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Polarization of Fucoid Eggs by Steady Electrical Fields[1]

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Pelvetia eggs were exposed to steady electric fields from 5 hr after fertilization until their rhizoids began to grow out some 6 to 10 hr later. Eleven batches of eggs responded by initiating rhizoids towards the positive electrode; two batches responded by growing towards the negative electrode; and three grew towards the negative one in small fields and towards the positive one in higher fields. Polarization, defined as the average cosine of the outgrowth directions, was proportional to field strength up to polarization values of 50% for the positive responses and 75% for the negative ones. A voltage drop of 6 mV/cell induced 10% polarization in the positively galvanotropic batches, while 3 mV/cell did this in the negative ones. We reason that *both* responses are mediated by faster calcium entry at the future growth point. It is supposed to be faster there in positively galvanotropic eggs because the membrane potential, hence the driving force, is highest; in negatively galvanotropic eggs because depolarization induces an overbalancing increase in calcium permeability there.

INTRODUCTION

The developing fucoid egg is an excellent system for studying the mechanism of localization. Immediately following fertilization, the eggs appear to be radially symmetric spheres, and the region of later rhizoid formation on each egg can be determined by a wide variety of physical and chemical agents (Jaffe, 1968).

Thus, as early as 1923, Lund showed that the rhizoid outgrowths of *Fucus inflatus* eggs would form towards the positive electrode if the eggs were exposed to an electric field of about 25 mV/egg throughout a period of about 10 hr prior to rhizoid initiation. In Lund's experiment, positive current was driven into the presumptive rhizoid pole of the egg and out of its other pole. The direction of this imposed current is the same as the endogenous one, i.e., one generated by the cell itself under natural conditions, as later measured by Jaffe (1966). Moreover, the magnitude of the total current driven through the cell by the applied field can be calculated from the tracer conductance data of Robinson and Jaffe (1973) and proves comparable to the endogenous one. These findings suggested that the endogenous current acts back on the cell to polarize it further and that an imposed field acts by means of the net current it drives through the cell.

However, other evidence raises serious doubts about this simple conclusion. First of all, Bentrup (1968) reported that *Fucus serratus* eggs, if exposed to comparable fields for comparable periods, actually form their rhizoids towards the negative rather than the positive pole. He proposed that an electric field can somehow determine the site of rhizoid initiation by shifting each cell's membrane potential either above *or* below its natural value. This conception received some further support from the later finding of Novàk and Bentrup (1973) that these eggs form their outgrowths along the axis of imposed *alternating* fields. Second, Robinson and Jaffe (1975) have recently shown that a substantial part of the endogenous current stimulated by unilateral light consists of calcium ions. Theoretical and comparative considerations strongly suggest that it is this calcium component which will dominate any action of the current (Jaffe *et al.*, 1974). These factual complexities and theoretical uncertainties have led us to reinvestigate carefully the effects of steady

[1] From H. Benjamin Peng's Ph. D. Thesis.

electrical fields on rhizoid initiation in fucoid eggs.

MATERIALS AND METHODS

We used ripe fronds of the monoecious alga *Pelvetia fastigiata,* which were collected at Pacific Grove, Calif., by Mrs. A. Phillips. Eggs and sperm from ripe fronds were obtained by the method of Jaffe and Neuscheler (1969). The fertilization time was considered to be 0.5 hr after shedding. At this time, about 50% of the eggs are released from their capsules. One hour after fertilization, eggs were collected and washed with natural sea water by letting the eggs fall to the bottom of a beaker and decanting the supernatant. This process was repeated several times. The eggs were then immediately distributed to Plexiglas culture chambers. Each contained a groove connecting two large reservoirs (Fig. 1). The eggs were distributed into the groove region. A #1 coverglass was glued to the chamber to form the bottom. After the eggs were poured into the chamber, the top of the groove was also covered with a #1 coverglass. Each reservoir was connected to a silver-silver chloride electrode by a low-resistance sea water-agar bridge as shown in Fig. 1. Each chamber was placed on an aluminum cooling block. The whole setup was placed on a large metal plate which was exposed to the environment and well ventilated. A wooden box placed on top of the metal plate served to keep the system from room light. Illumination was provided from a fluorescent glow box through a red plastic filter mounted on top of the box. The red light thus obtained (wavelength greater than 600 nm) did not effect any polarization of the eggs (Jaffe, 1958). The environmental temperature was kept at 15°C. The temperature in the egg region was never more than 1°C above room temperature even at the highest current used. Electric current to all chambers was provided by a power supply. Current was individually set at a desired value by a potentiometer. The measurement of current was done with a Keithley 600B electrometer. The current density was calculated by dividing the measured current by the cross-section of the groove.

The potential difference per egg diameter was calculated from the measured resistivity of natural sea water at 15°C (24 ohm · cm), and the diameter of the eggs (100 μm). Due to the high membrane resistance, the electric field around a single egg is distorted. This situation is similar to a nonconductive sphere in a conductive me-

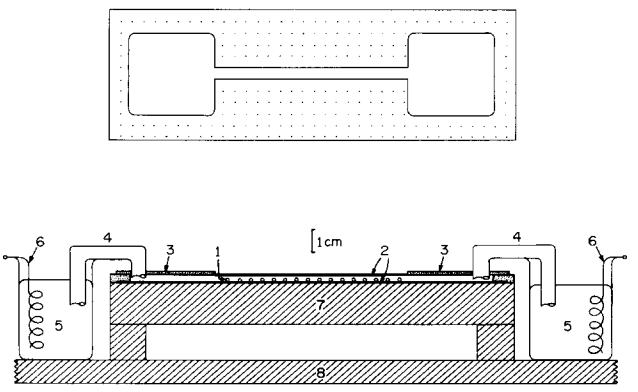

FIG. 1. The culture chamber and accessories. Top figure shows the top view of the chamber. Stippled area is made of Plexiglas. Bottom figure shows a cross-section through the middle of the chamber. The top and bottom of the egg groove are made of #1 coverslips as shown by thickened line. (1) Eggs in the groove (not to scale); (2) coverslips; (3) reservoir cover; (4) sea water-agar bridges; (5) beakers with seawater; (6) silver-silver chloride electrodes; (7) aluminum cooling stage; (8) ultimate metal heat sink.

dium. Hence the actual potential difference across an egg is larger than the value calculated by assuming a simple ohmic drop across an egg diameter. The factor of increase was calculated to be 1.5, following Abraham and Becker (1950, p. 80). The outgrowth directions were measured 1 day after fertilization when the orientation of the rhizoids could be clearly seen. The average cosine, V, was calculated from

$$V = (1/N)\Sigma_i\, n_i \cos \theta_i, \qquad [1]$$

where N = total number of outgrowths counted, n_i = the number of outgrowths whose projection in the plane parallel to the direction of the electric field originated at an angle θ_i to the direction of the anode.

For a distribution perfectly aligned towards the anode, V = 100%; for one perfectly aligned towards the cathode, V = −100%; for a uniform distribution, V = 0%. Eighteen categories, each of 10° on either side of the symmetrical axis, were used to characterize the outgrowth angle θ_i. These measurements were speeded by a device attached to the eyepiece consisting of a reticle and a ring of electrical contacts, each 10° wide, as described previously (Müller and Jaffe, 1965). The calculation of standard deviations was also described before (Jaffe, 1958).

RESULTS

The electric field was always turned on at 5 hr after fertilization and was kept on for 12 hr. When the field was shut off, more than 95% germination was usually observed.

Representative angular distributions of rhizoid outgrowth with respect to the direction of the electric field are shown in Figs. 2a–c. The corresponding average cosine of the angular distribution was calculated for each field strength according to Eq. [1] and is shown in Figs. 3a and b and 4a–c. In Figs. 2a and 3a, we pool data from three egg batches which gave an anodal response, i.e., tended to form rhizoids towards the positive electrode. The angular

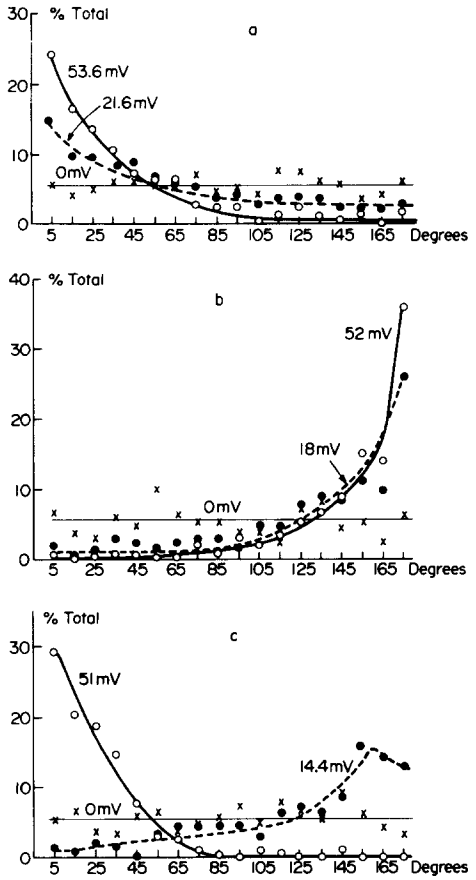

FIG. 2. Representative angular distributions of rhizoid outgrowths in response to an electric field. The abscissa shows the directions of outgrowth, characterized in 10° intervals on either side of the symmetric axis which is the line connecting positive and negative electrodes. Zero degree refers to the anode direction and 180° refers to the cathode direction. The points are plotted against the center of each angular interval. The ordinate shows the percentage of outgrowths falling into each category. Field strength, in millivolts per cell, is given for each curve. (a), Positive response. Results of three comparable experiments are pooled. Average of 1070 counts per curve. (b), Negative response. Average of 330 counts per curve. (c), Mixed response. Average of 194 counts per curve.

distribution (Fig. 2a) shows a single peak at 0°, the direction of the positive electrode. As the field strength is reduced, the magnitude of the peak becomes smaller and the outgrowth directions become more random. Figure 3a shows that the polarization increases linearly with field

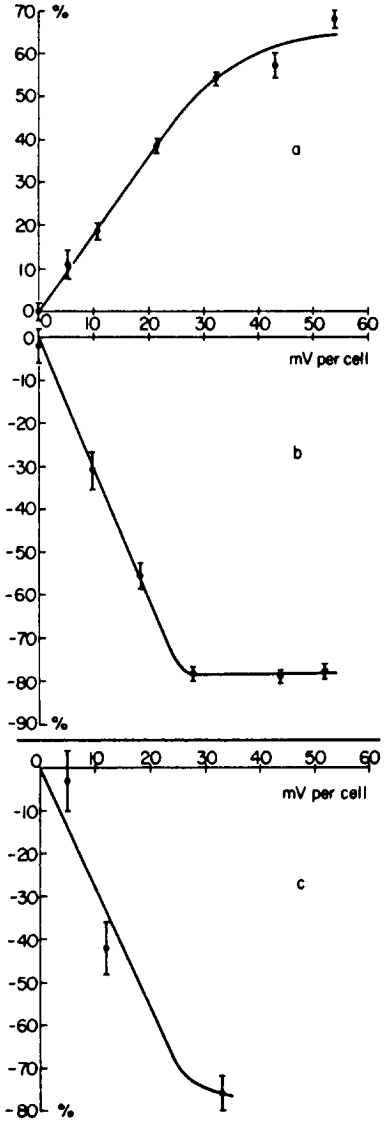

FIG. 3. (a) and (b), Galvanotropic responses of *Pelvetia* eggs. Ordinate: Average cosine of the direction of rhizoid initiation, Eq. [1]. Positive values indicate growth towards the positive electrode and vice versa. Abscissa, field strength expressed as the actual voltage drop across each cell. (a), Positive responses. The results of three experiments are pooled. Average of 975 counts per point. (b), A negative response. Average of 341 counts per point. (c), Bentrup's (1968) data on *Fucus serratus* are replotted here to show the similarity between the negative response of *Pelvetia* (b) and *Fucus*.

strength almost up to a value of 50% which is induced by a field of 29 mV/cell. Thus, the characteristic value of 10% polarization is induced by a field of 6 mV/cell. A threshold is notably absent.

Figure 2b shows the angular distribution of a single experiment which gave a cathodal response; i.e., the rhizoids tended to form towards the negative electrode. The response has a single, sharp peak at 180°, the direction of the negative electrode. The polarization is plotted in Fig. 3b. It is linear down to a polarization of −75% at 25 mV/cell. Thus the characteristic value of −10% polarization is induced by only 3mV/cell, which is half the value for the positive response.

In 3 out of 16 experiments, we observed responses of a mixed sort; i.e., at low field strength, the rhizoids tended to form towards the negative electrode and, at higher field strength, they tended to form towards the positive electrode. Figure 2c shows representative angular distributions of both positive and negative phases of a mixed response. For the negative phase the distribution peaks slightly away from 180°. In Figs. 4a–c the polarization of all three experiments giving mixed responses is shown. For all three the transition from negative to positive response occurs within a rather short voltage range, on the order of 10 mV/cell. The response curve crosses the axis, i.e., has a second null value, at fields of 18, 22, and 37 mV/cell in these three cases.

In Table 1 we have tabulated the frequency of each of the three responses, along with the voltage giving a 10% response for purely positive and negative responses. Although 70% of the batches gave a positive response, the outcome of each experiment is still completely unpredictable. In fact, even with identical experimental procedures we got opposite results a day apart with different fronds from the *same* collection of plants in one case. Moreover, we find that the age of the gamete-bearing fronds as judged from their size and physical appearance is not correlated with the type of galvanotropic response given by the zygotes.

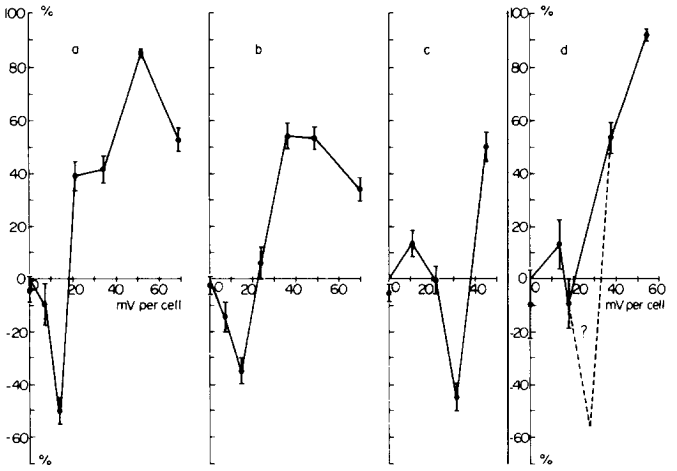

FIG. 4. (a)-(c), Mixed galvanotropic responses of *Pelvetia* eggs. The coordinates have the same meaning as in Fig. 3. Average of 160 counts per point. (d), Lund's data (1923) on *Fucus inflatus* are plotted here. This shows that what was described as a positive response by Lund might in fact be a mixed response similar to our results. Solid circles are actual data calculated from photographs published by Lund. The dashed line with a question mark shows a speculation of a possible mixed response based on (a)-(c).

TABLE 1

SUMMARY OF RESULTS

Type of response	Number of experiments	Field strength at 10% polarization (mV/cell)
Positive	11	5.5 ± 0.5 ($n = 8$)
Negative	2	3.1 ± 0.5 ($n = 2$)
Mixed	3	–
Total	16	

DISCUSSION

(1) In this study, we have shown that the direction of rhizoid initiation in *Pelvetia fastigiata* eggs can be determined by application of a steady electrical field. In 11 of 16 batches of eggs, these cells showed a tendency to initiate rhizoids towards the positive pole and this positive galvanotropic response increased steadily with increasing field strength (Fig. 3a). In 2 of 16 batches of the same species' eggs they showed just the opposite response. That is, they showed a tendency to initiate rhizoids towards the negative pole, and this negative response likewise increased steadily with increasing field strength (Fig. 3b). Moreover, in the 3 remaining batches, they showed a remarkable mixed response, negative at low field strengths and positive at higher ones with a transition occurring over a very small voltage range (Figs. 4a-c).

The literature shows two earlier studies of field effects on fucoid eggs, the pioneering study of Lund (1923) and the more recent one of Bentrup (1968). Lund did not present a quantitative analysis of the results but published some photographs of one experiment. Partly for its historical interest, we determined the average cosine of the outgrowth directions in these old photographs and have plotted them in Fig. 4d. Lund believed his results showed a distinct threshold voltage, but it seems quite possible that this batch of *Fucus inflatus* eggs actually exhibited a mixed response very similar to those we observed with *Pelvetia*. Bentrup published data on the responses of one batch of *F. serratus* eggs stimulated under conditions and during a period closely comparable to ours. These data are replotted in Fig. 3c and obviously show a negative response very similar to the ones we observed with *Pelvetia* eggs.

(2) As a first step in interpreting those complex galvanotropic responses, we have made a survey of the sparse literature on galvanotropism in various cells. A summary of what seem to be the most mean-

TABLE 2

VOLTAGE GIVING ONE-TENTH MAXIMAL RESPONSE IN KNOWN CASES OF GALVANOTROPISM[a]

Case[b]	Cell diameter (μm)	Genus	Cell type	Exposure time (hr)	Response	Direction	Millivolts per cell
1	~1000	*Griffithsia*	Shoot	70	Rhizoid starts	+	~4
2a	100	*Pelvetia*	Egg	12	Rhizoid starts	+	6
2b	100	*Pelvetia*	Egg	12	Rhizoid starts	−	3
3	70	*Fucus*	Egg	10	Rhizoid starts	−	4
4	65	*Equisetum*	Spore	10	Rhizoid starts	+	5
5	10	*Fucus*	Rhizoid	3	Curved growth	+	~0.3
6	9	*Ulva*	Egg	12	Rhizoid starts	+	0.4
7	6	*Funaria*	Chloronema	3	Curved growth	−	~0.2
8	1–10	Chick	Neurites	24	Growth speeded	−	~0.2[c]

[a] In cases 2, 3, 4, and 6 the one-tenth maximal response is taken as one where the average cosine of the outgrowth directions is 0.1; in cases 5 and 7, where the outgrowth curved by 10°; in case 1, where the response is just detectable; in case 8, where the difference in growth rates of neurites growing towards and away from the anode is 10%.

[b] Case 1 is from Schechter (1934); 2, from this paper; 3, 4, 5, and 7 from Bentrup (1968, Figs. 5, 6 (*E. limosum*), and 12 and Table 4, respectively); 6, from Sand (1973, wild type); 8, from Poo and Jaffe (in preparation).

[c] Since the neurites do *not* curve in response to a field, the field's effective component should be along rather than across these cells. So in this peculiar case the effective voltage difference is taken along a distance of 100 μm (the neurite's crudely estimated cable constant), rather than any cell diameter.

ingful results appears in Table 2. It shows the recorded responses falling into two distinct classes, namely, a low voltage and a high voltage group.

The low voltage responses require about 0.2 to 0.4 mV/cell (to yield a one-tenth maximal response) and involve four different cells each no more than 10 μm in diameter. While the high voltage ones require about 10 times this voltage, namely, about 3 to 6 mV/cell, and involve four much larger cells, actually cells from 70 to 1,000 μm in diameter.

The low voltage responses may well be mediated by perimembrane electrophoresis (Jaffe and Peng, 1975). That is, the tangential component of the electric field, along the periphery of the cell, could move certain mobile, growth-controlling plasma membrane components which have charged heads protruding from the lipid bilayer. Thus these components would be driven laterally, along the membrane towards one pole or the other.

The high voltage responses, on the other hand, involve voltage differences large enough to act across the plasma membrane, i.e., by introducing a significant gradient in the membrane potential around each cell; while these responses are exhibited by cells so large as to raise serious doubts as to whether membrane-bound components could be transported far enough along each cell's membrane in the times involved. Furthermore, according to Novàk and Bentrup (1973), the growth of fucoid eggs can be polarized by rapidly *alternating* fields with intensities comparable to the effective direct ones. It is difficult to imagine how net movement along the membrane could be produced by rapidly alternating fields, but equal membrane potential displacements in opposite direction often fail to have exactly opposite effects. The "second" field, of opposite polarity, may fail to reverse the effect produced by the "first" one, or it may even reinforce it. So we will interpret the high voltage responses, particularly the presently reported ones on fucoid eggs, as mediated by gradients in membrane potential rather than perimembrane electrophoresis.

(3) There is growing evidence that

growth localization in fucoid eggs involves an endogenous transcellular calcium current with growth occurring at the site of calcium entry (Robinson and Jaffe, 1975; Jaffe *et al.*, 1975). We would therefore propose that *both positive and negative* galvanotropic responses are mediated by increased calcium entry at the future growth site. In positively galvanotropic egg batches (or species?), calcium entry would be faster at the positive pole simply because the membrane potential is highest there, and thus the force driving calcium inwards is greatest there; while in negatively galvanotropic batches (or species), we suppose that calcium entry is faster at the negative pole because, in these cases, depolarization "excites" the membrane and opens enough extra calcium gates to effect faster entry despite the lower driving force there. We will now consider the evidence for this proposition.

(a) The shift in membrane potential at either pole of spherical cell in a uniform field is given by half the voltage drop across the cell. Thus a voltage drop, ΔV will raise the membrane potential by $\Delta V/2$ at the anodal pole and lower it by $\Delta V/2$ at the cathodal one. Between the poles, the shift in membrane potential will vary with $\cos \theta$. These conclusions depend only upon the membrane resistance being high with respect to the resistances of both the cytoplasm and the ambient medium. They do *not* require the membrane resistance to be the same at both poles. The membrane resistance for *Pelvetia* eggs at this stage has been estimated to be about 3000 ohm $\cdot$ cm^2 from both tracer flux measurements (Robinson and Jaffe, 1973) and intracellular microelectrode measurement (Weisenseel and Jaffe, 1974). The estimated cytoplasmic resistance per square centimeter across a distance of one cell diameter (100 μm) is about 2 ohms from high frequency impedance measurement (Jaffe, 1966). The medium's resistance per square centimeter across the same distance is only 0.2 ohm. Thus the above conditions certainly hold for fucoid eggs.

(b) Using point (a), we estimate how much extra calcium is *driven* into the positive poles of eggs by the field and find it comparable to the extra calcium *induced* to enter the dark (presumptive growth) poles by strong unilateral light. The 65% growth polarization of positively galvanotropic egg batches produced by a field of 55 mV/cell (Fig. 3a) involves an increase in membrane potential at the externally positive (and presumptive growth) pole of 28 mV. The natural membrane potential at the relevant stage is about 70 mV (Weisenseel and Jaffe, 1972). Increases in the membrane potential of *Pelvetia* eggs produced by reductions in external potassium are found to yield a proportionate increase in ^{45}Ca influx (Chen and Jaffe, in preparation). Hence the calcium influx at the future growth pole is increased by about 28/70 or 40%.[2]

The increased calcium influx into the presumptive growth pole of photopolarized eggs can be obtained from the nickel screen experiment of Robinson and Jaffe (1975). Illumination did not seem to change the net calcium influx but redistributed it so that during the relevant pregermination stage it was two to five times greater at the dark (and future growth) poles than the light ones. From this one can calculate that the calcium influx rose about 30% to 70% at the future growth poles. When one further considers that the light-polarized outgrowth of these screen-borne eggs has a net polarization somewhat more than the 65% effected by the largest field in the present experiments, it can be concluded that the extra calcium influx at the future growth pole is closely comparable in the two cases. This similar-

[2] It is true that the level of free cortical calcium will also be affected by calcium efflux rates. Unfortunately, ^{45}Ca efflux data on *Pelvetia* are ambiguous, since it is impossible to distinguish the efflux mediated by passage through the membrane from efflux mediated by the secretion of calcium-containing vesicles. Hence we must neglect possible effects of the membrane potential on efflux rates for now.

ity supports a calcium entry theory.

(c) When the membrane potential of *Pelvetia* eggs is *reduced* by raising external potassium, about 80% of the egg batches tested responded with an actual *fall* in ^{45}Ca influx (Chen and Jaffe, in preparation). This fall was relatively small, only about 12% for a 47-mV reduction, thus only 7% for a 28-mV reduction. Nevertheless it was a repeatable result and indicates that the externally negative, i.e., cathode-facing, egg pole would not compete with the positive one as a site of calcium entry in most egg batches.

However, about 20% of the egg batches studied by Chen and Jaffe responded to a tripling of external potassium (and thus a 30% reduction in membrane potential) by an *increase* in ^{45}Ca influx of about 20%. For a number of reasons, no quantitative comparison of these limited results with negative galvanotropic responses is possible. Nevertheless, they do show that a minority of egg batches respond to small depolarizations with marked increases in calcium influx despite the fall in driving force; and it seems reasonable to assume that it was such anomalous egg batches which exhibited negative galvanotropism.

(d) The mixed responses remain puzzling, but we note two findings that may well prove to be useful leads: First, while a tripling of external potassium raised the influx of ^{45}Ca in the anomalous egg batches studied by Chen and Jaffe, the larger depolarizations produced by raising potassium 10-fold actually lowered the influx in these same batches. Second, *Pelvetia* eggs show a dramatic "hyperpolarizing response" (Weisenseel and Jaffe, 1972); i.e., they rapidly close most of their potassium gates in response to increases in membrane potential which are comparable to the potential shifts that give galvanotropic reversal (Figs. 4a-c).

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