask if Na\(^{+}\) influx would continue to increase as additional sperm entered each egg. Two of three such experiments are shown in Fig. 7b. In all cases, a linear relation between sperm/egg and Na\(^{+}\) uptake was observed; however, beyond a certain point (>20–80 sperm/egg in different egg batches) Na\(^{+}\) uptake per additional sperm was slightly less. Fig. 7b shows a photograph of a highly polyspermic egg such as those studied in these experiments.

**Calcium Uptake As a Function of the Number of Sperm Entering per Egg and As a Function of External Sodium Concentration**

The purpose of these experiments was to determine the pathway by which Ca\(^{++}\) enters the egg following insemination. We wanted to distinguish between Ca\(^{++}\) entry through the action potential channels and Ca\(^{++}\) entry through channels gated by sperm. Therefore, we asked, if many sperm fertilized a single egg, would additional Ca\(^{++}\) channels be opened, as is the case for the Na\(^{+}\) channels? Fig. 8a shows four such experiments. Eggs in normal seawater were inseminated with either a moderate sperm concentration, in which case almost all eggs were monospermic, or with a high sperm concentration, in which case many eggs admitted two or more sperm. A plot of Ca\(^{++}\) uptake as a function of the average number of sperm entering the egg under each of these conditions shows that entry of the second sperm causes no significant increment in Ca\(^{++}\) uptake. The same results were obtained when uptake was measured during either a 5-min or 1-min interval. Since 1-min uptake is less than 5-min uptake, the failure of the
second sperm to increase uptake cannot be explained as an isotope saturation effect. We conclude from the data in Fig. 8a that not more than 10% of the Ca²⁺ uptake occurs by way of a sperm-gated channel. On the other hand, Ca²⁺ entry by way of the action potential channel would be expected to show the observed characteristics. The first sperm would bring the egg membrane potential to the action potential threshold, causing an influx of Ca²⁺; the second sperm causes no significant increase in average fertilization potential amplitude (see above) and therefore would cause no large increment of Ca²⁺ influx.

Measurements of Ca²⁺ uptake in 50 mM Na⁺ also indicated that Ca²⁺ entry is probably by way of the action potential channel. In this solution, the fertilization potential maximum amplitude, −11 ± 5 mV (SD, n = 10), is sufficient to activate the first action potential, with a threshold of −30 mV, but not the second, with a threshold of +10 mV. Since the duration of the second action potential is much longer than that of the first, it is the second action potential which we would expect to cause significant Ca²⁺ uptake during the fertilization potential. Fig. 8b compares Ca²⁺ uptake in 50 and 490 mM Na⁺. For each Na⁺ concentration, Ca²⁺ uptake was measured for a 5-min period in the unfertilized egg as well as for a 5-min period in the fertilized egg exposed to varying sperm concentrations. Ca²⁺ uptake per initial sperm entry for the 5 min after

![Figure 8](image-url)

**Figure 8.** Ca²⁺ uptake as a function of the number of sperm entering per egg and as a function of external Na⁺ concentration. (a) ⁴⁵Ca uptake in unfertilized eggs and during the first 5 min after insemination (○, ■) or during the first 1-min period after insemination ([△], □) in artificial seawater as a function of average number of sperm entering per egg. Sperm dilutions for fertilization were 1:2,500 or 1:25,000. 5-min and 1-min data were obtained on different days, so their relative magnitudes should not be compared quantitatively. (b) ⁴⁵Ca uptake in unfertilized eggs and during the first 5 min after insemination in (○) 490 and (□) 50 mM Na⁺ is plotted as a function of the average number of sperm entering per egg. Sperm dilutions for fertilization ranged from 1:2,500 to 1:100,000. 490 mM Na⁺ data are replotted from (a).
insemination in 490 mM Na⁺ is 105 ± 19 (SD, n = 3) times that in 50 mM Na⁺. The simplest explanation of these results is that in 490 mM Na⁺, most of the Ca²⁺ enters by way of action potential No. 2. However, some Ca²⁺ uptake does occur in 50 mM Na⁺, and this is proportional to the number of sperm which enter. This residual Ca²⁺ uptake may be by way of the sperm-gated Na⁺ channel.

DISCUSSION

At fertilization, the egg of the marine worm, Urechis caupo, produces a fertilization potential much like that previously reported in echinoderms and amphibians (see references in Introduction). The membrane potential shifts in a step from the unfertilized resting potential of ~ -30 mV to a peak potential near +50 mV with a rise time of ~ 1 s. The most rapid responses occur within 2 s after insemination; longer delays probably result from the time required for the sperm to reach the egg. After reaching the maximum, the potential falls to a plateau phase at ~ +30 mV. Finally, the potential becomes negative again, reaching the level of the unfertilized egg resting potential at ~ 10 min, and then continuing to ~ -60 mV at 20 min.

The potential change at fertilization results from the opening of channels in the egg membrane and not from the addition of channels to the egg by the sperm, since in the absence of sperm, artificial activating agents, and (or) electrode penetration can elicit activation potentials in Urechis eggs as well as those of amphibians (Maeno, 1959; Ito, 1972) and echinoderms (Higashi, 1971; Steinhardt et al., 1971; Chambers et al., 1974; Steinhardt and Epel, 1974). How sperm cause the channels to open is not clear; possibly they open as a result of sperm binding to a receptor in the plasma membrane, or perhaps as a consequence of fusion of the egg and sperm membranes. The precise time of sperm-egg fusion in Urechis is not known; according to Tyler (1965) it has occurred by 30-60 s after insemination (temperature not specified). In the annelid, Hydrodoides, fusion is reported to have occurred by 9 s (Colwin and Colwin, 1967).

Sodium Dependence of the Fertilization Potential

Our results show that during the fertilization potential, the Urechis egg plasma membrane is primarily permeable to Na⁺. The evidence is: (a) fertilization potential amplitude varies 50 mV per 10 times change in external [Na⁺] (Fig. 5); (b) the amplitude is reduced only slightly in 1/10 Ca²⁺ seawater, and only during the first 15 s (Fig. 4); (c) Na⁺ influx during the fertilization potential (0-10 min after insemination) is much larger (16 times) than the Ca²⁺ influx (Fig. 6); and (d) K⁺ permeability, if significant, is smaller than Na⁺ permeability according to the following argument. If K⁺ and Na⁺ were equally permeant, the 0-mV intercept of a plot of fertilization potential amplitude vs. log external [Na⁺] should occur at an external [Na⁺] close to 240 mM since the estimated internal [K⁺] is 240 mM (Table III). However, the observed 0-mV intercept occurs at ~ 90 mM external Na⁺ (Fig. 5a, plot of fertilization potential maxima excluding the first 15 s), indicating that K⁺ is less permeant than Na⁺.

The sea urchin fertilization potential has also been reported to be Na⁺-
dependent (Steinhardt et al., 1971; Chambers and de Armendi, 1978). However, it would be desirable to extend this investigation to (a) exclude the possible difficulty of variation of fertilization potential amplitude among individual eggs (see Jaffe, 1976; Jaffe and Robinson, 1978), and (b) test fertilization potential amplitude in low Ca ++ as well as low Na + solutions. (Fertilization potentials in low Ca ++ were described briefly by Chambers and de Armendi, 1978, but a more detailed analysis would be useful, particularly because Okamoto et al. [1977] have reported a Ca ++-dependent action potential in sea urchin eggs.)

Consistent with the evidence for a Na +-dependent fertilization potential, increases in Na + influx and efflux after fertilization of sea urchin eggs were reported (Chambers, 1968, 1972; Johnson et al., 1976). Increases in Ca ++ influx and efflux were also observed (Azarnia and Chambers, 1969; Chambers et al., 1970; Nakazawa et al., 1970; Steinhardt and Epel, 1974; Paul and Johnston, 1978). The magnitude of the Na + influx is greater than that of the Ca ++ influx, but data are not available to make a quantitative comparison.

Our result that Na + influx is proportional to the number of sperm entering *Urechis* eggs indicates that during normal monospermic fertilization, only a fraction of the available Na + channels open. Electrophysiology gives additional evidence for a correspondence between sperm entries and channel opening: (a) the fertilization potential amplitude in 50 mM Na + is more positive-going if more sperm enter the egg (Fig. 5); (b) in dispermic fertilization potentials in normal seawater, there is sometimes a second step in the initial voltage rise. This latter property has also been observed in sea urchin eggs (Jaffe, 1976).

**Action Potential Contribution to the Fertilization Potential**

Two voltage-dependent “action potential” channels are present in the membrane of the unfertilized *Urechis* egg. When the sperm opens the egg's Na + channels and shifts the egg's membrane potential, the action potential channels are opened as well. At least one of these channels (action potential No. 2) is a Ca ++ channel; our results indicate that this action potential channel provides the major pathway by which Ca ++ enters the egg at fertilization: (a) the kinetics are appropriate in that the amplitude of the Ca ++ action potential is greatest during the first 10 s after a current pulse is applied; after 10 s, a steady-state voltage is reached (Fig. 1 c). Correspondingly, the fertilization potential is Ca ++-dependent only during its first 15 s (Fig. 4 a). This is consistent with Johnston and Paul's (1977) report that Ca ++ influx is greatest during this period; from their data, we calculate the Ca ++ influx during the first 10 s to be ~ 4 times the average influx during the fertilization potential. (b) Ca ++ influx is independent of the number of sperm incorporations in normal seawater; in other words, Ca ++ influx is not by way of a sperm-gated channel (Fig. 8 a). (c) Ca ++ influx in normal seawater is 100 times the influx per sperm in 50 mM Na + seawater, in which solution the fertilization potential amplitude is subthreshold for the action potential (Fig. 8 b).

In summary, our results indicate that Na + entry into *Urechis* eggs after insemination is by way of a sperm-gated channel, whereas Ca ++ entry is primarily by way of a voltage-gated channel. This two-channel system is analogous to the receptor potential plus action potential system found in a
variety of chemo- and mechanosensitive cells. The fertilization potential can be thought of as a sum of a Na⁺-dependent "sperm receptor potential" and a superimposed Ca²⁺-dependent action potential.

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