Proc. Nat. Acad. Sci. USA
Vol. 71, No. 12, pp. 4855–4859, December 1974

Spontaneous Current Pulses Through Developing Fucoid Eggs

(membrane/localization/polarity/self-electrophoresis/*Pelvetia*)

RICHARD NUCCITELLI AND LIONEL F. JAFFE

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Communicated by Anton Lang, September 20, 1974

ABSTRACT **Using a newly developed, extracellular vibrating electrode, we can now measure the electrical currents that traverse a single developing cell. We have studied the eggs of the common seaweed, *Pelvetia*, during their first 2 days of development and find that the endogenous electrical current through them includes a pulse component as well as a relatively steady component. Both of these enter the egg's growing tip and leave the rest of the embryo. The current pulses first appear a few hours after growth begins and have a characteristic shape that is independent of amplitude. They have a duration of about 100 sec, an average frequency of 1–5 per hr, and enter with peak surface intensities of 3–10 (and rarely up to 30) $\mu A/cm^2$. By the two-cell stage they account for about a fourth of the total transembryonic current. Since they may overlap to any degree and (as is documented elsewhere) are generally accompanied by peak membrane depolarizations of only 2–6 mV, their course does not seem to be voltage-controlled. Thus, they seem essentially different from action potentials. We also find that the rate at which the egg grows in length is roughly proportional to the size of the steady current traversing it.**

The developing fucoid egg has long been considered to be a prototype of the central developmental phenomenon of localization (1–3). In the course of a day the essentially apolar zygote becomes differentiated into two grossly different regions and then cells: the tip-growing rhizoid cell and the initially quiescent thallus cell (Fig. 1). It has been argued that this process starts with a localized membrane change which then acts to localize internal components (4). In the pursuit of this concept, one of us found these eggs to drive a substantial (and no doubt membrane-driven) electrical current through themselves (3, 5). This current was found to enter the growing tip (or prospective growing tip) and to leave at the opposite end of the cell. It first became measurable at about the time of irreversible determination of this growth point. This current is of particular theoretical interest since it may feed back to further polarize and differentiate the embryo via self-electrophoresis; that is, it may generate a sufficient intracellular field to pull key components towards the growing region of the membrane and perhaps even repel them from the membrane in the nongrowing regions (6, 7).

These currents were first measured by a multicellular method. About 200 developing eggs were lined up in series within a long, loose-fitting, sea water-filled capillary. In this way the voltage drop along the tube was made 200 times larger than that generated by the current moving past a single egg, and was thus large enough to be measured by conventional means (5). While this multicellular method is simple and reliable, it obviously has minimal spatial resolution. Moreover, it has very low temporal resolution since the development of an egg population is poorly synchronized. To improve both spatial and temporal resolution, we have developed a new ultrasensitive vibrating electrode system that is at least

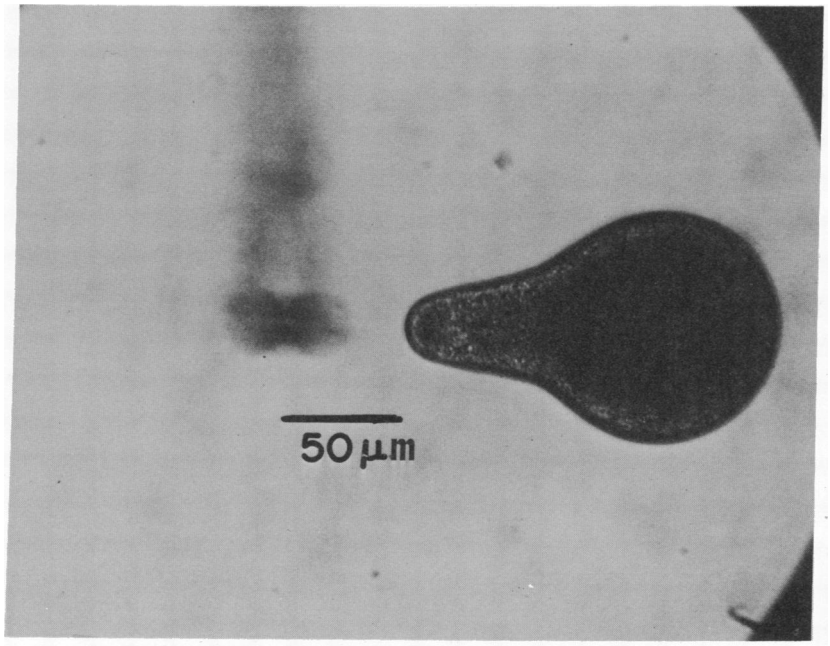

FIG. 1. Vibrating probe in front of a growing *Pelvetia* embryo.

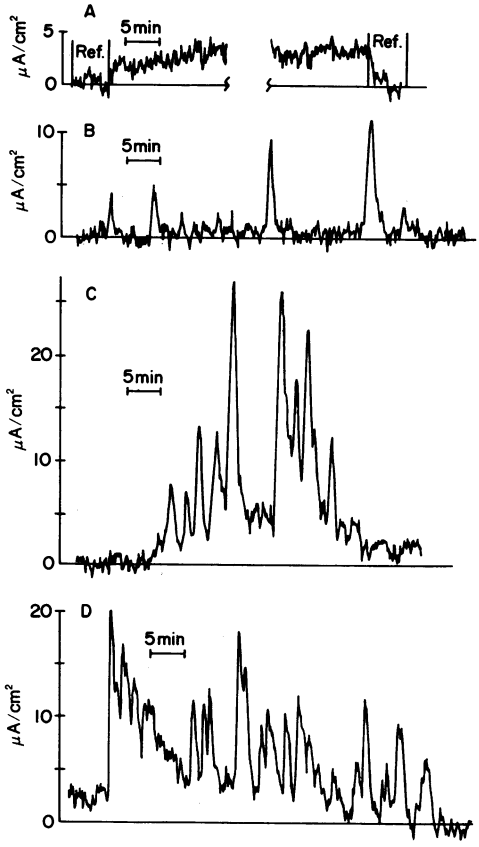

FIG. 2. Representative measurements of current traversing the growing tips of two-cell *Pelvetia* embryos. Probe position is as in Fig. 1 except for Ref. (reference) periods 250 μm away from the surface. Ordinates: Current densities entering the growth tip (1 μA/cm² is inferred from an actual measurement of 10 nV). Abscissas: Time (scale shown in figure). A. Steady component present alone (from exp. 2 at 11 hr). Note 30-min break in record. B. Relatively isolated pulses (from exp. 4 at 26 hr). C and D. Overlapping and merging pulse groups (from exps. 3 and 2 at 31.5 and 37 hr, respectively). Complete records are shown in Fig. 5.

100 times more sensitive than conventional means of measuring slowly changing extracellular fields (8). Using this system we find it possible to measure and study the current-generated fields around *single* developing fucoid eggs. This paper reports our first findings obtained in this way.

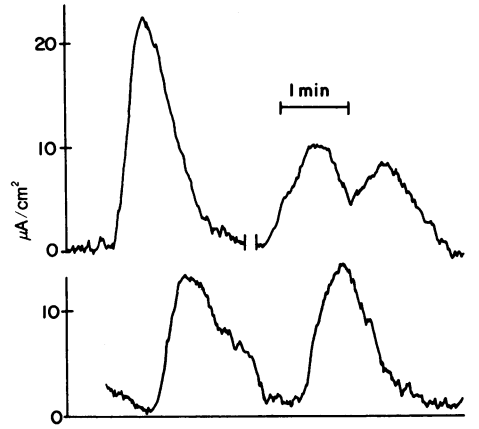

FIG. 3. Representative current pulses shown on expanded time axes. Same as Fig. 2 except for time scale. Top: an isolated pulse and two overlapping pulses. Bottom: twin pulses.

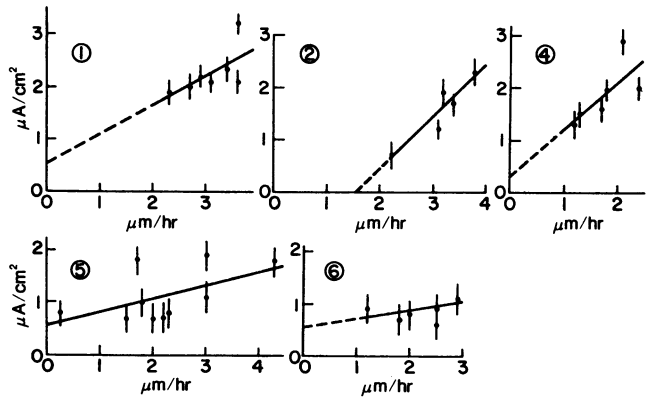

FIG. 4. Current density versus elongation rate for five separate eggs throughout the periods of illumination shown in Fig. 5. Each point represents the average value over a 2-hr interval for both current density and elongation rate. Bars indicate empirical standard errors. Solid lines are least squares fits. Experiment number 3 is not included due to unreliable growth measurements.

PROCEDURES

Zygotes of the common West Coast seaweed, *Pelvetia fastigiata*, were obtained as described (9), and cultured at 15°C in natural sea water under a 12-hr day beginning at the time of fertilization. In two experiments the sea water's pH was lowered from the natural value of about 8.0 to 6.2 with the aid of a 4 mM phosphate buffer. "Day light" was a horizontal beam of white, infrared-free light with an intensity of 250 footcandles (2700 lux). This illumination served to orient growth in the horizontal plane needed for both current and growth measurements. Observations during the dark periods were done with dim green, vertically directed light passed by a 546-nm interference filter. Growth was monitored by measuring the embryo's length every 1–2 hr with a Vickers image-splitting eyepiece, giving values reproducible to within ±0.1 μm.

The sensor of our current-measuring system is a vibrating probe with a spherical, 30-μm platinum-black-coated test electrode at its tip and a large reference electrode several millimeters behind this point. (The reference electrode is thus well out of the fields being studied.) The probe is vibrated at about 200 Hz in a horizontal plane between two extracellular points 30 μm apart. Fig. 1 shows a probe's tip vibrating in front of a growing two-cell embryo. Vibration between these points converts any steady voltage difference between them into a sinusoidal output with a peak-to-peak amplitude equal to that difference and measurable with the aid of a lock-in amplifier. Since the electric field will be nearly constant over this small distance, it is approximately equal to the voltage difference divided by this distance. The current density, i, in the direction of vibration and at the center of vibration, is then given by this field multiplied by the medium's conductivity. One main advantage of this system is provided by the low impedance and hence low noise of a platinum black electrode at 200 Hz; the other, by its self-stirring action, which practically eliminates any possible polyelectrolyte gradient and thus the diffusion potential that would be produced by such a gradient (8).

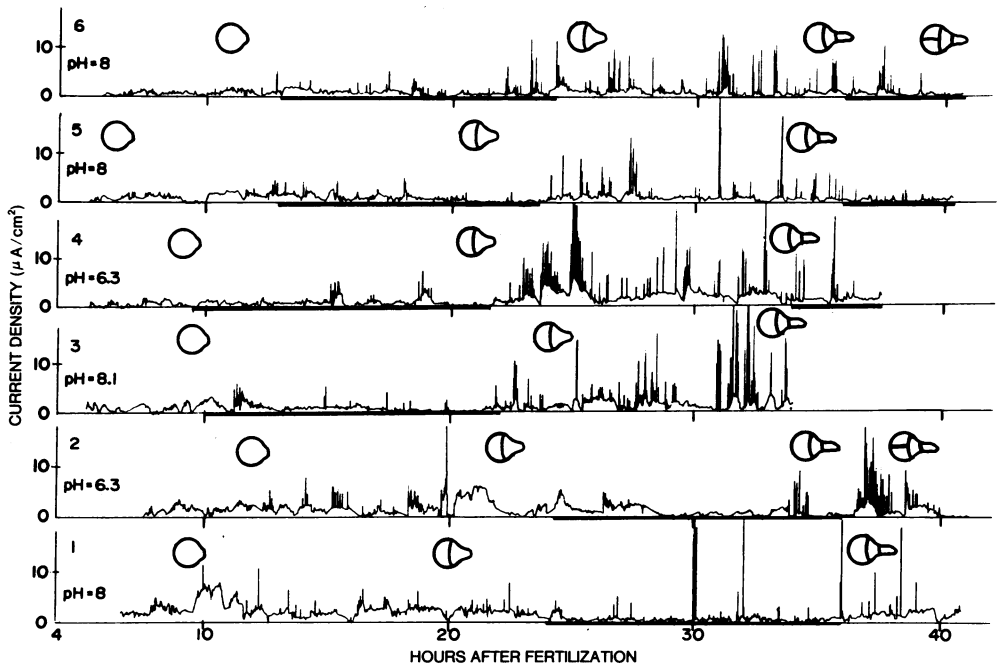

FIG. 5. Complete records of current traversing the growing tips of six separate *Pelvetia* embryos cultured under a 12-hr light-dark cycle. Ordinates: Current densities entering the growth tips in $\mu A/cm^2$. Abscissa: Hours after fertilization. The black bars indicate the dark periods, and the drawings show the embryos at the times of germination, first cell division, and second cell division.

In principle, the size and pattern of any transcellular currents can be obtained by extrapolating a group of extracellular current measurements to the cell's surface. In practice, the spontaneous current pulses reported here are too erratic for us to determine the variation of i with position during such pulses. However, very similar pulses can be stimulated by slight acidification of the medium, and a study of these stimulated pulses shows that the densest extracellular current is in front of the growing tip. Moreover, it shows that the axial current density falls off with the inverse cube of the distance from an apparent dipole center about 50 μm behind the growing point*. Accordingly, the present measurements of spontaneous current pulses (as well as those of relatively steady endogenous currents) were all made as in Fig. 1, i.e., with the probe's tip vibrating along the egg's axis and as close to the cell surface as was safe†. This put the center of vibration about 50 μm from the egg's plasma membrane and about 100 μm from its apparent dipole center. So the significant current densities, which are those at the plasma membrane, were inferred to be $(100\ \mu m/50\ \mu m)^3$ times the measured values; i.e., eight times greater. *All of the current density values given in this paper are thus eight times the measured ones.*

The rapid changes in current density found in a pulse could thus be measured with the probe in a single position, but steady current measurements require comparisons with a current-free reference position. So the probe was shifted to a point a few hundred μm out from its test position for a few minutes every 1–2 hr. For a control, we killed the egg in 2% glutaraldehyde after each experiment and again compared the potential at the test and reference positions.

* Using this inverse cube relation, we corrected for the small ($\leq$ 5%) errors introduced by assuming a constant field in the range of probe vibration.

† The smallest safe gap between the vibrating probe and the plasma membrane is about 20 μm. If the probe gets much closer, it may hit the intervening cell wall and yield artifactual signals.

RESULTS AND ANALYSIS

We have measured the current traversing the growth tips (or future growth tips) of six separate *Pelvetia* eggs in the period between 5 and 40 hr after fertilization. During this time the egg polarizes irreversibly, germinates or starts to grow, and divides at least once, forming a cross-wall between the rhizoid and the thallus cell. Throughout this period, a positive current was found to *enter* the growing tip (or prospective growth tip) and never to change direction.

As Fig. 2 illustrates, this growth current has both a pulse component and a relatively steady one. The pulses (shown on an expanded time scale in Fig. 3) rise to their peak current density of up to 10 or even 30 $\mu A/cm^2$ in 15–30 sec, then fall back to the starting level in another 60–90 sec. The pulses may be isolated (Fig. 2B) or may overlap to any degree up to near coincidence, or may even merge into a relatively steady current (Fig. 2C, D), though such ambiguous periods are only a minute fraction of the total.

The "steady" component also varies, going from 3–6 $\mu A/cm^2$ down to values of $<0.5\ \mu A/cm^2$ (although it varies 100 to 1000 times more slowly than the pulse component). As Fig. 4 shows, the embryo's growth rate consistently tends to increase with increases in the steady current density. It may also be noted that in four of the five plots, an extrapolation to zero growth yields a zero growth current value of about $0.5\ \mu A/cm^2$. There is some independent reason to suspect that the fifth plot was shifted by a so-called barrier artifact (8).

Fig. 5 illustrates both the pulse and the steady components of our total 205-hr record of spontaneous tip current, and Table 1 shows some summary values obtained from this record. We could not make measurements until five hours after

TABLE 1. *Currents entering growing tip of Pelvetia eggs**

Stage	Before growth† (Light)	One-cell stage		Two-cell stage		Dead‡ control
		Light	Dark	Light	Dark	
Growth rate (μm/hr)	0	3.1 ± 0.3	2.2 ± 0.3	2.0 ± 0.3	1.7 ± 0.6	0
Pulses per hour	0	1.5 ± 1	0.8 ± 0.7	5 ± 2	1.5 ± 1	0
Average pulse height§	0	4 ± 1	4 ± 1	7 ± 2	6 ± 3	0
Maximum pulse height§	0	12	12	27	10	0
Average i in pulses§	0	0.06 ± 0.03	0.06 ± 0.06	0.5 ± 0.2	0.3 ± 0.2	0
Average steady i§	0.9 ± 0.5	1.8 ± 0.4	0.8 ± 0.1	1.5 ± 0.5	0.7 ± 0.4	0.1 ± 0.1
Average total i§	0.9 ± 0.5	1.9 ± 0.4	0.9 ± 0.2	2.0 ± 0.7	1.0 ± 0.6	0.1 ± 0.1

* The indicated ranges are all standard deviations of the means of six experiments.
† During an average period of 4 hr before germination.
‡ Killed with 2% glutaraldehyde immediately after measurements on the live egg were ended.
§ In μA/cm^2.

fertilization because the eggs had not yet stuck tightly enough to the substratum. Between the first measurement and germination—a period that averaged four hours in duration—we recorded no pulses but did measure a steady current which averaged 0.9 μA/cm^2 in the light. Under this same illumination in the stage from germination to first division, the total average current density had risen to about 2 μA/cm^2. Pulses first appeared a few hours after germination and are thus included in this total, but they were initially so small and infrequent as to make up less than 5% of the total current in this one-cell stage. Under this same illumination in the two-cell stage, the total current still averaged 2 μA/cm^2, but the pulses had increased in both frequency and size so as to account for a fourth of it. In the dark the average steady current falls to half of the light value and is accompanied by a smaller fall in the growth rate. The pulse frequency also falls in half in the dark; however, the average size of the pulses appears to be independent of illumination.

Altogether, the 395 spontaneous pulses recorded in Fig. 3 varied continuously in peak height from a few giant pulses nearly 30 μA/cm^2 in amplitude down to ones just big enough to be clearly seen amidst the noise and less than 3 μA/cm^2 high. When we consider the obscuring effects of noise, we discern no significant dependence of total pulse duration, rise time, or fall time upon peak height over this 10-fold range. (It is as if amplitude were changed by just changing the ordinate's scale.)

Despite this large range, immediately successive pulses are often nearly equal in height. Indeed, "twin" and even "triplet" pulses‡ are a striking feature of the record, and a count shows that 37% of all the pulses were included in twins while 10% were included in triplets. Beyond this short time order, one sees an obvious tendency in Fig. 5 for the pulses to appear in larger groups which occur at apparently erratic intervals within which the frequency as well as the amplitudes tend to rise. Table 2 shows the characteristics of four particularly prominent pulse groups, all of which occurred in the light and in the two-cell stage. The pulse frequency within them was 4 to 6 times greater than the average frequency in the same light and stage.

‡ Twins and triplets are defined as successive pairs or trios that occur within a 30-min period and whose heights differ by less than one peak-to-peak noise value (about 2 μA/cm^2).

DISCUSSION

These first single-cell current measurements confirm the conclusion, drawn from population data (3, 5). that current enters the growth tip or prospective growth tip of the fucoid egg. They also confirm the rough proportionality between current density and elongation rate. Specifically, they show this relationship between the steady component of the current and the growth rate. This correlation supports our broad working hypothesis that local growth depends upon local current entry. The single cell measurements also confirm the (somewhat lucky!) estimate drawn from these data that the current density entering the growth tip is about 2 μA/cm^2 (4), and they also confirm the speculation suggested by the fluctuations in these data that "if measurements of single cell currents were possible, one should observe periods of little or no current flow alternating with ones of relatively large flow" and help confirm the associated speculation that "the membrane. . . undergo(es) episodes of depolarization" (ref. 3, p. 318).

With extracellular recording near single *Pelvetia* eggs we now, in fact, observe characteristic 100-sec-long pulses of current. Moreover, *intracellular* recordings in *Fucus* eggs show episodes of transient depolarization of 2–6 mV that have the same duration, shape, frequency, and twinning tendency as the externally recorded current pulses through *Pelvetia* eggs (10). So there is little doubt that they represent the same current flow.

Some of the pulse characteristics reveal information concerning the factors determining their time course. The intracellular depolarizations observed in *Fucus* have peak amplitudes of only 2–6 mV, and this change in membrane potential seems too small to control the time course of each pulse. Another and more compelling argument against membrane potential control of their time course is the fact that the pulses may overlap to any degree approaching coincidence.

TABLE 2. *Prominent pulse groups*

Exp.	Period (hr)	No. of pulses	Pulses per hr	% of all pulses
2	36.6–38.0	33	24	50
3	30.9–32.6	30	18	43
4	24.8–25.4	19	32	19
6	30.7–31.4	14	20	18

This overlapping strongly suggests that each pulse represents a separate event occurring in a different patch of the growth tip's membrane, since each pulse in an overlapping group still seems to have its own characteristic time course. This, in turn, argues strongly against membrane potential control since changes in membrane potential could only remain localized within one part of the growth region for less than a microsecond. We infer, rather, that their time course is controlled by some localizable change(s). These might include the changing concentration of some relatively immobile substance (calcium ion?), or the changing state (maturation?) of a patch of membrane derived from a recently inserted vesicle. We further infer that these local changes must involve the opening and closing of many ion channels or pumps in a membrane patch, since a single one could certainly not carry enough current.

Since the current pulses through these eggs do *not* seem to have their time courses controlled by changes in membrane potential, they indicate phenomena essentially different from the action potentials well known in nerve, muscle, and giant algal cells. If an analogous nomenclature were desired, we would suggest simply calling them *growth pulses*.

These growth pulses show striking similarities to ones recently observed along regenerating anucleate posterior stalk segments of *Acetabularia* (11). These segments are thread-shaped structures about 2-cm long by 0.03-cm wide. If kept in the dark for about a week they become depolarized, i.e., when they are then returned to the light they eventually resume growth and regenerate apical structures (but not a rhizoid) at the former apical end, the former rhizoid end, or occasionally at both ends. Such depolarized segments were placed in a chamber divided into 3 to 5 sea water compartments by well-greased partitions; illumination was begun, and the voltage between the end compartments was monitored for 50 hr. Eight to 10 hr after illumination began (and 36 hr before growth resumed at one end), a 0.1-V high pulse appeared, then reappeared at intervals throughout the study. These signals *always* indicated current pulses that enter the segment in the region that includes its future (or present) growth end and in this sense are in the same direction as those through fucoid eggs. Moreover, they have a remarkably similar duration, shape, and frequency. However, they do differ in never overlapping, in involving membrane potential shifts of >100 mV, and in having relatively constant amplitudes. These differences certainly suggest that the pulses through regenerating *Acetabularia* segments, unlike those through fucoid eggs, are under the relatively unlocalized voltage control typical of action potentials.

While the time course of a fucoid pulse seems to be under relatively localized control, its amplitude seems to be under some quite separate, more slowly changing, and less localized control. These inferences are suggested by several considerations. First, while the amplitudes of the current pulses vary over a 10-fold range—peak densities vary from 3 to 30 μA/cm^2—their time courses are independent of their amplitude. As we have commented above, it is as if amplitude were varied by changing the scale of the ordinate or current density coordinate. This clearly suggests that changes in one factor control the time course of a pulse while the level(s) of some quite separate factor(s) determine(s) its amplitude. Second, successive pairs (or even triplets) of pulses separated by 5- to 10- or even 30-min periods show a strong tendency to have nearly equal amplitudes. This would indicate that one supposed amplitude-setting factor(s) (chloride concentration?) may remain nearly constant over such periods. Third, when these twin (or triplet) pulses overlap, as they often do, this level-setting factor must have the same value over a region composed of at least two component regions with independent time course controls, and thus be relatively unlocalized.

Finally, we should consider the function of these current fluctuations. Our working hypothesis remains that the transcellular current produces a cytoplasmic field large enough to pull some critical components (such as wall-precursor vesicles) towards the growing membrane, and perhaps repel them from other regions of the surface. We would like to point out that if these key components were tethered by some weak bonds, then a fluctuating current might transiently establish a field strong enough to snap those bonds and move these components, while a steady current of the same average density might never exceed the required threshold.

This research was supported by the National Science Foundation. R. N. was supported by a U.S. Public Health Service predoctoral traineeship.

1. Rosenvinge, M. L. (1889) "Influence des agents exterieurs sur l'organisation polaire et dorsiventrale des plantes," *Rev. Gen. Bot.* **1**, 53–62, 123–27.
2. Whitaker, D. M. (1940) "Physical factors of growth," *Growth* **4**, 75–90.
3. Jaffe, L. F. (1968) "Localization in the developing *Fucus* egg and the general role of localizing currents," *Advan. Morphogenesis* **7**, 295–328.
4. Jaffe, L. F. (1969) "On the centripetal course of development, the *Fucus* egg, and self-electrophoresis," *Develop. Biol. Suppl.* **3**, 83–111.
5. Jaffe, L. F. (1966) "Electrical currents through the developing *Fucus* egg," *Proc. Nat. Acad. Sci. USA* **56**, 1102–1109.
6. Jaffe, L. F., Robinson, K. R. & Nuccitelli, R. (1974) "Local cation entry and self-electrophoresis as an intracellular localization mechanism," *Ann. N.Y. Acad. Sci.* **238**, 372–383.
7. Jaffe, L. F., Robinson, K. R. & Nuccitelli, R. (1974) "Transcellular currents and ion fluxes through developing fucoid eggs," in *Membrane Transport in Plants and Plant Organelles*, ed. Zimmerman, U. (Springer-Verlag, New York), in press.
8. Jaffe, L. F. & Nuccitelli, R. (1974) "An ultrasensitive vibrating probe for measuring steady extracellular currents," *J. Cell Biol.* **63**, 614–628.
9. Jaffe, L. F. & Neuscheler, W. (1969) "On the mutual polarization of nearby pairs of fucaceous eggs," *Develop. Biol.* **19**, 549–565.
10. Weisenseel, M. H. & Jaffe, L. F. (1974) "After microelectrode impalement the *Fucus* egg's membrane returns very slowly to a normal state," *Exp. Cell Res.*, in press.
11. Novák, B. & Bentrup, F. W. (1972) "An electrophysiological study of regeneration in *Acetabularia mediterranea*," *Planta* **108**, 227–244.