DEVELOPMENTAL BIOLOGY 27, 555–574 (1972)

Membrane Potential and Impedance of Developing Fucoid Eggs

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Accepted December 16, 1971

Within 7 hr after fertilization, the fucoid egg's membrane potential rises from about −20 mV to −80 mV (Fig. 4) and within 12 hr after fertilization its conductance rises 8-fold (Fig. 7). Its changing responses to rapid individual ion concentration changes (Table 1) imply permeability changes which help explain these electrical ones: Thus the resting potential and conductance of the unfertilized egg seem to be mainly explained by pathways jointly permeable to K^+, Mg^{2+}, and to a lesser degree Na^+; those of the developing egg by potassium channels in parallel with less conductive chloride channels. In the unfertilized state the egg's membrane is passive; by a few hours after fertilization it responds sharply and within 1 msec to imposed currents sufficient to shift its potential by more than 20 mV; responding to outward current by reducing the resultant voltage change and to inward current by increasing it (Figs. 6 and 7). It is speculated that these responses may respectively serve to start and stop natural transcellular current surges. Finally, the scattered literature on comparable electrical changes in developing eggs, from algae to amphibians, is culled and organized (Table 7); the main features shown by the fucoid egg are remarkably general.

INTRODUCTION

We have come to believe that development is a generally centripetal process; cell surfaces change first and cell nuclei last. In the fucoid egg, this view is particularly supported by inferences from polarotropism, from polarized plasmolysis, from transcellular currents, and from gross changes in ionic composition (Jaffe, 1970; Allen *et al.*, 1972). We have now explored the electrical changes across this egg's periphery. This approach has important advantages: It records from the living, developing cell; it can show very fast changes; and it indicates the radial fields which should be a large factor in organizing the cell cortex.

As a first step we have used one or two conventional KCl filled micropipettes and a simple current clamp. It proved easy to record rather stable membrane potentials for up to about a half hour after impalement, and such eggs subsequently developed normally despite some visible injury. However, since we were interested in developmental changes that extended over more than a day, most of the conclusions had to be drawn from measurements of many hundreds of separate eggs.

Despite this, much was learned. Indeed, these eggs really surprised us.

A concurrent study on another fucoid egg has been reported by Bentrup (1970). It is restricted to one electrode measurements with quite limited media changes; but within these bounds our results agree.

MATERIALS AND METHODS

Most of our experiments were done with *Pelvetia fastigiata*, a monoecious fucoid alga from the California coast; some were performed with the dioecious *Fucus vesiculosus* from the New England coast.[2] Zero time for development was taken to be a half hour after shedding began. In the experiments with fertilized eggs, 90–100% of these cells germinated. In experiments with unfertilized eggs, 40–80% germinated after later insemination. The

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[2] *Pelvetia* was collected by Mrs. Arlene Phillips of the Hopkins Marine Station. *F. vesiculosus* was provided by Dr. John Torrey at Harvard University.

eggs were grown and studied in a room held at 15 ±1°C. Except during measurements they were grown under continuous white fluorescent light.

Unfertilized *Pelvetia* eggs were obtained in two different ways: (1) killing the sperm by adding 0.9–1.0% chloral hydrate to the sea water for 1 hr (Kniep, 1907) or (2) by shedding the gametangia into sea water acidified to pH 5 and mechanically separating the still closed antheridia (diameter 20–30 μ) from the oogonia within 1 hr. Because the gametangia are so sticky this separation proved difficult; it was done by repeatedly forcing large quantities of fresh pH 5 sea water past oogonia held on a nylon screen. The *Pelvetia* eggs have an average diameter of 92 μ and an average surface area of 2.7×10^{-4} cm^2; this area increases to 3.0×10^{-4} cm^2 in recently germinated zygotes about 12 hr after fertilization and to 3.3×10^{-4} cm^2 in day-old embryos. The diameter of a *Fucus* egg is 50–60 μ. These cells have no vacuole or other large compartment inside. Before fertilization, they are naked and easily deformed; soon afterward they secrete a tough, rigid, and sticky wall.

Measurement Techniques

The eggs were grown and impaled while lodged in the holes of woven nylon screens[3], which were stretched (like drumheads) over plastic rings. For the intracellular recordings and stimulations, we used glass micropipettes with sturdy tips made out of thick-walled capillaries (i.d.: o.d. = 1.3) on a vertical puller. The outside tip diameter was about 1 μ, and it increased to 3 μ within 5–10 μ behind the tip. The pipettes were filled with filtered 3 *M* or 1 *M* KCl solution before every experiment. They had a tip resistance of 10–20 megohms and 20–60 megohms, respectively. The tip potential ranged from zero to a few millivolts. The electrodes were Ag-AgCl wires. An Ag-AgCl wire in 3 or 1 *M* KCl agar (2%) with a contact area of about 3 mm^2 served as reference electrode. Each voltage recording pipette was pushed into an egg directly from above while viewed obliquely with a Leitz stereoscope. Unfertilized *Fucus* eggs were punctured with the help of a specially designed spring loaded "microelectrode-gun" whose forward movement could be precisely regulated up to 75 μ (Fig. 1). Fertilized eggs (as well as unfertilized *Pelvetia* eggs) were impaled via manually moved micropositioners.

The recording equipment consisted of a preamplifier with input capacitance neutralization (Bioelectric Instr.), a calibrator (Bioelectric Instr.), a dual-trace oscilloscope with camera (Tektronix), and a chart recorder. A Grass stimulator in series with a 10^9 ohm resistor served as a current source. For the connections, see Fig. 2.

Current was injected into a cell through a second micropipette pushed into the cell 1–3 min after the first and oriented at about 50–60 degrees to the voltage-measuring pipette (Eisenberg and Engel, 1970). The E_m depolarized by 10–40 mV in fertilized, and by about 10 mV in unfertilized, eggs upon penetration of the current-pipette. Unfertilized and recently fertilized eggs did not recover completely in most cases. Nearly all older zygotes recovered fully in a few seconds. Direct current pulses of different strength of 5–20 msec duration and a repetition rate of 10–20 per second were applied for 0.5 to 5 sec starting 0.5 to several minutes after penetration. The sequence of the current directions, inward-outward... or inward, inward... outward, outward... has no influence on the response to a few short pulses. The variability of the resting transmembrane potential during a 5-min puncture was ± a few milivolts in unferti-

[3] "Nitex" monofilament screen cloth obtained from Tobler, Ernst & Traber, 71 Murray Street, New York 10007.

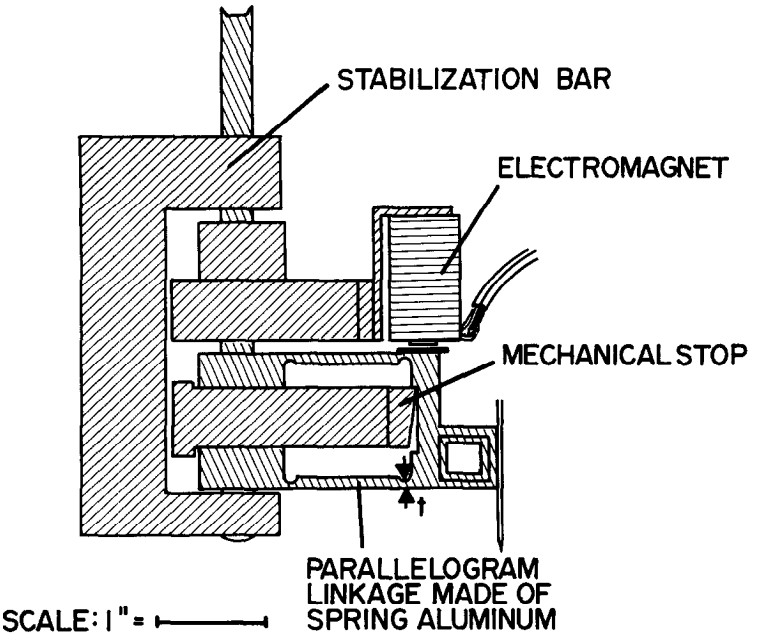

FIG. 1. Sketch of "microelectrode gun." Linkage and stabilization bar are $^{3}/_{8}$ inch thick. Stiffness of linkage depends largely on the critical thickness, t which is about 0.016 inch.

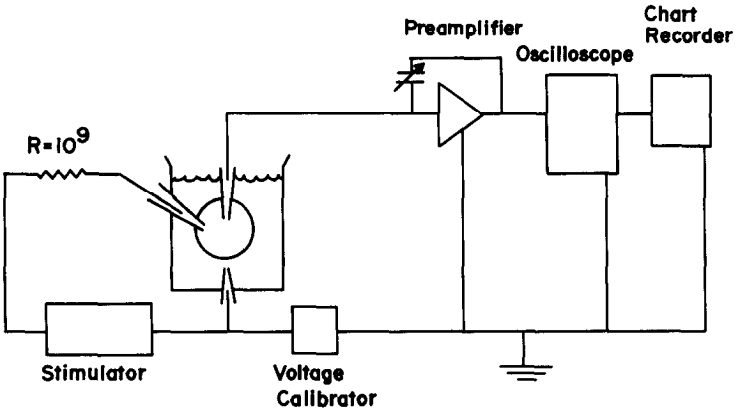

FIG. 2. Schematic representation of the measuring circuit.

lized and recently fertilized eggs; it fell to ± 1 mV during development. The final potential was reached in a few seconds after penetration in most cases; in some cells soon after fertilization it took 12 sec to 1 min to stabilize. The puncturing itself had no lasting adverse effect on the cells; zygotes impaled for 5–50 min developed completely normally. Whether they developed during puncturing is a very important question which cannot be answered yet. Zygotes could occasionally be penetrated for up to 1 hr before the resting potential showed any artifacts.

Media and Media Changes

The base medium for the development of *Pelvetia* and *Fucus* was artificial sea water (ASW) with the following composition (m*M*) (see Harvey, 1966): Na^+ 483, K^+ 10, Ca^{2+} 10, Mg^{2+} 55, Cl^- 564.5, SO_4^{2-} 28, HCO_3^- 2.5, Tris·Hcl 10; pH 8.0. In

media with high K^+ content, K^+ was increased at the expense of Na^+ or simply added as KCl or K_2SO_4; in media of low K^+ it was replaced by mannitol. The Na^+ content of ASW was changed by replacing it with choline or Tris. The pH of ASW was adjusted to different values with Tris·HCl or for pH 5 and 6 with Na_2HPO_4 + HCl. Ca^{2+} was decreased by replacing it with choline or mannitol and increased by adding $CaSO_4$. Mg^{2+} was decreased by replacing it with choline or Tris and increased by adding $MgCl_2$ and $MgSO_4$. The medium's Cl^- concentration was lowered by replacing it with propionate or SO_4^{2-}. SO_4^{2-} was replaced by Cl^- or propionate. Unfertilized eggs show no immediate change in E_m with an increase in the medium's osmolarity of up to 50%. Fertilized eggs were more sensitive; they showed no effect upon an increase of 10% but hyperpolarized 1–4 mV upon a 20% increase.

In the experiments in which the influence of the different ions in ASW on the E_m was tested the medium was always changed while a cell was impaled. Normally the procedure was started with a change from ASW to flowing ASW for 2 min to eliminate artifacts due to the flow. Then the test medium was applied with a constant flow rate of 2 ml/sec across the impaled cell. The medium change in the dish where the cells were resting in the holes of a stretched nylon screen was complete in 10 sec but the medium was kept flowing for at least 2–3 min. If the preparation allowed, the reference medium was brought back to check for reversibility. Temperature changes were produced by flushing cooled ASW through the dish and monitoring the temperature in the dish with a thermistor.

In cases where the E_m responded "instantaneously" to changes in the medium's composition, 90% of the full response was obtained in 10–30 sec after the change started; 10% of ΔE_m occurred during the next 2–5 min. We tried several methods to separate the rhizoid and thallus cell of a two-cell embryo in order to measure their membrane properties separately. These included short and long plasmolysis of various degrees, centrifugation, centrifugation combined with plasmolysis, application of ASW where Na was replaced by Li (Rose and Loewenstein, 1969), and mechanical destruction of the rhizoid.

RESULTS

Changes in Membrane Potential during Normal Development (Figs. 3 and 4)

The mean transmembrane potential (E_m) of an unfertilized *Pelvetia* egg is −16 ± 4.4 mV (mean ± standard deviation, σ, n = 254) that of an unfertilized *Fucus* egg −22 ± 6.6 mV (mean ± σ, n = 122). If the egg is not fertilized then E_m remains at this value for at least 1 day. Fertilization is possible for several hours after shedding. The membrane hyperpolarizes rapidly, showing an average increase in potential of 40–50 mV in an hour. In three cases, the change could be observed directly (Fig. 3). E_m hyperpolarized on the average 1.1 mV/min in the first 30 min. In a few instances a rise in potential like that which follows fertilization was observed by penetrating an unfertilized egg with a blunt pipette. After the first steep rise in potential, E_m increases more slowly over the next 6 hr and reaches a peak value of about 78 mV 7.5 hr after fertilization.

Then sometime *before* germination a remarkable depolarization of 8 ± 3.7 mV (σ, n = 6) occurs. This decline in potential was seen in many experiments where different individual zygotes were punctured for 5 min each. Only in one, not completely convincing, case could we observe such a depolarization during impalement of a single egg. It progressed slowly over 7 mV in 16 min. Then the membrane repolarized more or less. A similar development of E_m, including a transient depolarization at 9 hr, has been

reported to occur in *Fucus serratus* eggs by Bentrup (1970). Furthermore, *Pelvetia* germinates normally in a medium when 80% of the ASW is replaced by an approximately isoosmotic mannitol solution 1 hr after fertilization. In one experiment E_m's were measured in this medium. They increased from 2.5 hr to 9 hr and reached values of −90 to −95 mV; then the cells' membranes depolarized and germinated eggs showed E_m's of −80 to −85 mV.

During germination and first cleavage, E_m declines very slowly. The products of first cleavage, the thallus cell and the rhizoid cell, are isopotential at about −72 mV. Figure 4 shows two representative consecutive experiments with *Pelvetia*. We selected this kind of representation

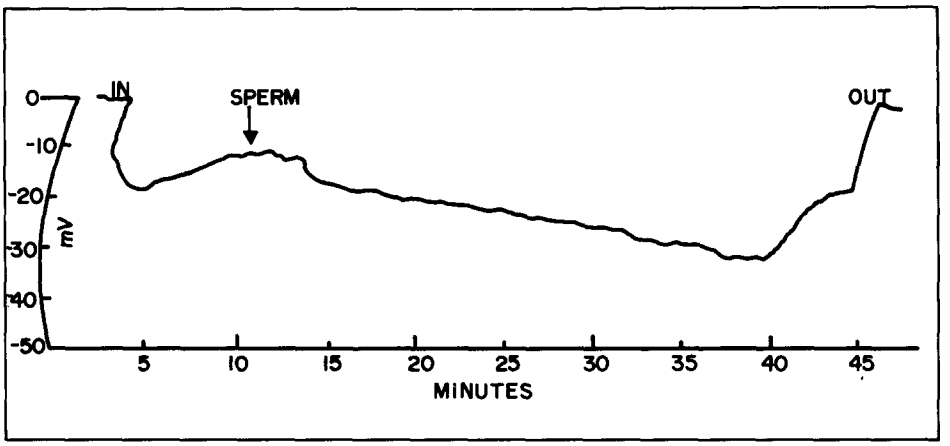

FIG. 3. Record of an experiment in which sperm were added (↓) to an unfertilized *Fucus vesiculosus* egg during a potential measurement. At IN and OUT, the probe penetrated and withdrew from the egg.

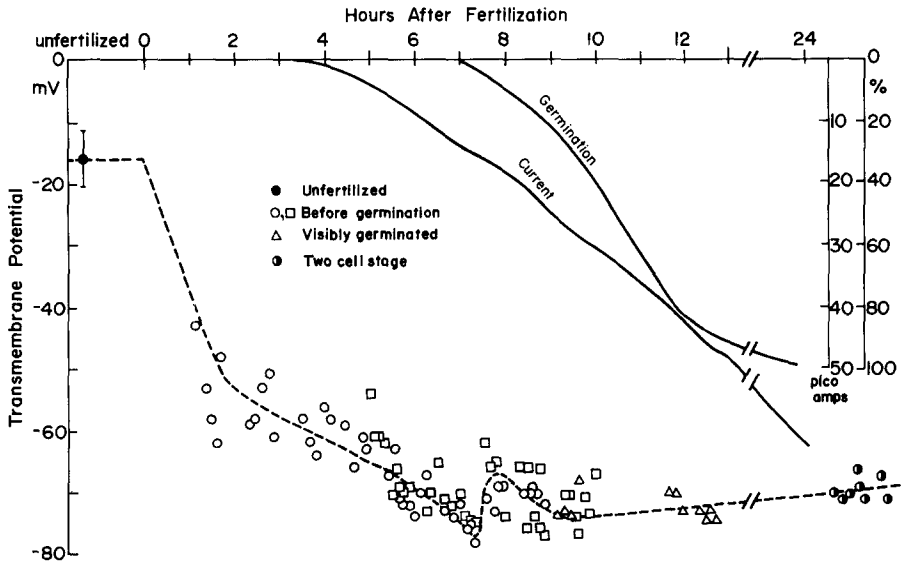

FIG. 4. Membrane potentials of developing *Pelvetia* eggs. Every measurement was made on a different egg during a 3–5 min puncture. The measurements on fertilized but ungerminated eggs are from two successive experiments (○, □). ● = Mean membrane potential ($\pm\sigma$) of unfertilized eggs. This does not change for at least 24 hr after shedding. The dashed line is drawn by eye through the experimental points. Germination and transcellular current curves are from Jaffe (1968).

over lumping measurements from different egg batches together because, as one might expect, different batches developed with somewhat varying velocities. We saw no significant difference in E_m between small and large cells. An electrode penetrating into the cell wall only became a few millivolts negative.

Changing the illumination from light to dark and vice versa has no immediate effect on the resting potential of unfertilized and fertilized eggs.

Effect of Temperature Changes on the Membrane Potential

We were interested to see whether the large changes in E_m during development involve electrogenic pumps. One way to investigate this question is to slow the pump through a substantial drop in the medium's temperature.

When the temperature was rapidly lowered from 15° to 3°–5°C for brief periods (2–5 min) during a potential recording the cells' response depended upon on their developmental stage (Fig. 5). Unfertilized eggs and zygotes more than 4 hr old show only the expected small depolarization (*RT/F* term in Nernst-equation). Zygotes 1–3 hr old, however, respond with a quite large and immediate *hyper*polarization of up to 7 mV until they are 3–4 hr old.

This temperature effect suggested that an electrogenic component was contributed by an unusual Na-K pump, one which drove more K^+ ions in than Na^+ ions out. Ouabain is the most specific known inhibitor of Na-K pumps; but even one millimolar ouabain failed to effect development, so its effect upon potentials was not explored.

Permeability Changes during Development

A large number of experiments were done to explore the changes in the membrane's relative ion permeabilities during development. In most of these, the concentration of only one ion was quickly changed and the immediate potential change recorded (Table 1).

The unfertilized egg's membrane shows little ability to discriminate between different ions. Those of both *Pelvetia* and of *Fucus* show small but rapid, reproducible, and comparable responses to changes in no less than five different ions, namely K^+, Na^+, Cl^-, Mg^{2+}, and H^+. Moreover, these responses are all in the direction expected for a diffusional potential. The small depolarization produced by low Ca develops only gradually over minutes and can be reversed in about the same time; moreover, this change is in the opposite direction to that expected of a direct diffusional effect. So we believe it unlikely that Ca^{2+} contributes directly to the unfertilized egg's potential. Finally, SO_4^{2-} changes yield no measurable response, and thus serve as a control indicating, the general absence of flow artifacts larger than about 1 mV.

A new pattern of permeabilities develops after fertilization. The main trend is toward a relatively higher permeability to K^+ (Fig. 5). By as early as 1 hr after fertilization, the membrane shows nearly two-thirds of the theoretical maximum response to a 10-fold increase in $(K^+)_o$. This selectivity for K^+ gradually increases, reaching a peak at 7–8 hr, and then slowly declines; the decline is clearest in the response to reducing K 100-fold. (This last response is raised by 10–30 mV at every stage if $[Ca^{2+}]_o$ is raised about 6-fold.)

The only other ion which demonstrably contributes to a diffusion potential is chloride. Indeed, the recently fertilized eggs show a greater response to chloride changes than do the unfertilized eggs. This sensitivity declines markedly, reaching a minimum at 7–8 hr and then slowly rises.

The responses to Ca^{2+} and Mg^{2+}

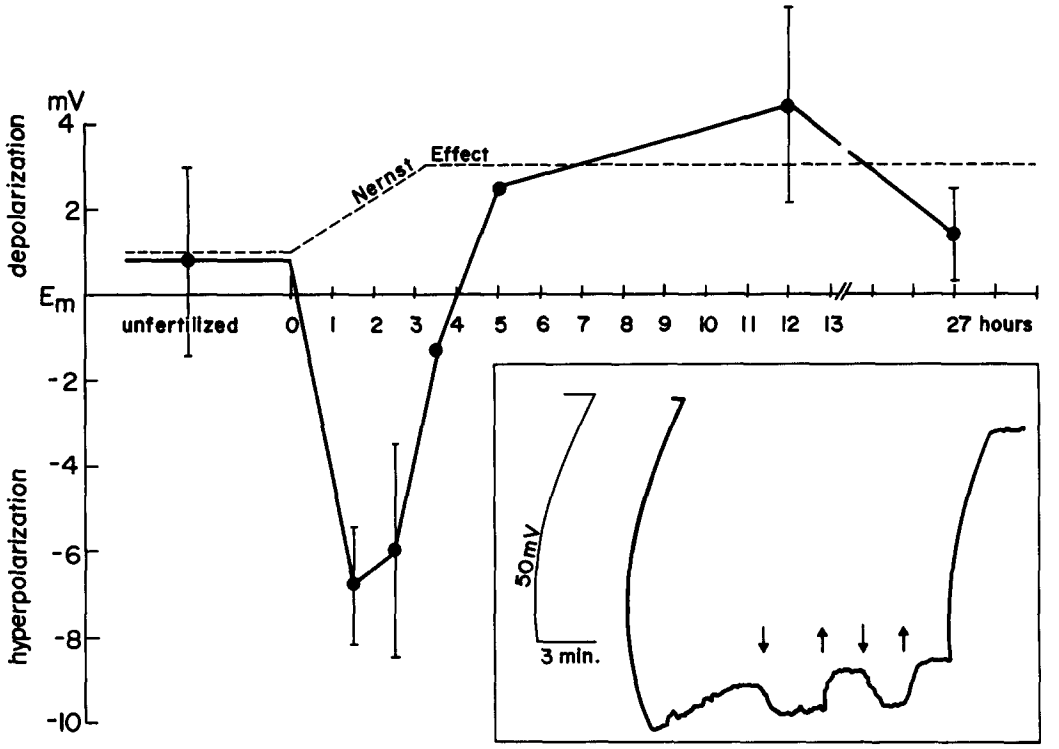

FIG. 5. Response of the membrane potential of developing *Pelvetia* eggs ($\pm\sigma$) to a rapid and brief reduction of the temperature from 15°C to 3-5°C. The measurements are grouped for 1-2, 2-3, 3-4, 4-6, 11-13, and 24-30 hr. Inset: Tracking of an actual recording (↓↑ falling and rising temperature, respectively) of a 2-hr-old zygote.

changes were variable, complex, and similar. Reducing their concentrations always causes a slow depolarization, which in turn is only slowly and sometimes only partially reversed when their normal concentrations are restored. In some cases a transient hyperpolarization of 1-3 mV appeared during the first seconds of a change of medium from ASW to low Ca^{2+} to low Mg^{2+} ASW. Transient hyperpolarizations of 2-4 mV and 3-5 mV, respectively were sometimes seen when Mg^{2+} was reduced to 1/100 in media containing 1/3 and 1/10 K^+, respectively. And transient hyperpolarizations of 10-30 mV were sometines seen when Ca^{2+} was lowered to 1/10 or 1/100 in a medium containing 1/100 K^+. These last events were always followed by a gradual depolarization of about 40 mV in 5 min.

The rapid transient hyperpolarizations which sometimes followed divalent ion reduction suggest that the state of the developing egg's membrane is variable: sometimes showing a very low permeability to divalent ions and at other times showing a substantial permeability to Ca^{2+} and Mg^{2+}. The slow depolarizations which follow no doubt indicate a rise in the permeability to various ions other than potassium.

Changes in SO_4^{2-} and in H^+, had no significant effects on the fertilized eggs membrane potential in the ranges tested. The rapid *depolarizations* which followed sodium reduction are difficult to interpret. While rapid and stable, these responses are nevertheless opposite in direction to those expected of a direct diffusional response. They plainly show that sodium permeability is relatively low. They may also indicate that external sodium nor-

TABLE 1
RESPONSES OF FUCOID EGG MEMBRANE POTENTIALS TO RAPID CHANGES IN INDIVIDUAL ION CONCENTRATIONS[a]

Ion	Change	Unfertilized		*Pelvetia*, hours after fertilization				
		Fucus	*Pelvetia*	1–2	3–6	7–9	10–13	24–30
K^+	49×			+71	+78	+88	+85	+82
	10×	+20 ± 1[b]	+9 ± 0.9	+34	+40	+47	+46	+50
	1/10×		+2 ± 0.5	−10	−24	−40	−32	−34
	1/100×	+1 ± 0.6	+2 ± 0.6	−11 ± 5	−34 ± 2	−52 ± 1	−43 ± 2	−31 ± 1
Na^+	1/3×	−8 ± 1	−10 ± 1	+9	+2	−2	+3	+2
	1/10×	−5 ± 1	−7 ± 1	+7 ± 1.0	+6	+4 ± 0.8	+5	+5 ± 0.7
	1/100×		−8 ± 1					
Cl^-	1/3×	+4 ± 1.4	+2 ± 0.8	+11 ± 1	+2 ± 0.5	+2 ± 0.4	+4	+3 ± 1.0
	1/10×	+3 ± 0.7	+6 ± 0.7	+12 ± 1	+12 ± 2	+2 ± 1.0	+6	+7 ± 3
Mg^{2+}	3×			(−6 ± 3)	(−1)		(−1)	(−2)
	1/10×	−7 ± 1	−3					
	1/100×		−2 ± 0.5	(+10)	(+3 ± 0.7)	0	(+3)	(+3 ± 1.2)
H^+	pH 9→6	+9 ± 1	+5 ± 0.6		0	0	0	+2
	8→6	+8 ± 0.6	+3					
	8→9	−2	−3 ± 1					
Ca^{2+}								
	5×		0 ± 0.9					
	1/10×	(+2 ± 0.5)	0 ± 0.4	(+4)	(+5)	(+5)	(+3)	(+1)
	1/100×	(+3 ± 0.6)	(+2 ± 0.5)	(+1)	(+13)	(+4)	(+9)	0
SO_4^{2-}	1/10×	0 ± 0.9	0 ± 0.5	0	+1	+1	+1	+1
	1/100×	0 ± 0.9	0 ± 0.4	0	0	+1	+2	+2

[a] Starting with artificial sea water. Changes are in millivolts; + indicates depolarization; −, hyperpolarization. Numbers not in parentheses indicate immediate responses; numbers in parentheses took a few minutes to develop. Standard errors are given for $n \geqq 8$. All responses were reversible upon returning to ASW.

[b] In some cases this response falls by half after 30 sec.

mally inhibits the influx of another cation. Some precedent for such an interpretation is furnished by evidence that internal sodium can inhibit potassium efflux in squid axons (Chandler and Meves, 1965).

The Membrane Response to Current Pulses during Development

One of the most striking changes in the membrane could be observed when we applied constant current pulses to unfertilized and to fertilized eggs. The membrane develops from a passive to a voltage sensitive state within several hours after fertilization.

Figure 7a shows the course of the voltage change of a representative unfertilized egg in response to 5-msec current pulses of different intensities. Figure 8 shows the steady voltage-current relationship taken from many such measurements on unfertilized eggs. For currents shifting the potential up to ±120 mV, the unfertilized egg membrane responds almost like a fixed resistor of about 1 kohm·cm^2 in parallel with a capacitance of about 1 μF/cm^2, and thus a time constant of 1 msec.

Figure 7b (made with a 12-hour-old zygote) illustrates the main features of the developed egg's response to 5-msec current pulses. It is completely passive, i.e., acts like a fixed resistor and capacitor in parallel, only for voltage displacements smaller than about 20 mV.

The resting resistance revealed by such small displacements is much lower than that of unfertilized or recently fertilized eggs. Figure 8 (inset) shows the course of membrane resistance (and capacitance) provided by many such measurements. After a few hours, this resistance falls

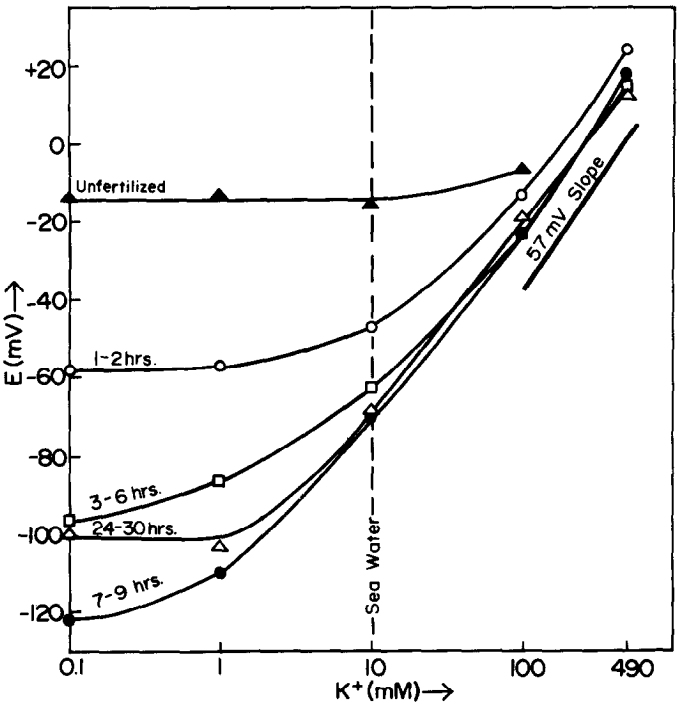

FIG. 6. Immediate responses of developing *Pelvetia* eggs to changes in potassium. Potentials in sea water are taken from Fig. 4. To these we added the rapid changes listed in Table 1.

rapidly, reaching a minimum value of only 135 ohm·cm^2 in the 12-hr-old, just germinated but still undivided, zygote. This is only one-eighth the value found in recently fertilized eggs. The capacitance, however, remains fixed at about 1 μf/cm^2 throughout the first day of development. Thus at 12 hr the egg's time constant has fallen to 1/6 millisecond.

The resistance to somewhat larger *inward* currents increases in the course of milliseconds and thus shows a so-called "hyperpolarizing response." The increase seems to occur in two stages: An initial rise whose rate increases rapidly with current density, and a second rise marked by the inflection at a displacement of 60–100 mV. (We assume that inward current evokes these delayed voltage changes by raising the membrane's resistance rather than its diffusion potential because no ions are present with potentials larger than the resting one.)

When the voltage displacement reaches 200–300 mV, a third response—perhaps a case of "punch-through"—occurs: The displacement falls back, often following an oscillating course which reaches a relatively steady value of about $\frac{1}{5}$ to $\frac{1}{2}$ of the peak displacement (Fig. 7c).

The lower half of Fig. 8 shows the peak voltages as a function of applied inward current as taken from a large number of measurements at different stages. After the first few hours, when the cells become voltage sensitive, the curves have two separated parts: The straight right-hand portion represents those cells which did not respond; the left-hand part, those which did. The arrows between the parts convey the idea that each responding cell began in a resting state having the voltage current relation shown in the right hand curve. The increase in so-called chord resistance in these transitions in 4- to 7-fold.

These resistances are calculated simply by dividing the final voltage displacement

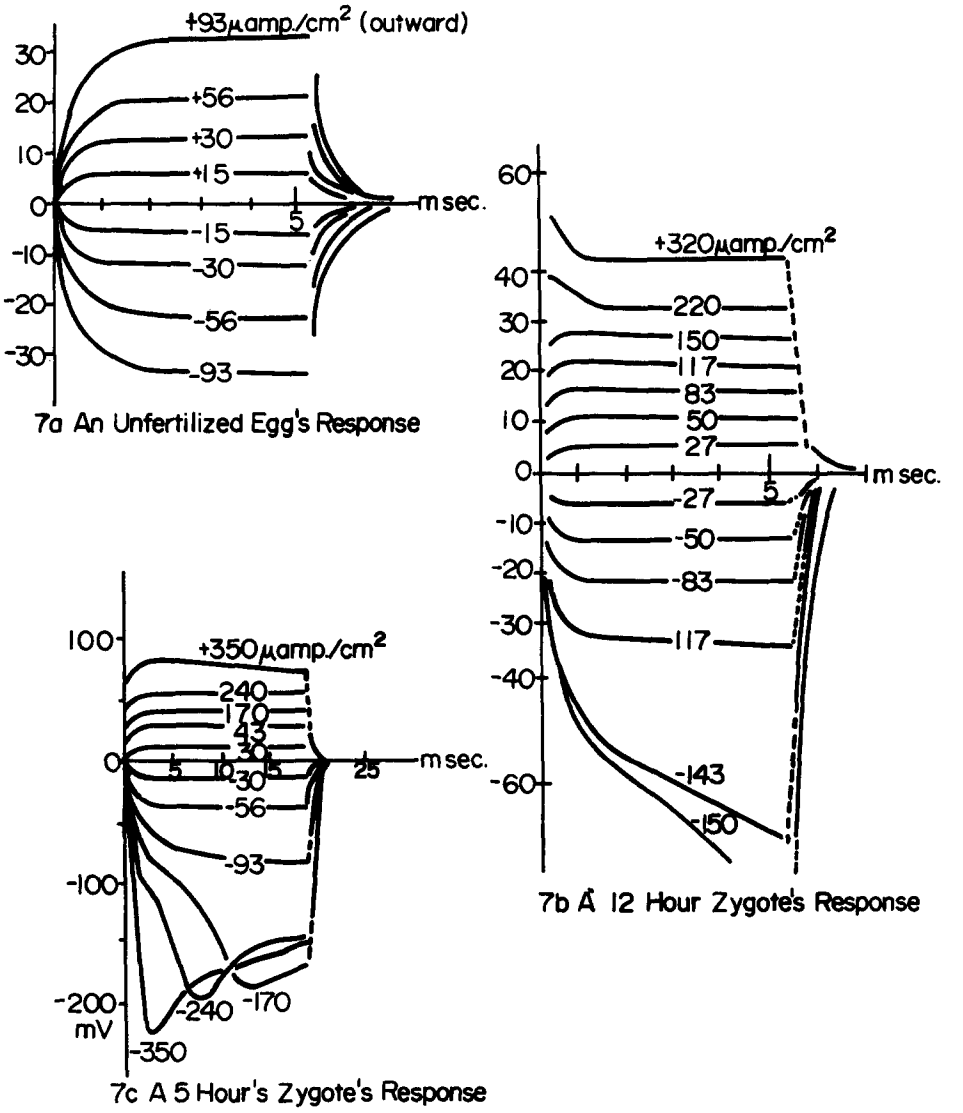

FIG. 7. Representative voltage responses of *Pelvetia* eggs to constant current pulses at different stages. Note coarser scale in 6c. (a) An unfertilized egg's response. (b) A 12-hr zygote's response. (c) A 5-hr zygote's response.

by the current strength. This is the best that can be done with the present data, but it should be noted that an accurate measure of the resistance change in such a rapidly responding system would require a voltage clamp technique.

The threshold voltages can have rather low values for a hyperpolarizing response: At 4–6 hr, a displacement of only −24 mV excited 13% of the cells; at 10–13 hr, 20% and even at 24–30 hr, in the two-cell stage (which seems a bit less sensitive), −28 mV excited 3% of the cells. The threshold currents, then, were about 100 $\mu A/cm^2$. The fraction of responding cells rose rapidly for larger displacements, reaching 100% for ones between −50 and −60 mV, which corresponds to about 150–300 $\mu A/cm^2$. It was further observed that repetition of subthreshold stimuli at 10–20 pulses per second can eventually excite a cell; repeated suprathreshold pulses

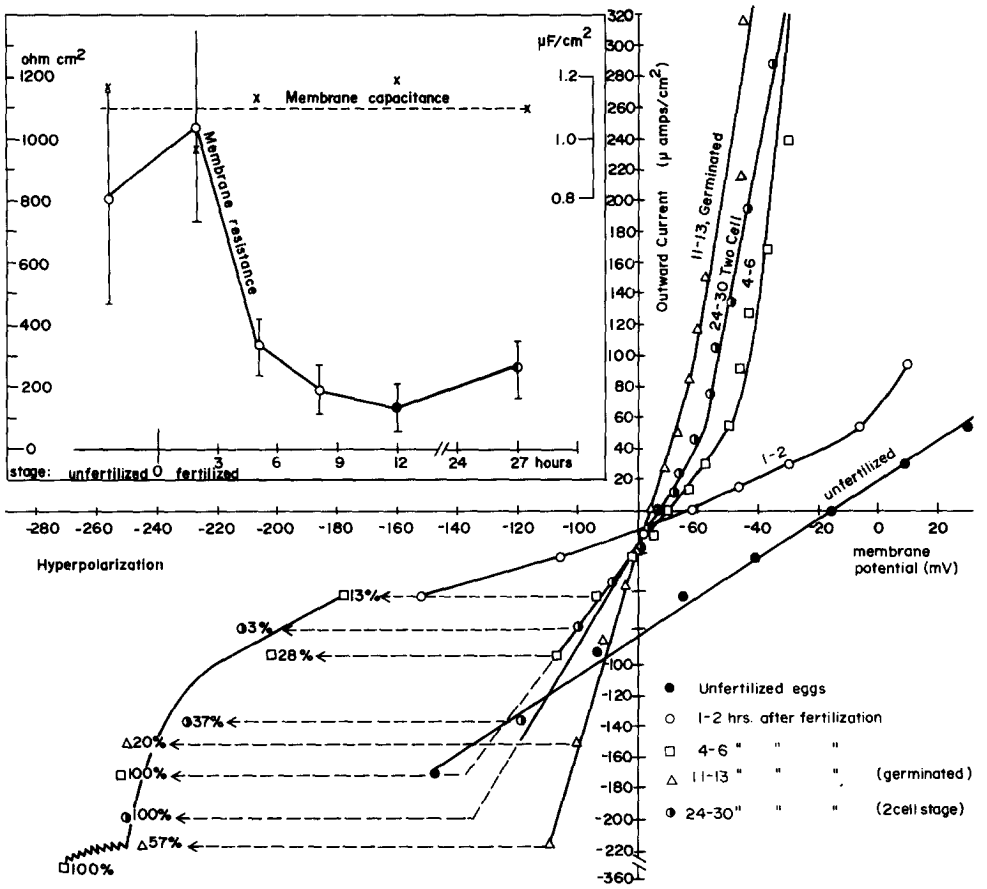

FIG. 8. Steady current-voltage relationships of the plasma membrane of developing *Pelvetia* eggs. Each point gives the mean of several measurements with different cells. The current pulses were 5–20 msec long. The numbers near the symbols give the percentage of cells giving the hyperpolarizing responses indicated by the arrows. Inset: Specific resistances (and capacitances) of the resting membrane of developing *Pelvetia* eggs ($\pm\sigma$).

elevate the threshold, but a few minutes rest restores it.

At the end of an inward current pulse, the voltage always returns rapidly to the resting potential. These return curves lie between those that would be given by a capacitor discharging through the (final) resting resistance of the membrane and through the higher resistence caused by inward current. (This point is illustrated in Fig. 9a.) These facts indicate that the resistance returns to the resting state after current stops in about the same time, about 1 msec, that it took to rise after current began. They further support our assumption that the voltage changes observed in response to inward currents represent changes in resistance rather than in the membrane's electromotive force.

The upper part of Fig. 8a shows the steady voltages reached by zygotes in response to *outward* currents. For zygotes more than a few hours old, the rate of voltage increase in response to current falls markedly for depolarizations above 20 mV. Figure 9a shows an example of the return of resting potential after large outward currents are stopped. This return curve is compared with those expected of a capacitor discharging through the resting value of the resistance of its apparent

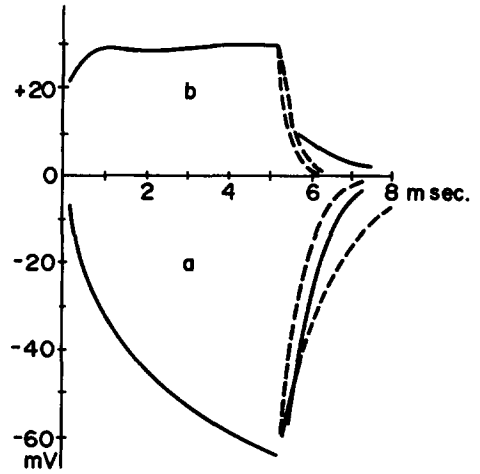

FIG. 9. Analysis of some discharge curves in a 4.5 hr-old *Pelvetia* egg. (a) The lower curve shows the membrane's response to a 5 msec long 56 $\mu A/cm^2$ pulse of inward current. A comparison with Fig. 6c indicates that a hyperpolarizing response had begun. When the current stopped, the voltage returned in a course between those which would be shown by a 1 $\mu f/cm^2$ capacitor leaking through the apparent resistance at the moment the current stopped (right-hand dashed line) and the resistance at the pulse's start (left-hand dashed line). (b) The upper curve shows the response to a 35 nA pulse of outward current. The "off" curve remains up to 5 mV above either capacitor discharge curve.

value at the end of the pulse. It is seen that the return is markedly slower than that of such a capacitor in this case; the membrane remaining some millivolts *depolarized* for about 1 msec. If the outward current response were entirely due to a fall in resistance, then the capacitative discharge would have fallen between the illustrated theoretical curves. It follows that some significant part of the out-current response must be due to a fall in membrane electromotive force rather than a change in resistance.

After cell division, we sought evidence for an electrical barrier between the thallus and rhizoid cells using a simple two electrode method. In some embryos, both the current and voltage electrodes were inserted into the thallus cell; in others, the current electrode was inserted into the thallus cell while the voltage electrode was put into the rhizoid cell or visa versa. The voltage shifts effected by current pulses did not differ significantly in these cases. Hence no very gross electrical barrier could be normally present between these cells.

In an effort to investigate differences in their membranes, we made many attempts to produce such a barrier; but they all failed. Some of the methods described earlier failed to separate them; the others did, but irreversibly depolarized the membrane.

ANALYSIS

Leak Artifacts Are Minor

As was noted before, the potentials of zygotes which are more than a few hours old recover rapidly and completely after insertion of a second micropipette. Their values are −40 to −80 mV, values far above those expected for a leak through the damaged membrane near the probe. Hence the gross features of the electrical behavior of older zygotes plainly could not be leak artifacts. Even the 5–10 mV drop in potential just before germination does not have the earmarks of a leak artifact, for a leak would be unselective and therefore show high conductance for chloride; yet the membrane potential is *least* responsive to chloride changes at 7–9 hr, when this voltage drop occurs.

A substantial role for a leak artifact in our measurements upon unfertilized and recently fertilized eggs is not so easily dismissed. It is necessary to quantitatively consider the effect of a leak. This analysis indicates that a leak could *not* have contributed much to the inferred conductance to K^+, Na^+, and Mg^{2+} in the unfertilized eggs, nor could it have contributed much to the inferred conductances to K^+, and Cl^- at 1–2 hr; but it *might* have caused much or all of the apparent Cl^- conductance in the unfertilized *Pelvetia* egg, or even in that of *Fucus*, though this latter is less likely.

To determine the supposed leak's behavior we consider it to be a shunt obeying a Goldman-type equation:

$$E = (RT/F) \ln \cdot \left[\frac{u_{Cl} Cl_i + u_{Na} Na_0 + 2u_{Mg} Mg_0 + u_K K_0 \cdots \text{etc.}}{u_{Cl} Cl_0 + u_K K_i + 2u_{(SO_4)_0} \cdots \text{etc.}} \right] \quad (1)$$

Where the u valves are relative ionic mobilities in sea water (since the shunt is assumed to be completely unselective). Introducing text book values for the mobilities (Moore, 1962, p. 337), and internal ion concentrations from Allen *et al.*, we obtain a leak potential of -13 mV in unfertilized eggs (which slowly declines to -8 mV in 27-hr eggs). This value is so close to the measured membrane potentials of -22 and -16 mV in unfertilized *Fucus* and *Pelvetia* eggs, respectively, that we applied a more refined test. In critical cases, we calculated the responses of such an unselective leak to the changes in various external ion concentrations. In Table 2 we show these results and compare them with the observed responses of the eggs. Such a leak would respond to a change in Mg^{2+} in the opposite direction to that observed (because the higher activities of the Tris and choline used as Mg^{2+} substitutes would have overridden their lower mobilities). Hence such a leak could not account for the magnesium responses at all. Moreover, it could account for only a negligible part of the H^+ and K^+ responses, and a small part of the Na^+ response.

To the extent that it were present, an unselective leak would decrease the responses, hence the inferred conductances, to K^+, and Mg^{2+} and increase those to Cl^- and Na^+. One can calculate the behavior of an egg's membrane shunted by a leak of comparable conductance using Eqs. (1) and (4), and one like (2). Then solving for the responses to $10 \times K^+$, $\frac{1}{3}$ Cl^-, and $\frac{1}{10}$ Mg^{2+} simultaneously, we

TABLE 2

IMMEDIATE RESPONSES TO CERTAIN ION CHANGES COMPARED TO THOSE EXPECTED FOR AN UNSELECTIVE LEAK

Ion	Change[a]	Leak (mV)	*Fucus*, unfertilized (mV)	*Pelvetia*	
				Unfertilized (mV)	1–2 hr (mV)
K^+	10×	+1½	+20	+9	+34
Na^+	1/3	−4	−8	−10	
Cl^-	1/3	+7	+4	+2	+11
Mg^{2+}	1/10	+1	−7	−3	
pH 9→6		0	+9	+5	

[a] K^+ replaced Na^+; Na^+ and Mg^{2+} were replaced by Tris·H^+ or choline; Cl^- by propionate. The smallness of the calculated leak responses results in good part from the substantial mobilities of the substitute ions.

calculated that if 20% and 45%, respectively, of the conductance of impaled unfertilized *Fucas* and *Pelvetia* eggs went through a leak, then the leak *alone* would account for all the chloride response. Therefore larger leaks would have necessarily produced larger chloride responses than those observed. So 20% and 45% are probably upper limits for a leak's contribution to the total conductance measured in unfertilized *Fucus* and *Pelvetia* eggs.

There are several other indications that *Pelvetia* leaks more than *Fucus*. (i) The unfertilized *Pelvetia* egg's responses to ion changes *differ* from those of *Fucus* in a leaky pattern: It was about half as responsive to K^+, H^+, and Mg^{2+}, but more responsive to Na^+ and Cl^-. (ii) Its -16 mV potential was closer to the estimated -13 mV leak potential than the -22 mV measured for *Fucus*. (iii) The procedure needed to block fertilization in the monoecious *Pelvetia* provided less viable and hence (we would guess) more easily damaged eggs than those directly obtained from the dioecious *Fucus*. (iv) We believe that the "gun" used to impale the unfertilized *Fucus* eggs was less injurious than the manual positioner used for *Pelvetia*.

An extension of this analysis suggests

that a leak could not contribute much to even the chloride conductance of the 1-2-hr egg. For a 34 mV response to a 10-fold increase in potassium limits the possible conductance of a leak to only 40% of the total; so such a leak could account for only a $0.4 \times 7 = 3$ mV response to lowering Cl^- to ⅓; thus a small part of the 11 mV observed.

Unfertilized Eggs

The potential of unfertilized eggs responds rapidly and in the direction expected of a direct diffusional effect to changes in K^+, Na^+, Mg^{2+}, Cl^-, and H^+. The relatively small responses to Cl^- may be leak effects while those to H^+ could be easily interpreted in many ways (e.g., they might arise from the concomitant changes in HCO_3^-). But there is much less reason to doubt that the larger K^+, Na^+, and Mg^{2+} responses are in fact direct diffusional effects.

For K^+, Na^+, and Mg^{2+}, the slopes, $\partial V/\partial \ln C$ decline rapidly with the concentration, C. (Compare the responses to 10× K and ⅒ K, ⅓ Na and ⅒ Na, ⅓ Mg and ⅒ Mg shown in Table 1.) Moreover, the conductance of the unfertilized eggs is voltage insensitive. So this indicates that these three ions share common pathways of low selectivity rather than traversing parallel channels of high selectivity; hence, too, the potential should follow a Goldman or common channel equation. (Jaffe, 1972)

$$V \cong (RT/F)\ln \cdot \left[\frac{P_K K_o + P_{Na}\,Na_o + 2P_{Mg}\,Mg_o}{P_K K_i}\right] \quad (2)$$

Their permeabilities, P, can then be estimated from the approximation:

$$P_j \cong \Delta V/z_j \Delta C_j \quad (3)$$

Its application indicates that $P_{Mg}/P_K \cong 0.8$ and $P_{Na}/P_K \cong 0.1$. Insertion of these values in Equ. (2) yields a membrane potential of −20 to −25 mV which agrees with the observed value for *Fucus*.

Fertilized Eggs

As a rough approximation, the 3- to 30-hr-old eggs show twice the response to ⅒ Cl^- as to ⅓ Cl^- (Table 1). So the data suggests that $\partial V/\partial \ln(Cl^-)$ is constant between 480 and 48 mM Cl^-. This in turn would indicate that K^+ and Cl^- traverse separate channels and hence V should follow a separate channel equation (Jaffe, 1972).

$$V = (RT/F)(\Sigma g \cdot E)/\Sigma g = (RT/F)\Sigma(t/z)(\ln C_o/C_i) \quad (4)$$

where the g's and E's are the channels' conductances and potentials. The transport numbers, t, are given by:

$$t = (zF/RT)(\partial V/\partial \ln C_o) \quad (5)$$

The results of applying this equation to the data in Table 1 are shown in Table 3. In a completely described multichannel system, the sum of the transport numbers should be one. The fact that these sums fall somewhat short of one, suggests that some other ion(s), perhaps Ca^{2+} and Mg^{2+}, make a significant contribution to the conductance of the fertilized eggs.

There are several indications that this other ion(s) share the potassium pathway. First, the decrease of $\partial V/\partial \ln K$ with decreasing K^+ in the vicinity of the natural potassium concentration indicates a

TABLE 3
ESTIMATED TRANSPORT NUMBERS OF IONS IN FERTILIZED *Pelvetia* EGGS[a]

Ion	3-6 Hrs	7-9 Hr	10-13 Hr	24-30 Hr
K^+	0.56	0.77	0.68	0.74
Cl^-	0.14	0.07	0.12	0.11
SO_4	0	0.04	0.04	0.04
Sum	0.70	0.86	0.84	0.88

[a] Estimated via Eq. (5) using data from Table 1. $\partial V/\partial \ln K^+$ was taken as the average for 10 K and 1/10 K; $\partial V/\partial \ln$ Cl was taken as the average for 1/10 Cl and 1/3 Cl.

shared pathway. (Compare data in Table 1 and Fig. 6 with Jaffe, 1972). Second, the greater immediate responses to divalent ion changes in low potassium (p. 560) suggests that these cations share a pathway. Third, the immediate depolarization upon sodium reduction (Table 1) may indicate that sodium blocks the entry of another cation.

Accounting for the Membrane Potentials

Insertion of the relative permeability values of K^+, Na^+, and Mg^{2+} for unfertilized eggs in a common channel equation for these ions alone yields a membrane potential of −22 mV. This compares satisfactorily with the observed values of −22 mV in *Fucus* and −16 mV in *Pelvetia*.

Insertion of the transport numbers of K^+ and Cl^- for fertilized eggs in a separate channel equation for these ions alone yields the results shown in Table 4. The fair agreement with the observed potentials indicates that K^+ diffusion (together with Cl^- diffusion) largely accounts for the observed potentials in fertilized eggs. Hence no large electrogenic contributions seem possible for either unfertilized or fertilized eggs.

TABLE 4

OBSERVED MEMBRANE POTENTIALS (mV) OF DEVELOPING *Pelvetia* EGGS COMPARED TO THOSE CALCULATED FROM TRANSPORT NUMBERS

	1–2 Hr	3–6 Hr	7–9 Hr	10–13 Hr	24–30 Hr
$t_K \cdot V_K$	−27	−46	−66	−60	−67
$t_{Cl} \cdot V_{Cl}$	−18	−2	−2	−3	−1
Sum	−45	−48	−68	−63	−68
Observed	−46	−63	−73	−73	−68

Pumps

Like most cells the fucoid egg must surely pump out Na^+, Mg^{2+} and Ca^{2+}. But the situation for K^+ and Cl^- is less obvious. So pertinent calculations are shown in Table 5. Note that the eggs must pump Cl^- out before fertilization, but must reverse this direction and start pumping Cl^- in by several hours after fertilization.

Temperature Effect

The unresponsiveness of the potential to large temperature changes (except at 1–3 hr) further indicates the absence of an electrogenic component. The anomalous hyperpolarization upon cooling during the first few hours might conceivably arise from the slowing of an electrogenic inward potassium pump or outward chloride pump. However, a more probable mechanism would be a relatively large decrease in the conductance of some ion other than potassium upon cooling. Such a mechanism has been proposed for the comparable temperature anomaly in *Dosidicus* axons (Latorre and Hidalgo, 1969) and established for mollusc neurons with blocked sodium pumps; in this last case cooling causes hyperpolarization by reducing sodium conductance (Marchiafava, 1970; Carpenter, 1970).

COMPARISONS

1. A clue to the mechanism of the hyperpolarizing response may be gained by considering the formally similar responses which are known in other systems. Usually they are much slower, taking the order of 100–1000 msec [e.g., lobster and frog striated muscle (Reuben *et al.*, 1961; Adrian *et al.*, 1970)] or even 10,000 msec [e.g., *Nitella* internodes in divalent ion-free media (Kishimoto, 1966)] rather than the 1 msec found in the *Pelvetia* egg. The presumably more relevant rapid hyperpolarizing responses of plasma membranes are listed in Table 6. Only one, that of the electrocyte of the fish *Hypopomus* shows this response in a physiological medium.

We are impressed by the fact that all four of these membranes have exception-

TABLE 5
MEMBRANE POTENTIAL, APPARENT POTASSIUM AND CHLORIDE POTENTIALS, AND IMPLIED PUMP DIRECTIONS IN DEVELOPING *Pelvetia* EGGS[a]

Hours	E_m (mV)	Cl_i (mM)	E_{Cl}	E_m-E_{Cl}	Chloride pump?	K_i	E_K	E_m-E_K	Potassium pump?
0	−16	103	−42	+26	Out	180	−72	+56	In
1	−38	83	−48	+10	Out?	170	−70	+32	In
2	−53	85	−47	−6	In?	170	−70	+17	In?
3	−58	103	−42	−16	In?	235	−78	+20	In?
7	−75	177	−28	−47	In	303	−85	+10	In?
11	−73	283	−17	−56	In	336	−87	+14	In?

[a] Internal potassium and chloride are from Allen *et al.* They are made uncertain by the apparent compartmentation of the cytoplasm.

TABLE 6
RAPID HYPERPOLARIZING RESPONSES OF PLASMA MEMBRANES

Cell	Medium	Initial			Time to double $\bar{R}$	Inflection voltage (mV)	Source[a]
		ΔV (mV)	δ ($\frac{\mu amp}{cm^2}$)	$\bar{R}$ (Ω cm^2)			
Pelvetia egg	Sea water	25–50	100–300	140–500	~1 msec	60–100	
Squid axon	Sea water 12 × K^+	30	500	60	3–6	70	*1*, Fig. 2
Toad node	Ringer, 30 × K^+	30	7,000	4	5–50	30	*1*, Fig. 7
Hypopomus electrocyte	Ringers	150	8,000	20	1–4		*2*, Fig. 10–11

[a] *1*, Tasaki (1959); *2*, Bennett and Grundfest (1966).

ally low resistances and thus are being stimulated by exceptionally high current densities. This suggests that inward current induces these resistance rises through exhaustion or accumulation of some ion near the membrane (i.e., a so-called transport number effect) rather than through a direct field effect. But which ion? As noted above most of the conductance is probably carried by potassium ions. Is it plausible then for observed currents to greatly deplete the potassium concentration outside the membrane? An order of magnitude estimate of the concentration of potassium removed from the boundary layer is given by

$$\delta t/F\cdot\sqrt{Dt}$$

where δ is the current density; t is the time it takes to act; F is the Faraday constant; D is the diffusion constant of potassium in water (2×10^{-5} cm^2/sec); $\sqrt{Dt}$ is the boundary layer thickness (5 μ after 1 msec). Substituting 200 μA/cm^2 and 1 msec yields about 10^{-5} *M*. That small a change of the K^+ within the boundary layers would surely be insignificant: $[K^+]_o$ is 1×10^{-2} *M*; $[K^+]_i$ is 2 to 4×10^{-1} *M*.

However, there *is* the possibility that enough Ca^{2+} or H^+ movement also occurs to markedly increase *their* activities in the peripheral cytoplasm within the times involved.

2. Between fertilization and first cleavage the membrane of the developing *Pelvetia* egg undergoes four main electrical changes: Its resting potential hyperpolarizes, changing from −16 to about −75 mV; its specific conductance rises about 5-fold; it becomes voltage sensitive (showing large, rapid resistance increases in response to moderate hyperpolarization by

inward current and substantial, rapid potential falls in response to comparable depolarizing currents); and it becomes sufficiently heterogeneous to drive a large current (order of 1–10 $\mu A/cm^2$) through the egg from rhizoidal to thallus pole [The first three points are documented here, the last in Jaffe (1968).] By first cleavage, this cell is differentiated, growing, developing, and functioning; in sharp contrast, the unfertilized egg is, if not dormant, only idling. We would like to know to what extent the large electrical changes which accompany this change from an idling to a functional state represent a general control mechanism; more exactly, we would like to know to what extent the peculiar electrical characteristics of the organism's unfertilized egg represent a general control which serves to keep cells in this idling state. Consequently, we have gathered together what reliable data is available on these electrical characteristics of various unfertilized eggs (Table 7a). While in Table 7b we show such data for the return to the functional state.

Some impressive similarities emerge from this compilation.

i. Most striking is the uniformly low but negative resting potential found in unfertilized (or just fertilized) eggs. In fifteen species in five extremely diverse groups of organism, this potential lies between −6 and −25 mV. In all 8 or 9 species that were followed further, this potential returns to at least −50 mV by an early developmental stage.

There have been several erroneous reports of zero to positive membrane potentials in vertebrate eggs. Kao (1956) obtained a zero potential by putting the probe into the yolk vesicle rather than the cytoplasm of the *Fundulus* egg (Bennett and Trinkaus, 1970). Hori (1958) obtained a positive value by distending the plasma membrane of the *Oryzias* egg without breaking through it (Ito, 1962). Morrill and Watson (1966) seem to have

TABLE 7a
ELECTRICAL PROPERTIES OF MATURE UNFERTILIZED EGGS

Group	Form	Medium	E_m (mV)	Ions entering	μMho/cm^2	Passive range (mV)	Source[b]
Fucales	*Fucus serratus*	Sea water	−19	Na, K			*1*
	F. furcatus	Sea water	−22	K≥Mg, >Na			*2*
	Pelvetia	Sea water	−16		1200	−130 to +100	*2*
Echinoderms	*Asterias forbesii*	Sea water	−25		300	±30	*3, 4*
	A. amurensis	Sea water	−15		1600	±40	*5*
	Hemicentrotus	Sea water	−9		2500	±30	*5*
	Peronella	Sea water	−13		250	±30	*5*
	Lytechinus	Sea water	−8	Cl and others			*6*
	Dendraster	Sea water	−7	Cl and others			*6*
Tunicates	*Halocynthia*[a]	Sea water	−19		5	−75 to +25	*7*
Vertebrates	*Fundulus*[a]	Sea water	−10		10	Moderate	*8*
(13)	*Oryzias*	Ringers	−6		1.6		*9*
	Bufo	Ringers	−12	K, Na, Cl	5	±40	*10*
	Triturus[a]	Holtfreters	−6		1.6	±40	*11*
	Xenopus[a]	Steinbergs	−6		16		*12*

[a] Zygote

[b] *1*, Bentrup (1970); 2, This paper; *3*, Tyler *et al.* (1956); *4*, Tupper *et al.* (1970); *5*, Hiramoto (1959); *6*, Steinhardt *et al.* (1971); *7*, Takahashi *et al.* (1971); *8*, Bennett and Trinkaus (1970); *9*, Ito (1962); *10*, Maéno (1959); *11*, Ito and Hori (1966); *12*, Palmer and Slack (1970); *13*, There have been several erroneous reports of zero or positive membrane potentials in vertebrate eggs (see text).

TABLE 7b
RETURN TO THE FUNCTIONAL STATE

Form	E_m Rises to at least 50 mV	Conductance rises x-fold, when	Becomes excitable
Fucus serratus	−55, 5 hr		
Pelvetia	−50, wall formed, 1–2 hr	6 polarized, 9 hr	Photopolarizable, 4 hr
Asterias forbesii	−50, 2 cell, 2 hr		
Lytechinus	−60, 20 min		
Dendraster	−70, 10 min		
Halocynthia	−70, gastrula, 10 hr	15 gastrula, 10 hr	Gastrula, 10 hr
Fundulus	−50, gastrula, 1–2 days		
Triturus	−54, morula, 2 days	12, 2 cell, 16 hr	
Xenopus	−57, blastula, 5 hr	4, 8 cell, 1 hr	Blastula, 4 hr

made a similar mistake with the frog egg. They record two potential jumps during penetration: First a rise, then a fall. They attribute the rise to penetration of the membrane and the subsequent fall to movement into an imagined second cytoplasmic phase 0.3–0.6 mm below the surface. A careful rereading of their paper convinces us that the first change represents contact with the plasma membrane while the *second* represents penetration. One strong indicator of this is that their Fig. 3 (bottom) shows only *one* jump upon pushing the probe *out* of the egg's far surface. Moreover, two other studies on amphibian eggs clearly show the first rise to result from contact not penetration (Ito and Hori, 1966, Fig. 2; Palmer and Slack, 1970, Fig. 2).

ii. The specific membrane conductances of the chordate eggs are the order of a hundred times lower than that of the fucoid and echinoderm eggs. Nevertheless, there are sharp *relative* rises in conductance, comparable to that in *Pelvetia*, in early stages of *Xenopus* and *Triturus* as well as of the tunicate, *Halocynthia*.

iii. At least for voltage displacements of up to ± 30 mV, the conductances of all the unfertilized eggs are voltage independent. In brief, these membranes are unresponsive over these ranges. Responses to such displacements appear not only in a very early stage of *Pelvetia's* development but also in the early gastrula of *Halocynthia* and the early blastula of *Xenopus*.

DEVELOPMENTAL SIGNIFICANCE

1. We conclude from the very general existence of a small membrane potential in unfertilized eggs that this is somehow connected to their idling state. We therefore wonder about the mechanisms which maintain the unfertilized egg's low membrane potential on the one hand, and its possible links to the idling state on the other.

In *Pelvetia*, this potential is about 60 mV smaller than it is some hours later. This is partly because the internal potassium concentration is less than half of that found some hours after fertilization (Allen *et al.*, 1971); but it is mainly because the potassium conductance is greatly reduced in the unfertilized egg without a corresponding fall in the conductances of other ions. There are indications of a similar mix in the amphibian egg: In the unfertilized egg of the toad, *Bufo*, a relatively high permeability to Na^+ and to Cl^- is at least partly responsible for the low membrane potential (Maéno, 1959); while the total potassium concentration of the unfertilized frog egg is only 20–50% lower than that in various other stages (work of Morrill and others discussed in Allen *et al.*, 1971). Likewise in unfertilized echinoderm eggs, there are indications of both low permselectivity and of undepressed

potassium activity (Steinhardt *et al.*, 1971).

We tentatively conclude that the low resting potential characteristic of unfertilized eggs is only in small part a result of their reduced internal potassium; in large part it results from their reduced permselectivity. This in turn suggests that this low potential is a partial *cause* of the arrested state. While it would be premature to speculate upon the mechanism of this suggested link, there certainly is no dearth of possibilities; for this low potential corresponds to a greatly reduced field across the membrane and thus could critically effect any or all of its important properties, whether these be transportive, catalytic, or binding properties.

2. We had supposed that the transcellular current is driven by a local increase in the conductance of some ion other than potassium. But our new results do not support this notion; for during the period when the average membrane potential falls and the transcellular current begins, the transport number of potassium is highest and that of chloride lowest. This suggests that the polar current may not be started by a local change in conductance but rather by a local change in some pump, either an acceleration of an inward cation pump at the rhizoid pole or a deceleration of an inward anion pump there.

3. The question of the developmental significance of the depolarizing and hyperpolarizing responses depends upon whether the egg drives enough current through itself to evoke them. The time averaged currents [inferred from the voltages developed across among eggs in series (Jaffe, 1966, 1968)] do not seem large enough; but, preliminary measurements of the fields around single eggs reveal 1-min long surges of transcellular current which do.

Whatever their exact mechanism may be, it seems clear that the depolarizing response involves positive feedback while the hyperpolarizing response involves negative feedback. In other words, outward current can regenerate itself while inward current can brake itself. Moreover, the response to outward currents tends to require a bit less time than that to inward currents as well as somewhat smaller voltage displacements and current densities. Putting these observations together, one can imagine that the surges which seem to make up much of the transcellular current may rise, boosted by a depolarizing response to outward current at the thallus pole and later fall, braked by a hyperpolarizing response to inward current at the rhizoid pole.

This work was supported by the National Science Foundation and also assisted by a travel grant to Dr. Weisenseel from the Deutsche Forschungs-gemeinschaft. We received excellent technical assistance from Mrs. Judith Seeley.

REFERENCES

ADRIAN, R. H., CHANDLER, W. K., and HODGKIN, A. L. (1970). Slow changes in potassium permeability in skeletal muscle. *J. Physiol.* (*London*) **208,** 645–668.

ALLEN, R. D., JACOBSEN, L., JOAQUIN, J., and JAFFE, L. F. (1972). Ionic concentrations in developing *Pelvetia* eggs. *Develop. Biol.* **27,** 538–545.

BENNETT, M. V. L., and GRUNDFEST, H. (1966). Analysis of depolarizing and hyperpolarizing inactivation responses in gymnotid electroplacques. *J. Gen. Physiol.* **50,** 141–169.

BENNETT, M. V. L., and TRINKAUS, J. P. (1970). Electrical coupling between embryonic cells by way of extracellular space and specialized junctions. *J. Cell Biol.* **44,** 592–610.

BENTRUP, F. W. (1970). Electrophysiologische Untersuchungen am Ei von *Fucus serratus:* Das Membranpotential. *Planta* **94,** 319–332.

CARPENTER, D. O. (1970). Membrane potential produced directly by the Na^+ pump in *Aplysia* neurons. *Comp. Biochem. Physiol.* **35,** 371.

CHANDLER, W. K., and MEVES, H. (1965). Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* (*London*) **180,** 788–820.

EISENBERG, R. S., and ENGEL, E. (1970). The spatial variation of membrane potential near a small source of current in a spherical cell. *J. Gen. Physiol.* **55,** 736.

HARVEY, H. W. (1966). The chemistry and fertility of sea waters. Cambridge Univ. Press, London and New York.

HIRAMOTO, Y. (1959). Electric properties of echinoderm eggs. *Embryologia* **4,** 219–235.

HORI, R. (1958). On the membrane potential of the unfertilized egg of the medaka, *Orzyias latipes* and changes accompanying activation. *Embryologia* **4,** 79–91.

ITO, S. (1962). Resting potential and activation potential of the *Oryzias* egg. II. Change of membrane potential and resistance during fertilization. *Embryologia* **7,** 47–55.

ITO, S., and HORI, N. (1966). Electrical characteristics of *Triturus* egg cells during cleavage. *J. Gen. Physiol.* **49,** 1019–1027.

JAFFE, L. F. (1966). Electrical current through the developing *Fucus* egg. *Proc. Nat. Acad. Sci. U. S.* **56,** 1102–1109.

JAFFE, L. F. (1968). Localization in the developing *Fucus* egg and the general role of localizing current. *Advan. Morphog.* **7,** 295–328.

JAFFE, L. F. (1970). On the centripetal course of development, the *Fucus* egg, and self-electrophoresis. *Develop. Biol.*, Suppl. **3,** 83–111.

JAFFE, L. F. (1972). Voltage-concentration relations indicate the number of kinds of passive ion pathways. *Biophysical Society Abstracts*, p. 262.

KAO, C. Y. (1956). Changing electrical constants of the *Fundulus* egg plasma membrane. *J. Gen. Physiol.* **40,** 107–119.

KISHIMOTO, U. (1966). Hyperpolarizing response in *Nitella* internodes. *Plant Cell Physiol.* **7,** 429–439.

KNIEP, H. (1907). Beiträge zur Keimungs-Physiologie und Biologie von *Fucus*. *Jahrb. Wiss. Bot.* **44,** 635.

LATORRE, R., and HIDALGO, M. C. (1969). Effect of temperature on resting potential in giant axons of squid. *Nature (London)* **221,** 962–963.

MAÉNO, T. (1959). Electrical characteristics and activation potential of *Bufo* eggs. *J. Gen. Physiol.* **43,** 139–157.

MARCHIAFAVA, P. L. (1970). The effect of temperature change on membrane potential and conductance in *Aplysia* giant nerve cell. *Comp. Biochem. Physiol.* **34,** 847.

MOORE, W. J. (1962). "Physical Chemistry." Prentice-Hall, Englewood Cliffs, New Jersey.

MORRILL, G. A., and WATSON, D. E. (1966). Transmembrane electropotential changes in amphibian eggs at ovulation, activation and first cleavage. *J. Cell Physiol.* **67,** 85–92.

PALMER, J. F., and SLACK, C. (1970). Some bioelectric parameters of early *Xenopus* embryos. *J. Embryol. Exp. Morphol.* **24,** 535–553.

REUBEN, J. P., WERMAN, R., and GRUNDFEST, H. (1961). The ionic mechanism of hyperpolarizing responses in lobster muscle fibers. *J. Gen. Physiol.* **45,** 243–265.

ROBINSON, K., and JAFFE, L. F. Manuscript in preparation.

ROSE, B., and LOEWENSTEIN, W. R. (1969). Depression of functional membrane permeability by substitution of lithium for extracellular sodium. *Biochim. Biophys. Acta* **173**/1, 146–149.

STEINHARDT, R. A., LUNDIN, L., and MAZIA, D. (1971). Bioelectric responses of the echinoderm egg to fertilization. *Proc. Nat. Acad. Sci. U. S.* **68,** 2426–2430.

TAKAHASHI, K., MIYAZAKI, S., and KIDOKORO, Y. (1971). Development of excitability in embryonic muscle cell membranes in certain tunicates. *Science* **171,** 415–417.

TASAKI, I. (1959). Demonstration of two stable states of the nerve membrane in potassium-rich media. *J. Physiol. (London)* **148,** 306–331.

TUPPER, J., SAUNDERS, J. W., and EDWARDS, C. (1970). The onset of electrical communication between cells in the developing starfish embryo. *J. Cell Biol.* **46,** 187–191.

TYLER, A., MONROY, A., KAO, C. Y., and GRUNDFEST, H. (1956). Membrane potential and resistance of the starfish egg before and after fertilization. *Biol. Bull.* **111,** 153–177.