LARGE ELECTRICAL CURRENTS TRAVERSE GROWING POLLEN TUBES

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ABSTRACT

Using a newly developed vibrating electrode, we have explored the electric fields around lily pollen germinating in vitro. From these field measurements, we infer that each wetted pollen drives a steady current of a few hundred picoamperes through itself. Considered as a flow of positive ions, this current enters an ungerminated grain's prospective growth site and leaves its opposite end. After a grain germinates and forms a tube, this current enters most of the growing tube and leaves the whole grain. The current densities over both of these extended surface regions are relatively uniform, and the boundary zone, near the tube's base, is relatively narrow. This current continues as long as the tube grows, and even continues when elongation, as well as cytoplasmic streaming, are blocked by 1 μg/ml of cytochalasin B.

After an otherwise indistinguishable minority of tubes have grown to lengths of a millimeter or more, their current comes to include an endless train of discrete and characteristic current pulses as well as a steady component. These pulses are about 30 s long, never overlap, recur every 60–100 s, and seem to enter a region more restricted to the growing tip than the steady current's sink.

In most ways, the current through growing lily pollen resembles that known to flow through fucoid eggs.

Some time ago, and with the use of a rather specialized technique, it was shown that developing fucoid eggs drive large electrical currents through themselves (7, 8). With the recent emergence of a vibrating electrode system for measuring extracellular currents (9), it has become possible to investigate the currents which may traverse a variety of developing cells. As a first object for this survey, we have chosen germinating lily pollen. Germinating pollen grains and fucoid eggs undergo a somewhat similar development. Both of them form a long process by tip growth. But they are cells from completely unrelated organisms, and they grow in a radically different ionic milieu and at very different rates. Thus, lily pollen can grow optimally in a solution of low millimolar salt concentration, and the only necessary inorganic ions of such salts are K^+ and Ca^{++}, while fucoid eggs grow in seawater, a half-molar salt solution bearing six major inorganic ions. Moreover, lily pollen tubes elongate at 6–10 μm/min, and their cytoplasm streams at about 50 μm/min, while fucoid rhizoids elongate at 0.03–0.05 μm/min and show no detectable streaming at all.

In view of these developmental similarities coupled with physiological and essentially genetic differences, we wondered if there would be an endogenous transcellular current, and if so, to

what degree the currents through pollen would resemble those through fucoid eggs, for a sufficient similarity might suggest a causal and general role for these currents in initiating and maintaining tip growth. Indeed, we have advanced the more specific thesis that one important mechanism of developmental localization lies in local cation (particularly Ca^{++}) entry, which could constitute part of a current loop, and the resultant establishment of a cytoplasmic field which pulls critical constituents towards the cation entry region (10). Here, we will present some information about the net electrical current and also briefly discuss the calcium theory of localization.

MATERIALS AND METHODS

Pollen and Growth Medium

Throughout this study we used fresh lily pollen obtained from fully opened flowers of *Lilium longiflorum* cv. *Arai*. These flowers were grown under 16 h of light per day in a greenhouse.

Our standard growth medium, no. 12d, has the following composition, all in glass-distilled water: 290 mM mannitol, 2.0 mM $CaCl_2$ and 1.3 mM $Ca(OH)_2$ to give a total of 3.3 mM Ca^{++}, 1.0 mM KNO_3, and 3.9 mM H_3BO_3. It has a pH of 5.3–5.5 and is held there by the buffering action of the borate-mannitol complex. (This curious component has a pK of ~5 [4]). It has a specific resistance of 1,530–1,600 Ω-cm at the 20°C temperature used in our study. This medium is similar to those used by several previous investigators of pollen physiology (1, 2, 19). The main innovation was the use of mannitol instead of sucrose. This change has two advantages. It greatly reduces the danger of bacterial or fungal contamination and thus obviates the use of antibiotics. Also, by forming a buffering complex with borate, it makes the use of another buffer unnecessary. However, the recent report of Sfakiotakis et al. (19) suggests that this air-equilibrated medium contains a suboptimal CO_2 concentration.

Field Measurements

Our newly developed vibrating probe system for measuring extracellular currents has been described in detail (9). In brief, its "... sensor ... is a vibrating probe with a spherical, 20–30-μm platinum-black electrode at its tip which measures voltages with respect to a coaxial reference electrode .... The probe is vibrated at about 200 cycles per second in a horizontal plane between two extracellular points 30 μm apart. Vibration between these points converts any steady voltage difference between them into a sinusoidal output measurable with the aid of a lock-in amplifier tuned to the vibration frequency. 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 in the direction of vibration is then given by this field multiplied by the medium's conductivitiy" (18).

Fig. 1 shows a simplified view of the probe. In the original description (9), it was reported that this system sometimes registered artifactual signals (so-called barrier artifacts). Certain improvements have eliminated such artifacts in the present study, as is best shown by the complete lack of apparent currents around glutaraldehyde-fixed pollen tubes (Figs. 2 and 8). The main improvement is a shift of the platinum-black reference electrode farther back from the test electrode. It is now deposited on the lower face of the inner boundary setter (part 2 of Fig. 1).

The main technical problem encountered in this study was that of supporting the growing pollen tubes so that they would grow horizontally and not be moved by the flow in turn generated by the vibrating electrode. We solved this support problem with the aid of a thin (3-μm wet thickness), yet tough and ion-permeable cellulose membrane (part 3 of Fig. 1).[1]

Pollen is grown *under* this membrane while the bulk of the medium, as well as the vibrating probe, is *above* it. The membrane in turn is supported by an appropriate nickel screen. The surface tension relations are such that fluid lies under the unsupported parts of the membrane only in shallow sleeves which surround each pollen grain and tube. The surface forces exerted by these fluid sleeves, together with the apparent negative geotropism of the tube, combine to keep the tubes in contact with the membrane throughout almost all of their growth. Thus, they are kept horizontal as they grow, and are not moved by even the closest approach (short of contact) of the vibrating probe to the other side of the membrane.

We apply pollen to the membrane by spreading out dry pollen on a clean dry surface, blotting excess fluid off the lower surface of the screen-supported membrane, then brushing the dry and separated pollen grains onto this blotted surface. Within a few minutes after deposition of a grain on the membrane, enough fluid oozes through to swell and activate the grain.

The subsequent development of the pollen grain on the membrane closely resembles Iwanami's description of lily pollen development in vitro (5). The cytoplasm within the pollen tube quickly differentiates into two regions: a

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[1] This material was generously provided by Dr. Robert J. Petersen of the North Star Research and Development Institute, Minneapolis, Minn. He reports that it has a measured NaCl permeability which indicates an internal mobility of NaCl (or actually of Na^+ ions, since their movement seems to limit NaCl transport) about one-fifth that in water; a molecular weight cutoff of about 1,500 daltons; a two-ply construction which makes it practically free of pin holes; and consists of pure cellulose regenerated from cellulose acetate.
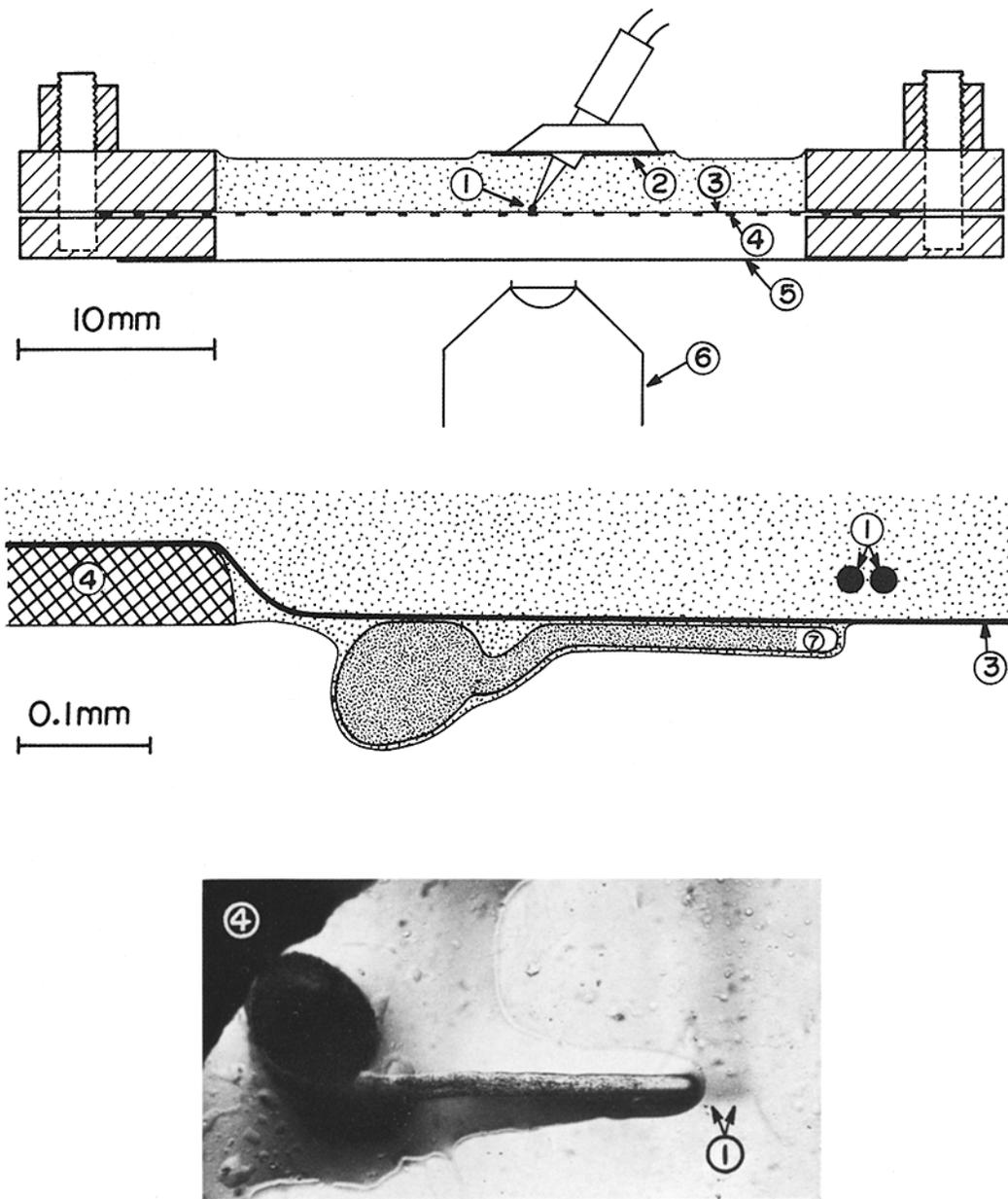
FIGURE 1 Membrane method of supporting growing pollen during field study. Top: Section of the measuring chamber. The probe is immersed in the 3-mm deep layer of medium lying above the membrane and vibrates perpendicular to the printed page. Middle: Magnified sectional view of one pollen tube growing under the membrane. Bottom: Photomicrograph of such a tube taken from below. (*1*) Vibrating test electrode. (*2*) Vibrating reference electrode. (*3*) Permeable, 3-μm thick, cellulose membrane.[1] (*4*) Nickel screen bearing closely spaced 1.4-mm diameter holes (Perforated Products Inc., Brookline, Mass.). (*5*) Cover slip which closes air-filled space under the membrane. (*6*) Objective of inverted microscope. (*7*) Clear cap behind tube's growing tip.

20-30-μm long terminal region (part 7, Fig. 1), variously called a "cap block" (5) or "clear cap" (3), and a subterminal region which extends from the clear cap to the grain. This clear cap region is also relatively refractile, and what particles can be seen within it show relatively little movement. By contrast, the coarse-grained subterminal region exhibits rapid streaming at rates of up to 50 μm/min or more. In the subregion just behind the clear cap, this streaming is of the reverse fountain type, i.e., towards the tip within a peripheral sleeve and back within a central stream. Farther back, this streaming often becomes asymmetrical, moving up one side and back the other. We studied no tubes old enough to have been blocked by callose plugs or grossly vacuolated.

A grain typically grows on a membrane at a steady 6-8 μm/min rate while lengthening from 0.2 to a few millimeters. (Other workers have reported steady-state growth rates of 10 μm/min and 6 μm/min for lily pollen in vitro [2, 15].) Both its initial and later growth tend to be somewhat slower.

The membrane blocks the probe from getting close enough to effectively explore the fields around ungerminated grains. To alleviate this problem, we cover the bottom of a glass dish with a thin layer ($\sim$10 μm thick) of silicone grease. Dry, isolated grains are sown on this surface and covered with a 3-mm deep layer of medium. The medium is changed several times to wash away loosely attached grains, and the field around the residual, well-attached grains is studied.

RESULTS

Steady Currents

AXIAL CURRENTS NEAR THE AXIS: We have explored the fields around approximately 200 growing pollen tubes. Every one of these, without any exception, had features indicating a large, steady current loop with current leaving the grain and entering the growing end.[2]

Fig. 2 presents a representative recording which shows these features. During this recording, the probe was vibrated parallel to the tube's axis in six positions slightly above this axis (as well as in a remote reference (REF) position). Thus we explored what may be called the axial component, E_z, of the field.

As the probe was moved from the REF position to position A, well in front of the growing tip, an easily measurable electric field appeared, indicating a current component moving towards the growing tip (and thus to the "right") with a density

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[2] We use the usual convention in which the current direction is that of positive charge movement.
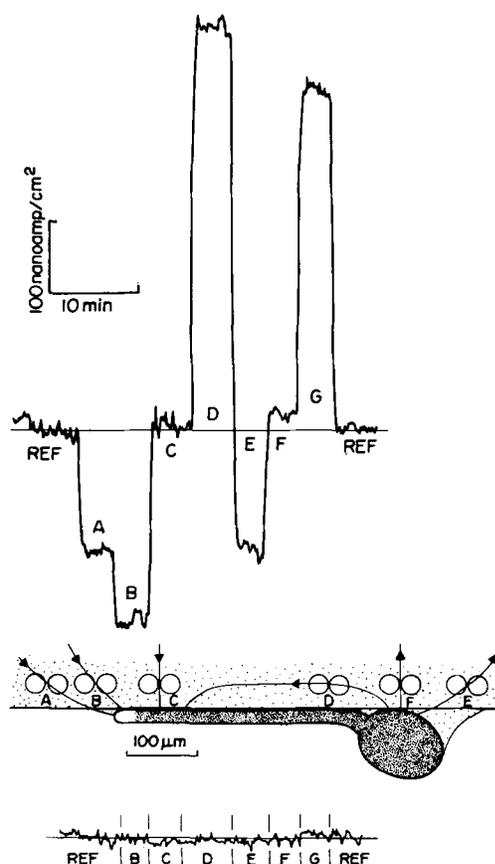
FIGURE 2 Representative recording of the axial field near a young, growing pollen tube and grain. Top: Actual recording; downward movement of the trace indicates rightward movement of the current in the diagram. Middle: Scale diagram of the pollen tube and grain in side view. Drawn near the end of the recording when the tube was 350 μm long; when the recording began, it was 150 μm long. It grew at 6-7 μm/min during the recording. The vibrating probe is represented by two circles. Pairs A-C are drawn to show their position with reference to the tip; D-F, the grain. Lines with arrows symbolize the inferred current. Bottom: Control recording from a similar tube after fixation in glutaraldehyde.

Probe vibrated at 205 Hz, with a 30-μm amplitude, and 37 μm above the membrane. System had a 10-s time constant. REF position 150 μm in front of and 300 μm to the side of the tip. 100 nA/cm^2 corresponds to a directly measured root mean square voltage of 164 nV.

of 170 nA/cm^2. As the probe was then moved rightwards to point B, E_z rose to a peak which indicated 270 nA/cm^2 of rightward current, rapidly fell to zero at position C, 60 μm behind the tip, and then reversed direction to indicate current

moving leftward and thus back towards the tip. Near the base of the tube, at D, ths left-ward current reaches a peak intensity of 570 nA/cm². The probe was then moved beyond the grain where E_z again changed direction to again indicate rightward current, thus current leaving the grain. At point E, this second rightward current reaches a peak value of 180 nA/cm². The probe was then moved back left to find the second null point at F, just above the grain. In this particular record, it was then moved to point G, 70 μm "behind" D (thus below the printed page in the diagram), and finally returned to the remote REF position.

The general features of the local currents inferred to underlie this recording are sketched in the middle section of Fig. 2, and a more exact picture of the whole current pattern inferred from a more extended study is shown in Fig. 7. In brief, current leaves the grain and enters the growing end of the tube. So the grain can be considered to be a "source" of the current, the growing end a "sink," and the whole system a "current dipole."

QUASINORMAL CURRENTS NEAR THE SURFACE: The ideal way of determining more precisely the pattern of current through the growing pollen's surface would be to explore the field normal and close to this surface. The necessary protective membrane precludes this. Nevertheless, as Fig. 3 shows, some further information was obtained by approaching this ideal as closely as the membrane allowed. These data confirm that the system's surface consists of one current source region along the grain's surface and one current sink region along the tube's surface; they also locate the boundary between source and sink regions relatively exactly. Repeated explorations of this sort yield a picture, shown in Fig. 4, of the movement of this boundary as a tube grows. It is seen to move forward during growth but at only a small fraction of the elongation rate.

Such explorations also suggest that the current density is fairly uniform over the source, with only a relatively short transition region between source and sink. However, there are serious uncertainties, particularly around the grain, in extrapolating from these measured current densities to those at the plasma membrane. Such data are therefore a rather poor basis for estimating the absolute value of the total current traversing the growing pollen system.

AXIAL CURRENTS THROUGH A BISECTING PLANE: Instead of using the data, we estimated this total current by integrating the current

returning through the plane—or rather halfplane above the tube's center—which bisects its dipolar field (Fig. 5). This was relatively easy since the field, E_z, normal to this surface, was found to fall off with the distance, r, from the tube's axis, according to a formula valid in the plane which
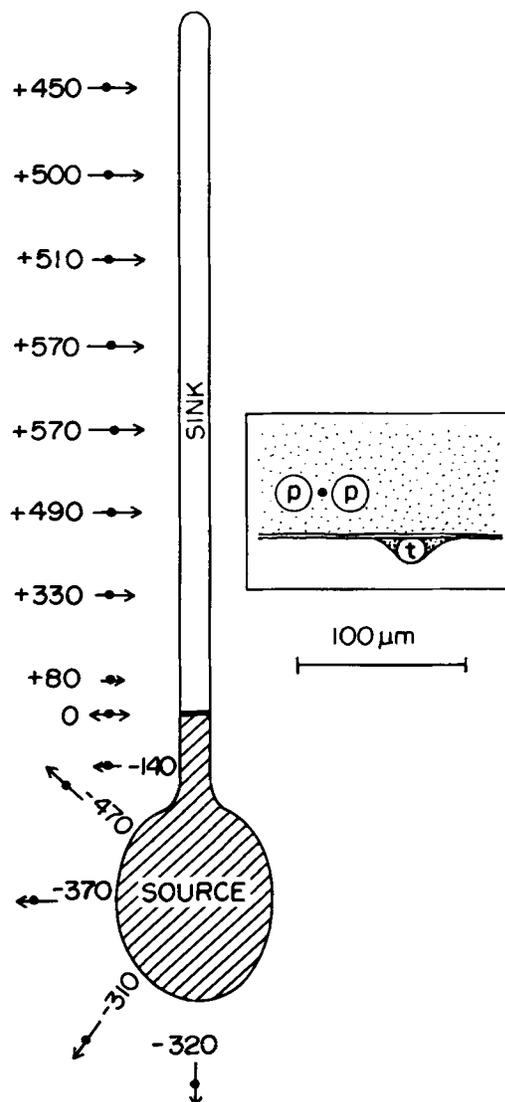

FIGURE 3 Representative exploration of the quasinormal field around a growing pollen tube. Each arrow indicates a current density value (in nanoamperes/square centimeter) measured in the indicated horizontal direction but in a plane 25 μm above the membrane. Insert diagrams the probe (P) vibrating about one of its measurement positions near the tube (t). Similar results were obtained from a total of four tubes at various stages from 100 to 600 μm long.
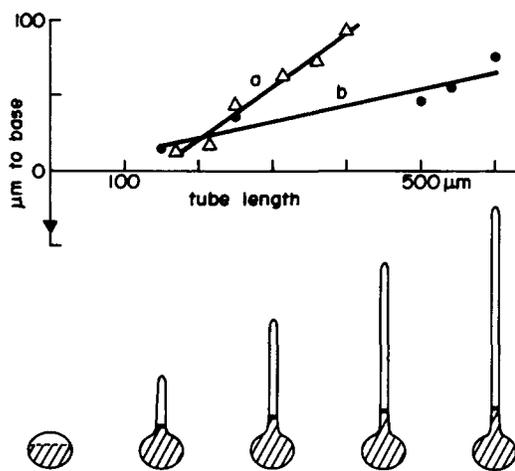
FIGURE 4 Movement of the boundary between the current source region at the grain end and the current sink region at the growing end of two pollen tubes (*a* and *b*). Abscissa, tube length, Ordinate, distance of the boundary from the tube's origin. Boundary positions in the tube are taken from repeated measurements exemplified in Fig. 3. The boundary's position in the ungerminated grain is based on observations exemplified in Fig. 8. The boundary's movement in case *b* is diagrammed below.

bisects a dipole made of one point source and one point sink:

$$E_z \propto (r^2 + H_2)^{-3/2} \qquad (1)$$

where *H* is this equivalent dipole's half length.

For the shorter tubes of 100–180 μm (which yielded the data shown in Fig. 5, left), this formula best fitted the data when *H* was taken to be 80 μm; for the 250–450-μm tubes which yielded Fig. 5 right, *H* was taken to be 85 μm. The 80-μm figure is easily understood, since the average half distance, *H'*, between these same short tubes' axial reversal levels, i.e. between points like C and F in Fig. 2, was actually *measured* as 80 μm. Among the longer tubes, *H'* averaged 140 μm which may seem discordant with the best fitting *H* value of 85 μm; but this can be understood by considering the fact, shown in Fig. 4, that the sink becomes much longer than the source in long tubes.

Once the validity of Eq. 1 was established, integration over the bisecting half plane was done as follows: First, it was used to obtain the axial current density, i_s, at the tube's surface, from a measurement of the axial current density just above the membrane. Then the total current *I*, was obtained by analytical integration:
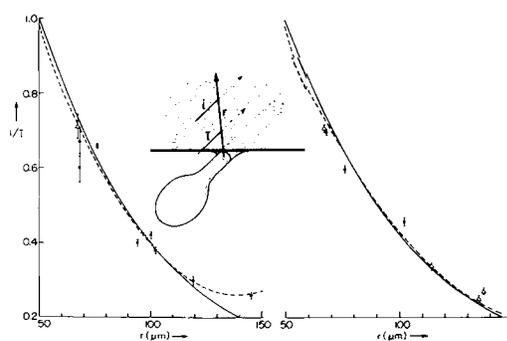

FIGURE 5 Decline of the axial field, hence current density with distance, *r*, from a tube. Insert: The plane in which these measurements were made. It was halfway between the tubes axial field reversal levels, thus halfway between points like C and F of Fig. 2. We measured the current densities, *i*, which pass through this plane. Left: Measurements on tubes 100–180 μm long. Right: Measurements on tubes 250–450 μm long. Abscissas, distances from the tube's axis in μm. Ordinates, relative current densities. All data points were obtained by repeated displacement of the probe between a standard point 50 μm above the tube's axis (to measure a standard current, *ī*) and a test point (to measure *i*). Circled data points were obtained from vertical displacements; triangled, from horizontal ones. Dashed lines are the least squares fit to the data with a second-order polynomial. The solid lines follow Eq. 1 for the field normal to the plane bisecting a point source and a point sink. The half lengths of these equivalent dipoles (80 μm [left] and 85 μm [right]) were selected to give curves which best fit the data.

$$I = i_s \int_a^\infty \left(\frac{r^2 + H^2}{a^2 + H^2}\right)^{-3/2} \cdot \pi r dr,$$

$$= i_s \cdot \pi(a^2 + H^2), \qquad (2)$$

where *a* is the tube's radius.[3]

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[3] This integration involves a simplification done to partially correct for the current through the sleeve of medium under the membrane. On the one hand, it falsely assumes that Eq. 1 holds within that aerial region which is up to one tube radius below the membrane *and* lateral to the medium sleeve. Obviously this should *overestimate* the current. On the other hand, it also assumes that Eq. 1 holds within that main part of the sleeve which is up to a tube radius below the membrane. This should *underestimate* the current since it should be constrained and concentrated within this latter region. Both errors should be relatively small since both regions have small cross sections, and their *balance* should be smaller still.
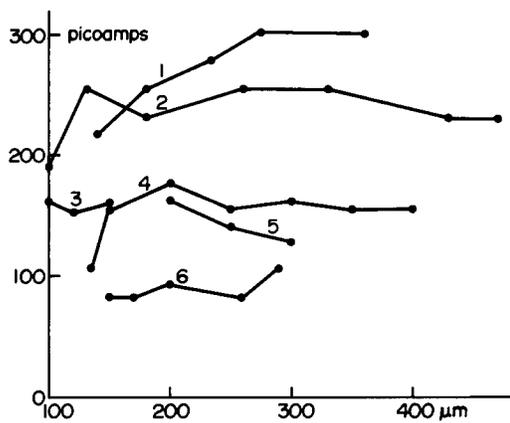
FIGURE 6 Total current through six growing pollen tubes. Abscissa, tube length in micrometers. Ordinate, total current in picoamperes.

Since H is 8–11 times greater than a,

$$I \cong i_s \cdot (\pi H^2). \qquad (2\ a)$$

The results of such determinations on six tubes at various stages from 100 to 470 μm are shown in Fig. 6. The total current is seen to vary considerably from tube to tube, to average about 200 pA, and to have some tendency to rise a bit as the tubes elongate.

OVERALL CURRENT PATTERN: It will be seen that we explored the field around growing pollen in three ways: (a) the axial field just above the axis (Fig. 2), (b) the quasinormal field just above the protective membrane (Fig. 3), and (c) the radial decline of the axial field in a bisecting plane (Fig. 5). By integrating such information, one can generate a picture of the whole current field both outside of and within a growing pollen tube (Fig. 7). We extended the current lines through the cell's interior by assuming that the internal conductance is uniform at the coarse level diagrammed and by using the well-known properties of "lines of force," viz, their continuity, their "elasticity" or tendency to have a minimal length, and their equally intense mutual "repulsion" or tendency to be evenly spaced (20, 21). (We were also guided by the fact that the tangential component [and hence the direction] of the current lines are usually discontinuous through the plasma membrane and by the additional fact that the current pattern within a battery-free region of uniform conductance [the cytoplasm] is uniquely

determined by the current pattern normal to its surface [the plasma membrane] [6].)

Our assumption of a uniform cytoplasmic conductance should be a good approximation since the conductance of the continuous cytoplasmic phase must be largely provided by the highly mobile and hence unlocalizable potassium ions,[4] while the patterns of cytoplasmic streaming within our relatively young pollen tubes seemed to preclude the existence of vacuoles or other inclusions gross enough to greatly distort their internal fields, except, perhaps, within the grain itself.

CURRENTS BEFORE GERMINATION: The discovery of a current through growing pollen tubes led us to explore the fields around wetted but still ungerminated grains. No attempt was made to explore current through grains wetted for less than 15 min. Limited explorations, which were begun 15–20 min after wetting, first showed no currents. Depending upon the batch, currents were first detected at 30–80 min. These earliest detected currents tended to be relatively small and somewhat shifty. So most explorations were done at 1–3 h when the currents were relatively large and stable.

All explorations were done by moving the vibrating electrode in (or somewhat above) the plane of a grain's equator as seen from below (Fig. 8, top). The field in this plane was first explored to find the points showing the highest densities of inward, outward, and tangential current. Then, more extended recording was done at these peak density points. Fig. 8 (middle) shows a representative recording at these points made near an ungerminated grain 2½ h after wetting. Current entered at C, left at A, and flowed back around the grain at B and D. That the inward current at C shows a characteristically greater density than the outward one at A indicates that the current sink was somewhat more concentrated than the current source.

6 h later, this same grain grew a tube close to the direction of greatest inward current density, as shown in the first drawing of Fig. 9. This same figure shows drawings of the relationship between the pregermination current direction and subsequent germination site in all the grains in which this was determined. In all 10 cases, the tube emerged quite near the site of densest inward current.

We find the concentration of potassium ions within young growing lily pollen to be about 0.1 M.

The absolute amount of current that we recorded varied considerably from grain to grain. Presumably, this variability resulted, in good part, from a variable tilt of the current dipole with respect to the horizontal plane. This assumption is supported by the fact that the tube usually emerged well above this plane, sometimes well below it, and only in rare cases well within it. The 12 largest inward current densities measured (which probably represented relatively horizontal and thus reliable dipoles) implied an average peak inward current density at the plasma membrane of 4 ± 0.3 μA/cm^2. This value was obtained by extrapolation using a simple formula appropriate for a spherical cell which generates a uniform internal current (12):

$$i_s = i(r/a)^3. \tag{3}$$

The total current traversing the grain may be estimated as 200 pA from this same model. To do this, we simply averaged the current densities at the protoplasts' two current poles, obtaining 2.9 μA/cm^2, and multiplied this figure by 65 × 10^{-8} cm^2 which is the average cross section of the protoplast.

CYTOCHALASIN EFFECTS: One may ask whether the rapid cytoplasmic streaming found in the pollen tube is somehow needed for generation of the electrical current through it. One way to answer this question would be to inhibit streaming with cytochalasin (3, 13). To do this, we used our standard medium supplemented with 1 μg/ml of cytochalasin B. (This drug was added via a di-

methyl sulfoxide (DMSO) solution to give a final DMSO concentration of 0.1%.) These additives change neither the pH nor the conductivity of the medium, while 0.1% DMSO alone changes neither the germination nor the growth of the pollen.

Five experiments with short (330–550 μm) tubes, one of which is shown in Fig. 10, all show the same result; streaming stops completely within 2–3 min of the moment when the exchange of medium above the membrane starts, yet the current's strength scarcely changes for up to 30–80 min.

Longitudinal growth also stops within a few minutes of adding cytochalasin, but the tip of the tube slowly swells (and eventually ruptures) in this medium. So cytochalasin cannot be said to immediately stop growth; rather, it seems to delocalize it. Thus, the continued current is apparently accompanied by continued, though delocalized, growth. As an accompaniment to this delocalization, we observed a slow lengthening of the terminal clear zone in three of the five experiments (Herth et al. have also noted this phenomenon [3]). It extended from a natural length of about 30 μm to one of about 100 μm within 2 h.

Current Pulses

PULSE TRAINS: When a pollen tube has grown to a length of about 1 mm or more, a new electrical phenomenon may suddenly appear. An endless train of spontaneous, monophasic pulses ride on top of the steady current. Fig. 11 (top) demonstrates the sudden onset of such pulsing while recording at position B of Fig. 2. We have
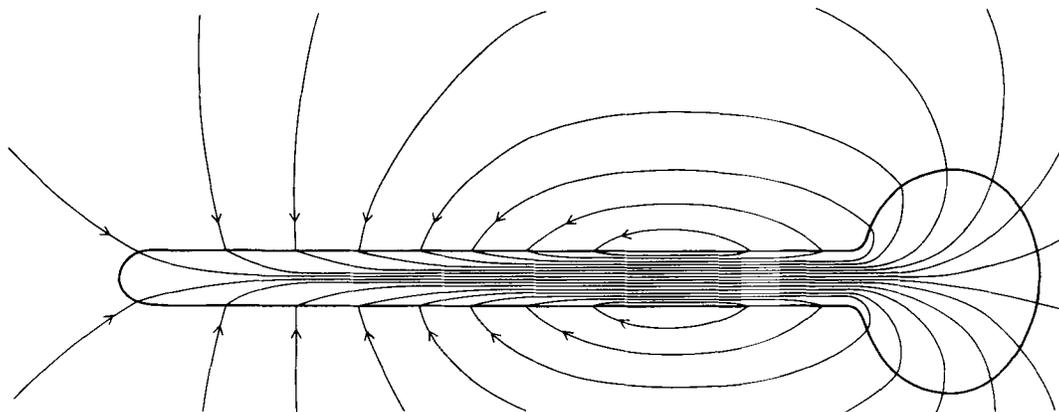
FIGURE 7   Pattern of current through a growing pollen tube. Note the concentration of current within the central region of the tube. A total of 200 pA concentrated within a 10-μm radius tube reaches the considerable density of 60 μA/cm^2.

found pulsing tubes as early as 3.3 h after sowing the pollen grains. However, the chances of finding such tubes increases in older tubes, so most of those studied were found between 5 and 7 h when the tubes are 1–3 mm long. During this stage, about 20% of the tubes are pulsing in some batches of pollen; in others, only a few percent. The growth rates of 11 pulsing tubes which were carefully investigated varied from 6 to 10 μm/min. Nonpulsing tubes during this stage seemed indistinguishable from pulsing ones in both growth rate and appearance under the light microscope.

FIGURE 9 Drawings of all ungerminated grains which grew out after current measurements began. Each dot represents the site (i.e., vibration center point) where the densest inward current (indicated by the arrow) had been measured before germination. At the site of germination, a short pollen tube is drawn depicting the shape and direction of the real outgrowth.
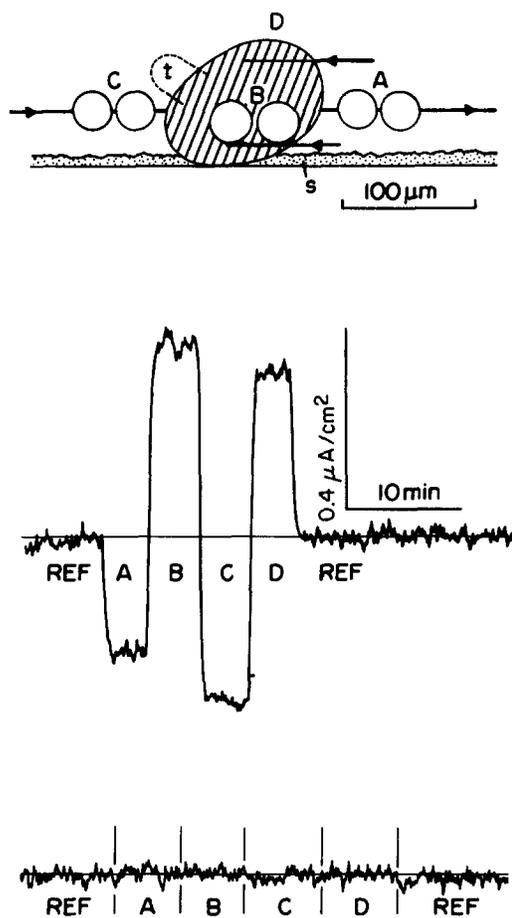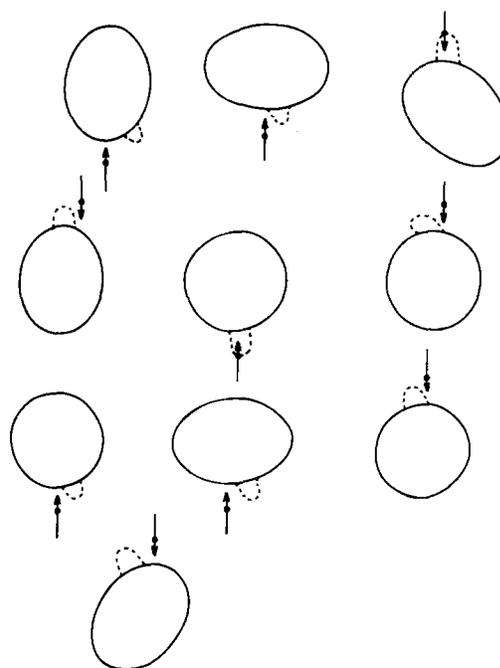

FIGURE 8 The electric field around an immersed but still ungerminated pollen grain. Top: Side view of the grain and the various probe positions. All are in the same horizontal plane. Position B is above the page; D, below it. Arrows indicate the current directions; t, the tube which emerged several hours later near C; s, silicone grease. Middle: Recording around this grain. Bottom: Recording around a similar grain after fixation in glutaraldehyde.

Once pulsing starts, it seems to continue until a tube dies. Pulsing was observed for as long a time as possible near 10 tubes. Four burst after 29–64 min of nearly uninterrupted spiking; in the other six, observation had to be stopped after 30–85 min of such pulsing. (Such observation is eventually cut off because the tube grows too close to the edge of the supporting screen hole or too close to other tubes). Fig. 11 (middle) shows a representative exploration of the axial component of current along the growing end of a long, pulsing tube, from 25 μm in front of it to 500 μm behind it. This pattern indicates that pulse current enters the terminal 30–100 μm of this 2-mm long tube, but gives no indicator of where pulse current leaves it. (Exploration closer to the grain, which might have yielded such information, was blocked by the nickel screen.)

It may also be noted that the pulse current's (axial component) pattern is shifted well in front of the steady current's pattern, reversing about 10 μm behind the tip instead of 100 μm behind it and

decreasing to the noise level at about 200–300 μm instead of 400–500 μm behind it. This would seem to indicate that the sink for pulse current is more concentrated towards the growing tip than is that for the steady current.

The record in Fig. 11 (top) shows 1.3 pulses/min and that in the middle, 1.1 pulses/min. The rate in a total of 11 records varied from 1.0 to 1.6 ml and averaged 1.3 pulses/min. In all of these records, there are occasional gaps in the otherwise regularly periodic pulse trains. These gaps are 1–2 or even 3 min long and follow relatively large pulses. The current densities at the peaks of the largest pulses are 4–7 times those of the steady current at the time. Finally, a typical train of pulses recorded with an expanded time scale (Fig. 11, bottom) shows that each pulse is about 30 s long, of nearly symmetrical shape, and clearly separated from the others.

ISOLATED PULSES: Some current measurements were made near pollen tubes growing in an otherwise standard medium acidified to pH 3.7–3.9, instead of the standard 5.3–5.5. Occasionally an isolated giant pulse entered the tip of such a tube, and four such cases were observed carefully. All four pulses were followed within 15 s by a cessation of longitudinal growth and a swelling of the tip. Then, growth resumed after 5, 5, 6, and 40 min with shifts of 0°, 45°, 30°, and 135°, respec-
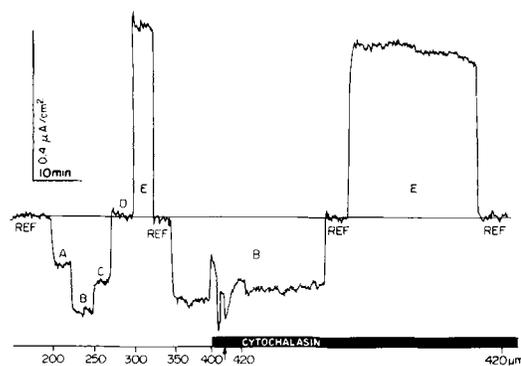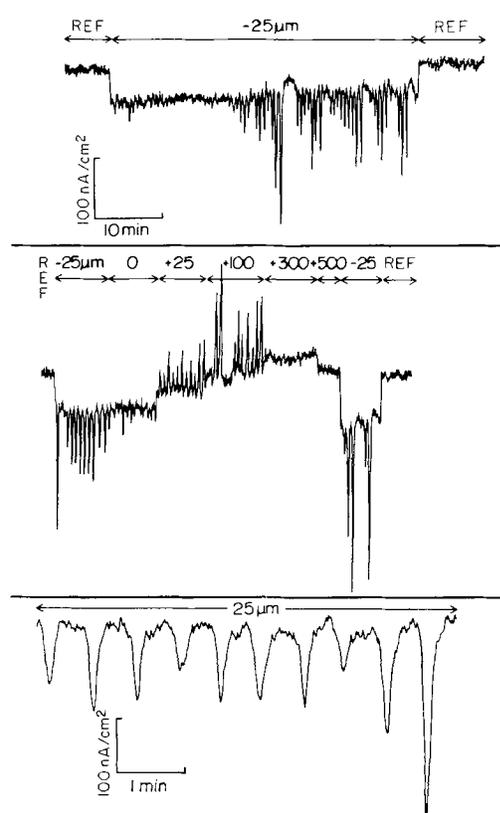

FIGURE 11 Representative recordings of current pulse trains near growing, 1–2-mm long pollen tubes. Top: Sudden onset of pulsing in front of a 1-mm long tube. Middle: Pattern of pulsing along a 2-mm long tube. Bottom: Pulsing recorded on an expanded time scale. Indicated test positions are in μm behind the growing tip, so minus means in front of it; REF, 200–300 μm in front of and 100–300 μm to the side of the tip. Other data as in Fig. 2 except for a 3-s time constant.

FIGURE 10 Representative recording of the effects of cytochalasin on the steady current. The regular medium was replaced by a 1 μg/ml cytochalasin B medium during the period indicated by the heavy bar. At the arrow all streaming had stopped. Apparent current fluctuations during and soon after medium exchange are believed to be artifacts. Probe positions in A, 100 μm in front, in B, 25 μm in front, in C, 50 μm behind, in D, 110 μm behind the tip; in E, 100 μm in front of the grain; REF 350 μm in front and 400 μm to the side of the tip. The tube's lengths are shown below. Other data as in Fig. 2.

tively, in growth direction. The steady endogenous current was *not* interrupted during these changes. Thus these pulses preceded episodes of growth delocalization which did not interrupt the steady current. They were about twice as long and three times as high as the largest ones found during the trains of pulses described above.

DISCUSSION

Developing lily pollen, like developing fucoid eggs, drive steady currents of the order of 100 pA through themselves. As in fucoid eggs, this current enters their growing ends and leaves their non-growing ends, is present well before growth starts, indicates where it will start, and continues as long

as growth continues. We have yet to see growth without current. Again, as in fucoid eggs, this steady current may be supplemented by large and repeated pulses of current which first appear some hours after growth starts and last on the order of a minute. Finally, it may be noted that application of 1 μg/ml of cytochalasin B completely stops streaming in lily pollen tubes without changing the steady current. Thus, steady current flow does not require streaming, and in a sense fucoid eggs may again be considered similar since they show no streaming even in natural seawater.

The field around lily pollen is easier to study than that around fucoid eggs since the standard pollen medium has only 1/60th the conductivity of seawater. We have therefore been able to find evidence that steady cureent enters the whole rather extended sink region along the growing pollen tubes with a rather uniform density, and likewise leaves the source region extending over the grain and tube base with a rather uniform density, with only a rather narrow transition zone left between sink and source regions (Fig. 3). This suggests that the membrane tends to differentiate into two discrete states: what is probably an ion-leaky state in the sink region and an ion-pumping one in the source region (11). The low conductivity of the pollen medium also made it possible to show that the last site of most intense current entry before germination is quite close to the site of outgrowth initiation some hours later. In these two regards, fucoid eggs may also prove similar when they can be studied.

In one regard, however, there is a distinct qualitative difference between the currents through lily pollen and those through fucoid eggs; the current pulses through pollen tubes never overlap (and, we may note, nor do the apparently similar voltage pulses across regenerating *Acetabularia* sections [16]). In sharp contrast, those through fucoid eggs frequently overlap (17). Such overlapping pulses must indicate partially coincident and hence somewhat independent episodes in different patches of the fucoid egg's membrane. Their absence in pollen tubes presumably reflects greater coordination between the parts of this system which in turn may be somehow related to its 140-fold faster growth rate.

Current enters almost the whole pollen tube uniformly and thus must accumulate within the cell to reach a maximum density, of about 60 μA/cm², at the tube's base (Fig. 7). This current density multiplied by the cytoplasmic resistivity

(which is probably a few hundred Ωcm) yields an estimated field within the tube's base of 10^{-2} V/cm. Most isolated cellular constituents have a negative charge, and a mobility of about 1 μm/s per V/cm in water. Thus, most of those constituents which are small enough and free enough to move through the cytoskeleton would be pulled forward at about 1 μm/min. In the absence of other flows, these inevitable electrophoretic movements might well have important physiological consequences. In fact, however, these electrophoretic movements are superimposed in this region of the cell on a filament-generated bulk streaming movement with velocities on the order of a 100 times faster. It is therfore difficult to imagine how the field produced by this current density could have much effect on the cell's growth, and we would therefore be inclined to view it only as an interesting epiphenomenon. Nevertheless, several considerations suggest that the pollen current, or really one of its components, acts to localize growth at the tube's tip. First, we have so far failed to find growth without current. Second, our unpublished work on the dependence of the current upon the ionic composition of the medium indicates that most of the inward current consists of potassium ions. Thus, the pattern of net current entry reported here only indicates that *potassium* ions enter the whole pollen tube uniformly. Autoradiography, on the other hand, indicates that *calcium* ion entry is concentrated at the tube's terminal 20–30 μm (11). Thus a calcium-generated field should be in the right place to first accumulate wall precursor vesicles near the growing membrane at the tip and then to fuse them with this membrane. Note that bulk cytoplasmic flow is *not* present in the terminal "cap block." Furthermore, even a relatively small calcium current is likely to produce larger cytoplasmic gradients and thus larger field effects (as well as larger chemical effects) than a potassium current (10, 11). Third, pollen tubes from three other species (*Antirrhinum, Narcissus*, and *Clivia*) are reported to start or curve towards a calcium source (14). Since curvature of a tip-growing system like a pollen tube is mediated by bulging rather than bowing, this means that local application of high calcium favors initiation or local expansion of these species' pollen tubes. However, any direct action of high calcium on their walls would be expected to *increase* their rigidity[5] (by cross-linking acidic

[5] The only systems in which this expectation has actually been tested are ones in which the cells elongate via

polysaccharides) and thus inhibit local expansion. Hence, local application of high calcium may well act to favor local expansion of these cells by crossing the plasma membrane to refocus entry of an endogenous current.

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relatively uniform, but anisotropic, expansion rather than localized growth; Dr. Robert Cleland (University of Washington, Seattle) tells us that experiments of D. Rayle and himself on oat coleoptiles as well as pea stems show no direct effects of increased external calcium on wall stiffness one way or the other.