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ELECTRICAL CONTROLS OF DEVELOPMENT

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> Wonderful as are the laws and phenomena of electricity when made evident to us in inorganic or dead matter, their interest can bear scarcely any comparison with that which attaches to the same force when connected with the nervous system and with life.
>
> Michael Faraday, 1839 (1)

INTRODUCTION

Scope

This review focuses on the natural, relatively steady electrical fields involved in the control of growth and development of cells and tissues. It is divided into two main inquiries. What natural electrical fields are inside or across cells and organisms and what the consequences are of modifying these fields. The emphasis is on relatively steady transcellular fields and field effects, as opposed to the transient fields normally associated with membrane potential changes. Among the important topics not covered are the following. (*a*) There are some important cases in which natural membrane potential changes control development. For example, the large reduction (or even reversal) of membrane potential that follows fertilization of the sea urchin egg somehow acts as the natural fast block to entry of additional sperm (2). (*b*) There is some information bearing on possible piezoelectric effects in bone development, as well as possible developmental effects of action potentials in animals and plants. This, too, is beyond our scope here. (*c*) Finally, we do not discuss the possible effects of electrostatic fields on organisms, but it appears to us that they involve entirely different factors than the dynamic fields to be discussed here.

History

As early as 1892, Wilhelm Roux had imposed electrical fields on a wide variety of animal eggs and observed gross stratifications of the cytoplasm in many cases (3). However, he found no consistent effects of these fields on development and made no comment on possible natural cellular electrical fields. The first investigator to propose that natural fields might influence growth was Albert Mathews in 1903 (4). He measured potential gradients along the surface of regenerating hydroids, which seemed to indicate that current entered the regenerating polyp end, and suggested that this current polarized the protoplasm or cells. However, the most significant and influential early investigations of developmental fields were surely those of E. J. Lund (5, 6). These studies, begun in about 1921 (5) and culminating in his book *Bioelectric Fields and Growth* in 1947 (6), seem to have stimulated a considerable number of contemporary investigators, such as Spek (7), Went (8), Burr (9), and Costello (10), and remain as substantial primary sources. However, this wave of interest ended in the late 1940s and there was a distinct decline in progress in this area until quite recently.[1] The revival of interest and investigation, combined with the new ideas and advances in electrophysiological techniques over the past 20 years, is likely to lead to a rapid increase in our knowledge in this area. Therefore we feel this is an appropriate time to review the older literature from a modern viewpoint, both to clarify what is already known and to point out the gaps in our understanding to guide future investigation.

The last general review in this area was done by Crane in 1950 (12), and more specialized reviews include those of Schrank (13) and Smith (14). Lukiewicz wrote an historical review (15) and Rosene wrote a comprehensive bibliography of the early work (see 6).

ENDOGENOUS CURRENTS AND FIELDS

Theory

Theoretical consideration of cytoplasmic fields has been dominated by Ohm's law. In continuous media this is given by

$$E = I\rho, \qquad 1.$$

where E is field strength, I is current density, and ρ is resistivity.

This simple formula should be essentially correct as long as the current involved is of an ion with relatively little tendency to bind to fixed charges in the cytoplasm. The spectrum of cytoplasmic binding tendencies among the six major inorganic physiological ions goes from K^+ and Cl^-, which have almost no tendency to bind to fixed charges in the cytoplasm, to Ca^{2+} and H^+, which bind very strongly. In between, but much closer to K^+ and Cl^-, lie Na^+ and Mg^{2+}. So Ohm's law should yield an excellent approximation for the cytoplasmic fields generated by K^+ and

[1]This recent revival of interest is indicated by the New York Academy of Sciences Symposium of Electrically Mediated Growth Mechanisms in Living Systems (11).

Cl^- currents and a reasonably good one for Na^+ and perhaps even Mg^{2+} currents but a grossly incorrect value for ones generated by Ca^{2+} and H^+ currents. A theoretical treatment of this problem appears elsewhere (16).

Methods for Measuring Electrical Currents and Fields Through Developing Systems

There have been three main approaches to the study of steady bioelectric fields and currents: (*a*) direct measurements of the field within (or across) the developing system; (*b*) measurements of the currents driven through its medium by the system; and (*c*) measurements of the voltage gradients along the surface of a system that has been removed from its natural medium.

(*a*) In direct field measurements, steady potentials across developing epithelia can be reliably measured with conventional KCl-filled micropipettes (cf Table 3). A few efforts have also been made to directly measure fields within the continuous cytoplasmic phase of cells with such microelectrodes (e.g. see 18). Uncertainties introduced by puncture injury currents as well as by tip potentials have generally rendered such measurements very questionable. An important exception, made possible by the system's favorable dimensions, is Woodruff & Telfer's apparently reliable measurements of the potential across the cytoplasmic bridge between the oocyte and its nurse cell in an insect follicle (19).

Grahm & Hertz determined the field developed across an aerial structure (actually the coleoptile) of growing plant seedlings by measuring the alternating voltage induced in a capacitor, separated by an air gap from the seedling, and vibrating (at 250 Hz) so as to vary this gap (20, 21). Their "method uses the well-known fact that if an electric field exists between the plates of a plane condensor and the distance between the condensor plates is changed (without changing the charge on the plates), a corresponding change is observed in the voltage across the condensor because of the change in its capacity." In this elegant way, the serious artifacts generally produced by contacting an aerial organ were entirely avoided.

(*b*) External current measurements have the important advantage of minimizing any disturbance of the system being studied; moreover the current that traverses the source system can be inferred from the current through its medium. Both static and vibrating electrodes have been used to measure voltage differences between various external points. Combined with a measurement of the resistance or resistivity, these yield the current or current density, respectively, between these points. Examples of the static method include measurements of the voltages at various points in an extended medium near a growing root (22) and of the voltage drop across a fine tube bearing several hundred developing fucoid eggs in series (23). External currents can also be measured by vibrating a platinum-black microelectrode near a cell or tissue (at a few hundred hertz) and measuring the voltage difference between the extremes of the probe's oscillation, with the aid of a lock-in amplifier tuned to the vibration frequency (24). Such a vibrating probe system has a signal-to-noise ratio about 1000 times that of comparable size static probes; this is because the effects of drift and of low-frequency noise generated at the probe's surface are filtered out and because probe impedance (hence thermal or Johnson noise) is radically reduced at the

frequency of vibration. A good example of this technique's power is the successful exploration of the currents around growing pollen tubes (25). Local current densities as small as 0.1 $\mu A/cm^2$ were easily measured with a spatial resolution of about 30 μm.

(*c*) The third and oldest approach to bioelectric field detection is via surface potential measurements. This technique measures neither the natural field nor current values because the electrode contacts the surface of the cell or tissue in a more or less unphysiological condition. It measures the voltage generated between two surface points by the extracellular current passing through the surface fluid layer. This voltage is maximized by maximizing the surface resistance, usually by drying the cell surface somewhat. Since the surface resistance is usually variable and not measured, the current magnitude cannot be calculated from these voltages, but only the current direction can be inferred. The degree of disturbance of the tissue depends both on the surface drying and the contact fluid in the electrodes. Often an unnatural contact fluid has been used, such as a strong solution of NaCl without the other ions found in the normal growth medium. Such unnatural contacts could grossly perturb the electrical behavior of the outer cell membrane beneath it. Examples of good surface potential technique can be found in Lund's work (6, 26).

In addition to the above, several methods have been used to measure specific ion currents through developing systems. One uses a vibrating probe to monitor the immediate effects of rapid changes in specific external ion concentrations on the current through a system (27). Two others use tracers. Local entry into individual cells may be detected with low-temperature radioautography of whole (unsectioned) cells in favorable cases (28), whereas local effluxes from, as well as influxes into, large populations of cells may be measured using the so-called nickel screen method of Robinson & Jaffe (29).

Measurements of Currents Through Single Plant Cells

The available measurements of the endogenous currents through single plant cells are summarized in Table 1. In all five cases, cellular elongation occurs in a localized region at one end of the cell, and in each case, with the possible exception of *Acetabularia,* current seems to enter the prospective or actual growth region. However, some uncertainties should be noted about the two oldest reports. The first is taken from Lund (6), who published three measurements of surface potential profiles along filaments of the fresh water alga, *Pithophora.* All three show the surface potential to be highest (i.e. most positive) somewhat behind the tip of the terminal cell (actually 0.2–0.6 mm behind it). These would seem to indicate external current flow towards the tip from this potential peak and therefore current entry into the tip. However, it is uncertain whether *Pithophora* will grow in the tapwater medium employed or even whether it shows the tip growth, which is typical, though not universal, in plant filaments. Case 2 is taken from the measurement of Slayman & Slayman (18), with conventional intracellular electrodes, of an enormous (600 mV/cm) distally positive voltage gradient in the terminal cell of a growing fungal filament. If reliable, this would indicate intense current flow into the growing tip; however, it seems possible that the relatively low membrane potentials measured

Table 1 Natural currents through single plant cells

Case	Cell type		Measurement method	Current characteristics	Inferred current direction at growth region	Current density ($\mu A/cm^2$)	Elongation rate (μ/hr)	Year	Ref.
1	*Pithophora* sp.	green alga apical filament	extracellular surface potential tapwater contact	steady	inward	–	–	1947	6
2	*Neurospora crassa*	fungal hypha	intracellular microelectrode	steady	inward	?[a]	3000	1962	18
3	*Acetabularia mediterranea*	green alga posterior stalk segment	extracellular current measurement	steady	variable	1–2	–	1972	30,31
				pulses	inward	10–20	–		
4	*Pelvetia fastigiata*	brown alga germinating egg	extracellular vibrating probe	steady pregermination	inward	2	–	1974–76	32–34
			^{45}Ca tracer	steady pregermination	inward	0.03	–	1975	29
			various	steady postgermination	inward	1–2	2	1966–75	23,32
			extracellular vibrating probe	pulse postgermination	inward	4	–	1974–75	32,35
5	*Lilium longiflorum*	lily germinating pollen grain	extracellular vibrating probe	steady pregermination postgermination	inward	4	6–10	1975	25
				steady	inward	1.3			
				pulse	inward	10			

[a]It is difficult to know how much of the measured potential difference is due to current from a fixed charge gradient or a lower apparent membrane potential at the relatively fine tip where the penetration injury leak could be greater. If the entire observed 600 mV/cm field were generated by a current loop, the intrahyphal resistivity of 300 Ωcm would indicate a density of 2000 $\mu A/cm^2$.

near the tip were the result of greater puncture injury there rather than a growth current.

In the three recently investigated cases, local current entry has been shown to precede and predict local growth. In both the unilaterally illuminated *Pelvetia* egg (29, 32–34) and the lily pollen grain (25) a relatively steady transcellular current begins hours before germination, i.e. before the initiation of local growth, and such growth begins at or close to the center of current entry. Similarly, enucleated stalk segments of *Acetabularia* may be morphologically depolarized by an appropriate pretreatment (with light), in the sense that their subsequent regeneration of a cap occurs with nearly equal frequency at either end. Thirty hours before cap generation begins in such segments strong 100-sec-long current pulses begin to enter one end; it is this end that then regenerates (30, 31). These facts suggest that local current entry somehow causes vesicle secretion and local growth, perhaps as a result of the current's Ca^{2+} component. There is growing evidence that Ca^{2+} influx stimulates vesicle secretion.

This inference is supported by evidence that calcium ions constitute a significant part of the current through *Pelvetia* eggs. First, tracer measurements on polarizing eggs directly indicate a calcium current (29). This current was actually largest when it could first be measured, at 6 hr after fertilization, and thus 1–2 hr before final commitment to growth in a particular direction. At this time it was estimated to be 2 pA per egg. If the current were uniform within the egg it would have had a density of about 0.03 $\mu A/cm^2$, about 5% of the total transcellular current. Second, despite this relatively small Ca^{2+} component, the early transcellular current is remarkably sensitive to the Ca^{2+} concentration: a threefold increase in external Ca^{2+} yields an immediate two- to threefold (and occasionally up to tenfold) increase in the whole transcellular current (R. Nuccitelli, manuscript in preparation). This strong Ca^{2+} dependence suggests that the relatively small calcium current establishes a calcium gradient that in turn acts to induce a steady current of other ions. Third, direct evidence of a calcium gradient across the ungerminated egg is still lacking, but low-temperature $^{45}Ca^{2+}$ autoradiography indicates that the total Ca^{2+} concentration is 40% higher in the growing end of the two-celled *Pelvetia* embryo than in the opposite end (37). The free calcium concentration may also be proportionally higher at this end. Furthermore, vibrating probe and tracer evidence indicate that the current pulses that appear after germination are each triggered by calcium entry (38). Thus, altogether there is little doubt that calcium ions are an important component of the steady current (as well as the pulsed current) through *Pelvetia* eggs.

It may be added that low-temperature $^{45}Ca^{2+}$ autoradiography also suggests that calcium ions are a significant component of the current entering the tips of growing pollen tubes (28). The relatively immediate responses of the pollen current to systematic changes in the concentration of various ions in the medium indicate that the bulk of the steady pollen tube current is carried inwards by K^+ ions (and outwards by H^+ ions) (27). Nevertheless, autoradiography shows striking accumulations of ^{45}Ca in the growing tips of lily pollen tubes, and this same result is obtained when the pollen is exposed to labeled medium for times as short as 1 min or as long as 5 hr. The 1- to 5-hr labeling experiments show that calcium is relatively concen-

trated within the cytoplasm of the growing tips and the 1- to 3-min labeling experiments suggest that calcium may enter the tip faster than it enters other regions. However, there is still doubt as to whether the short-time accumulations might not represent calcium absorbed on some special component of the tip wall rather than calcium that has crossed the tip plasma membrane into its cytoplasm (28).

Measurements of Transcellular Currents and Fields in Animal Eggs

The measurements of transcellular currents and fields made on animal eggs are summarized in Table 2. In compiling this data we included only those measurements made on one- or two-celled embryos. We chose not to include measurements on multicellular embryos at later stages of development (see 43–46) because of the complexity of these systems and the difficulty of interpreting such data. There are three very interesting observations apparent from this summary that deserve discussion. (*a*) In all four animal eggs studied between fertilization and first cleavage, a steady current was found to enter the animal pole while leaving the vegetal pole or equator. (*b*) In the silkmoth oocyte-nurse cell complex, or so-called ovarian follicle, the oocyte cytoplasm is 10 mV more positive than that of the nurse cell cytoplasm despite their connection by a broad cytoplasmic bridge. Moreover, as discussed later, there is evidence that maintenance of this remarkable potential difference is necessary for maintenance of unidirectional transport of macromolecules across the bridge and into the growing oocyte (19, 41). (*c*) A steady current enters the prospective cleavage furrow in both frog and sea urchin eggs during the 10 min prior to cleavage initiation, and about 8 min after initiation this current reverses and leaves the furrow region.

The most interesting and remarkably consistent result is the steady inward current at the animal pole of the two fish eggs, *Fundulus* and *Oryzias,* as well as the two amphibians, *Hynobius* and *Rana.* This observation by four independent investigators using both contact electrodes and the vibrating probe suggests that these animal-vegetable transcellular currents are both reliable and general. Thus it would be interesting indeed to learn whether or not these currents play a role in the determination of the animal-vegetal axis in these eggs. In this regard it is worth noting that in the ovarian follicles of the *Cecropia* moth Woodruff & Telfer have also measured a steady current entering the animal pole (41).

However, in these same follicles, intracellular measurements show a large voltage difference across the bridge(s) from the oocyte to the nurse cells, which implies current flow in the opposite direction through the bridge, i.e. towards the animal pole. We would like to suggest a possible explanation for this apparent paradox (Figure 1). We suggest, since the whole oocyte-nurse cell complex arises by the incomplete cleavage of a single precursor cell, that the membrane properties around each of the cells in this complex changes continuously and in the same way in going from pole to pole. Hence, current should leave the posterior or vegetal end of each nurse cell, cross the incomplete cleavage furrow that partially separates it from the oocyte, and then enter the anterior or animal end of the oocyte. We suggest that it is such furrow currents that make the animal end of the oocyte electropositive with regard to the vegetal end of the nurse cell and thus drive a back current through

Table 2 Natural currents (and fields) through developing animal cells

Case	Cell type		Measurement method	Contact medium	Inferred steady current direction	Surface current density ($\mu A/cm^2$)	Year	Ref.
Unphysiological[a]								
1	*Chrysamys*	turtle egg	extracellular surface potential cut camel hair	0.13 M NaCl	leaves animal pole, enters vegetal pole during maturation	–	1905	39
2	*Fundulus*	fish egg	extracellular surface potential	0.13 M NaCl	enters animal pole, leaves vegetal pole during 45 min before 1st cleavage	–	1905	39
3	*Rana* (?)	frog egg	extracellular surface potential	insufficient description	enters animal pole, leaves equator between fertilization and 1st cleavage	–	1941	9
Physiological								
4	*Hynobius nebulosus*	urodele egg	extracellular surface potential	pond water	enters animal pole and sperm entry point, leaves vegetal pole between fertilization and 1st cleavage	–	1935	40
5	*Oryzias latipes*	fish egg	extracellular vibrating probe	–	enters animal pole, leaves vegetal pole after fertilization during cytoplasmic segregation	1–5	1977	–[b]
6a	*Hyalophora cecropia*	silk moth oocyte-nurse cell complex	extracellular voltage across unknown resistance	–	enters animal pole, leaves vegetal pole	–	1974	41
6b	*Hyalophora cecropia*	silk moth oocyte-nurse cell complex	intracellular microelectrode	–	internal current flows across bridge from oocyte to nurse cells	–[c]	1973	19
7	*Strongelocentrotus purpuratus*	sea urchin egg	extracellular vibrating probe	–	enters prospective cleavage furrow 10 min before each cleavage	0.2	1977	–[d]
8	*Xenopus laevis*	frog egg	extracellular vibrating probe	–	enters as in sea urchin; leaves at furrow beginning 8 min after cleavage begins	0.5	1977	–[e]
9	Rat visual rod		extracellular voltage gradient (known resistance)	–	enters outer segment and leaves inner segment	60	1970	42

[a] We have not included the measurements of Dorfman (47) in this summary because he used gross 20- to 30-μm microelectrodes that permanently damaged the egg so that it could not be fertilized.

[b] R. Nuccitelli, manuscript in preparation.

[c] The oocyte cytoplasm was 10 mV more positive than the nurse cell cytoplasm. If this difference was entirely produced by the steady flow of a highly mobile ion, such as K^+, it can be estimated to correspond to a density of the order of 10^6 $\mu A/cm^2$ across the bridge (!) and the order of 1000 $\mu A/cm^2$ across the plasma membrane. To the extent that it arose from a calcium ion (or relatively immobile ion) flow it could correspond to far lower current densities.

[d] R. Nuccitelli and K. R. Robinson, manuscript in preparation.

[e] Robinson and Nuccitelli, manuscript in preparation.

the bridge. If this explanation is correct, it may indicate the more general development of back currents across the bridges that join cells that undergo incomplete cleavage across their axes.

The third interesting observation concerns recently found cleavage currents in both sea urchin and frog eggs. The first few cleavages in both of these eggs exhibit a similar current pattern: current enters the prospective cleavage furrow during the 10 min preceding cleavage initiation and then a few minutes after cleavage has begun there is a 5–10 min interval of reversed current leaving the furrow region. We suspect that the precleavage inward current may be involved in generating the contractile ring. The outward furrow current during cleavage may result from the insertion of new membrane that is highly potassium permeable. Such membrane insertion has been demonstrated (48, 49) based on membrane hyperpolarization during cleavage as measured with intracellular microelectrodes in *Rana* and *Amby-stoma.*

The last case in Table 2 is the dark current through rat visual rods. This is, of course, a mature, functioning, and highly differentiated cell. However, in an important sense it is also a developing one. Every 7–9 days, the entire outer segment is

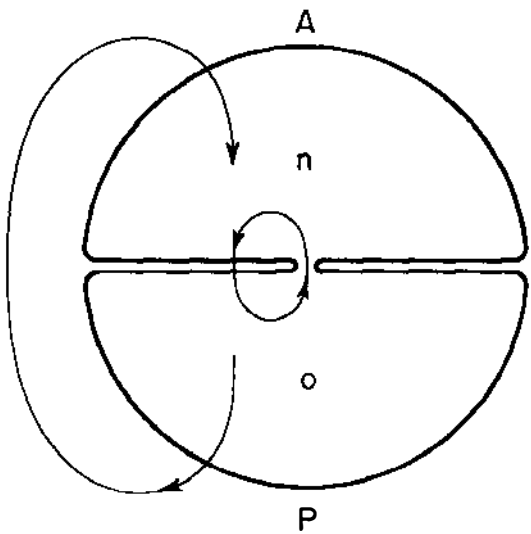

Figure 1 Proposed furrow current to explain Woodruff & Telfer's (41) observations on an oocyte-nurse cell complex. A diagrammatic complex containing only one nurse cell (n) plus the oocyte (o) is shown. The membrane of the complex is postulated to bear a gradient of properties going from the anterior pole (A), where current has the greatest tendency to enter, to the posterior pole (P), where it has the greatest tendency to leave. Incomplete furrowing thus juxtaposes membrane regions with markedly different properties and yields the internal furrow current loop shown.

apparently replaced by a process of continuing basal growth and apical degeneration (50). On the basis of the rod's dark current, shape, and internal conductance, Hagins et al (42) estimated a steady internal voltage drop of about 2 mV along the rod, with the distal end of the interior positive. However this may be a significant underestimate for reasons discussed above.

Potentials Across Developing Epithelia

The most obvious place large steady endogenous potentials exist across metazoan cells is in epithelia. Various mature, functioning epithelia are well known to maintain potentials of the order of 30–100 mV or more across themselves (51). To the extent that the charged components on the lateral membranes of these cells are free to move, the usual large transepithelial potentials would maintain them in a highly polarized distribution (95). However, one may well ask whether sufficient epithelial potentials to effect such redistribution appear while these epithelia are developing; thus, before their structures and functions are so grossly polarized, when the polarity of the epithelial cells may not yet be fixed, and membrane components may be more mobile. Table 3 summarizes the limited data available on potentials across epithelia quite early in development. In all cases the values tabulated were obtained at the earliest developmental state studied. The four potentials recorded lie between 3 and 8 mV, an order of magnitude lower than those typical of mature epithelia. Nevertheless they should be large enough to grossly polarize the distribution of some membrane components via laterial electrophoresis. We may add that Morrill et al (57) claim to find potential differences of about 40 mV (inside negative) across the frog's blastocoel wall but, within the limits of their measurements (a few millivolts?), Slack & Warner could find no measurable blastocoel potential in the frog at all (58).

In the best studied case, that of the chick chorioallantoic membrane, certain additional facts may be noted. First, according to Stewart & Terepka (53), during 6–10 days of incubation this potential increases in an exponential way from –3 to –18 mV. Second, even at 9–10 days this membrane still shows little sign of structural or functional polarization (53, 59). Third, dissociated and reaggregated cells undergo relatively organized development when explanted to this membrane (60), and Scott (61), who first measured the chorioallantoic potential, raised the interesting question of whether it helped organize the development of explants. Fourth, as its

Table 3 Potentials across developing epithelia

Case	Epithelium	Number of cell layers	Potential[a]	Year	Ref.
1	Coelenterate (*Obelia*) body wall	2	-1 to -3 mV	1925	26
2	1- to 2-day fish (*Fundulus*) gastrula	1	-7 ± 1 mV	1970	52
3	6-day chick chorioallantoic membrane	2	-3 mV	1969	53,54
4	Early mouse blastocyst wall	1	-5.0 ± 0.5 mV	1973	55
5	5-day rabbit blastocyst wall	1	-7.6 ± 0.6 mV	1969	56

[a]Negative sign means the inner region is electronegative.

name implies, this membrane is actually a compound structure including two separate epithelial layers, and a recent study of Kyriakides & Simkiss (54) indicates that, at least at a somewhat later stage, most of the potential is developed across the outer chorionic component and is very sensitive to the chloride ion concentration.

Natural Currents and Fields Across Higher Plant Organs

Using noncontacting vibrating electrodes, a Swedish group has shown that corn coleoptiles, which are stimulated to bend upward by horizontal placement, develop potential differences of 80 mV or more (!) across themselves, with their lower (and faster growing) side the positive one. These remarkable geoelectric potentials develop after a 15-min latency period and may last for an hour or more. It should be emphasized that unlike most surface potential measurements these directly indicate the natural potentials developed across the growing tissue (21, 62; also see 63). Very similar potentials accompany phototropic responses (64, 65). It seems clear that these geoelectric and photoelectric potentials are some consequence of a gravitation- or light-induced auxin gradient; the tissue becomes electropositive on the high auxin side. They begin with about the delay known necessary for auxin redistribution, are completely dependent upon a supply of auxin within the tissue, and can be simulated by application of an auxin gradient.

One may ask how an auxin gradient generates a field across the shoot. It develops rather slowly, with a half time of about 15 min, which might suggest the slow creation of an extracellular concentration gradient that in turn would generate a diffusion potential in the extracellular space. However, the final potential differences reached are so high—up to 90 mV—as to make such a mechanism unlikely. Presumably, cell membranes are more directly involved. There is a growing evidence that physiological increases in extracellular auxin somehow act to hyperpolarize plant cell membranes (66–68), though this hyperpolarization may not be a direct (i.e. electrogenic) effect but rather an indirect consequence of the secretion of hydrogen ions in turn induced by auxin (69, 70). We suggest that auxin gradients somehow induce fields by way of this hyperpolarization. This could imaginably occur in two ways: via the sum of currents loops induced in each of a series of electrically isolated cells or via one large current loop through a symplasm of electrically connected cells. The degree and direction of coupling between high plant cells is still quite obscure. (71).

Another piece in this puzzle is recent observations that slow calcium and potassium redistributions accompany the tropistic responses of corn coleoptiles (72). By an hour or two after stimulation, the faster growing side has 10–20% less calcium[2] and 10–15% more potassium on either a fresh or dry weight basis. The potassium is presumably largely intracellular and might well be driven in by a higher membrane potential on the faster growing side; however, the potentially more interesting calcium difference is harder to interpret since it could easily be either intracellular or extracellular.

[2] Note that the abstract of Goswami & Audus's paper (72) seems to have inadvertently implied that calcium is higher on the faster growing side.

A consistent pattern of surface potentials has been measured by several investigators on a variety of roots moistened with tap water or other physiological media, particularly onion roots (73, 74), broad bean and *Lupinus albus* roots (75), and maize roots (76). It can be inferred from these surface potential patterns that growing roots generally drive currents through their interiors from base to apex and that such acropetal currents flow through the terminal centimeter or so of the root. Longitudinal voltage differences of 30–60 mV were measured in these studies. Since roots can (and perhaps often do) grow in environments electrically comparable to those used during the measurements, these surface potential gradients (as well as the inferred inner fields) may be natural control factors (see Table 8, below).

More recently, Scott & Martin (77) have measured potential gradients along bean roots growing in more extended media, i.e. fully immersed rather than just moistened. This method makes it easier to estimate the size of the currents involved, and in various media total currents of 0.1–0.4 μA were inferred (77). The exact pattern of these currents within the roots is unknown; however, considering that their diameter is about a millimeter, internal longitudinal current densities could easily reach 10 $\mu a/cm^2$ or more. However these measurements were made on roots growing in unnatural, single salt solutions incompatible with extended growth, so the more or less aberrant current patterns found are of doubtful significance as natural growth controls. Other aspects of these studies, such as the 5-min oscillations of current density sometimes observed, are reviewed by Scott (78).

Natural Currents Through Regenerating Invertebrates

The literature shows a number of older reports of surface potential measurements on regenerating annelids and coelenterates. The results of these studies, which employed physiological contact fluids and are therefore relatively reliable, are summarized in Table 4. As an earthworm regenerates posterior segments, its posterior end remains relatively electropositive indicating that current is emerging from (or perhaps near) the caudal, regenerating surface (79, 80). Current also tends to emerge from the regenerating end or ends of a hydroid stem section. In all of these hydroid studies, it appears that a hydranth (rather than a stolon) was regenerating (81, 82).

The voltage measured across a caudally regenerating earthworm rises in proportion to the number of new segments rather than the absolute increase in the animal's length. Moment has interpreted this to mean that segment addition normally stops when a critical longitudinal voltage difference is reached (79). One difficulty with this idea is that the animal seems likely to be able to regenerate over a wide range of surface conductances and to generate a relatively constant current rather than a constant external voltage as this surface conductance is varied.

Natural Currents Through Regenerating or Healing Vertebrate Limbs, Skin, and Bone

The surface potential and current measurements made on regenerating vertebrate limbs as well as healing skin and bone are summarized in Table 5.

The pioneering investigation of the natural surface potentials along regenerating limbs was conducted by Monroy in 1941 (83). Although he used an unphysiological

Table 4 Natural currents through regenerating invertebrates

Group	Genus	Source of regenerate	What is regenerated	Inferred steady current direction	Year	Ref.
Annelids	*Eisenia*	anterior end	posterior segments	leaves regenerating end	1949–55	79, 80
Hydroids	*Obelia*	stem section	hydranth	leaves regenerating end	1921–25	5,26, 81
	Tubularia	stem section	hydranth	variable	1934	82
	Eudendrium	stem section	hydranth	leaves both regenerating ends	1934	82
	Pennaria	stem section	hydranth	leaves regenerating proximal end, but enters regenerating distal end	1934	82

contact solution of 0.8% NaCl, his finding that current leaves the regenerating stump and enters proximally is in agreement with the two other independent measurements, at least during the first 5 days after amputation. Moreover, he found by surgical denervation of the limb that this current was completely independent of the nerve supply. After determining that the current also persisted up to 24 hr after removing the heart, he concluded that the skin must be considered a possible source of the measured surface potentials. The most recent and reliable studies using the extracellular vibrating probe indicate that in fact the skin is driving this regeneration current (Borgens et al, manuscript in preparation). These first true current measurements confirm Monroy's finding that current consistently leaves the regenerating stump and the probe measures outward current for at least 10 days after amputation. This current is unaffected (or slightly increased) by surgical denervation and appears to be generated by the Na^+ pump in the skin since the current is greatly reduced in low Na^+ (less than 100 μM) and greatly increased by an increase in external Na^+ (8 mM), and because it is quickly shut off by application of 0.3 mM amiloride, a compound that blocks transcutaneous Na^+ uptake. Using this more reliable and physiological vibrating probe method, the 5-day-postamputation current reversal reported by Becker (84) was not observed, but rather the current continued to leave the entire blastema with the largest current in the postaxial region. About 10 days postamputation the current became erratic but did not exhibit a consistent reversal.

Interestingly, currents have also been measured leaving a lesion in human skin during healing (86, 87). It is extraordinary that no further investigation into this extremely intriguing finding has been done. Finally, healing bone fractures in frog, rabbit, and humans drive current into the periosteum and skin above the fracture (88, 89).

Table 5 Natural currents through regenerating vertebrate limbs, skin, and bone

Case	Organism		Measuring method	Inferred current direction	Year	Ref.
1	*Triturus cristatus*	regenerating newt limbs and tail	surface potential 0.8% NaCl contact	leaves regenerating end of limb or tail, enters proximal end throughout regeneration	1941	83
2	*Triturus viridescens*	regenerating newt limb	surface potential 0.8% NaCl contact	leaves regenerating end of limb, enters proximal end during first 5 days of regeneration, then reverses	1961	84
3	*Triturus viridescens*	regenerating newt limb	surface potential 10% Holtfreter contact	enters regenerating end of limb, leaves proximal end 10 to 18 days after amputation	1974	85
4	*Triturus viridescens*	regenerating newt limb	vibrating probe	30–100 $\mu A/cm^2$ leaves regenerating end of limb, enters proximal end and shoulder throughout regeneration	1977	-[a]
5	Human	healing skin lesions	insufficient data	leaves healing skin lesion, enters surrounding skin	1952 1972	86,87
6	New Zealand white rabbit	healing tibial fracture	surface potential isotonic saline contact	enters skin above fracture during healing period	1966	88
7	Human	healing tibial fracture	surface potential isotonic saline contact	enters skin above fracture during healing period	1966	88
8	*Rana pipiens*	healing tibial fracture	surface potential ringer contact	enters intact periosteum above fracture during 7 days after fracture	1970	89

[a] Borgens et al, manuscript in preparation.

EFFECTS OF MODIFYING NATURAL FIELDS

Theory

The direct target of steady field action on a single, relatively isodiametric cell should be the plasma membrane since practically all of the resistance across such cells is generally across this membrane. Thus, unless field strengths large enough to greatly reduce membrane resistance are involved, only a very small fraction of a steady voltage drop across a cell will be exerted across its interior. This point is most easily illustrated by considering a spherical cell such as an egg cell.

Consider such a cell placed in a conductive medium (e.g. sea water or serum) in a uniform field, E. If the resistivities of membrane and interior are uniform, then the field across the interior is known to be uniform, too (90). The voltage drop from pole to pole across the whole cell is given by

$$\overline{V} = 1.5\ Ed, \qquad 2.$$

where d is the cells diameter (90). The drop is divided between the resistances (per unit area) of the plasma membrane (σ) at the two poles and that of the cytoplasm in between. If the cytoplasm has a resistivity, ρ, then the resistance of a unit cross-section will be ρd. So the fraction of the total voltage drop across the cytoplasm (c) will be

$$f_c \leq \rho d/\sigma. \qquad 3.$$

Two relatively well-investigated cases are those of the sea urchin egg and the seaweed egg. For unfertilized eggs of the sea urchin, d is about 10^{-2} cm, ρ is 200 Ωcm (17), and σ is 150 kΩ cm^2 (L.A. Jaffe and K.R. Robinson, manuscript in preparation); hence, $f_c = 10^{-5}$. For fertilized *Fucus* eggs, d is 8×10^{-3} cm, ρ is 200 Ωcm, and σ is 1400 Ωcm^2 (91, 23); hence, $f_c = 10^{-3}$. We know of no (more or less isodiametric) cells in which f_c falls outside the range of 10^{-5}–10^{-3}.

Thus the most obvious mode of steady field action on cells is to act across the plasma membrane, i.e. by introducing a significant gradient in the membrane potential around the target cell. Since the interior will be essentially equipotential, this gradient in membrane potential will depend only upon the gradient external to the membrane. For a highly resistant sphere in an otherwise uniform field, the potential just outside of it is given by

$$V = 1.5\ D \cos\theta, \qquad 4.$$

where θ is the sphere's latitude (90). If the membrane does not actively respond to the field, the preexisting and imposed potentials will simply add to give

$$V_m = \bar{V}_m + 1.5\ E \cos\theta, \qquad 5.$$

where $\bar{V}_m$ is the membrane potential before imposition of the field. (Note that equation 5 does not require membrane resistance to be uniform; it need only be large compared to that of the cytoplasm to keep the cytoplasm equipotential, as well as large compared to the medium to make the cell as a whole relatively resistant.)

Provided that relatively small fields are employed (i.e. fields evoking essentially linear responses below the region of inevitable saturation) the degree of growth polarization to be expected should be about

$$P = 1.5\ Ed/\bar{V}_m, \qquad 6.$$

where P is defined as the average cosine of a population of cell outgrowth directions. The reason for suggesting this simple equation lies in the empirical rule that governs the polarization of cell growth by vectors (such as light gradients and chemical concentration gradients) whose size can be meaningfully given by a percentage (i.e. fraction) of difference per cell: the percentage of growth polarization approximates the percentage imposed gradient (92). Thus for cells with typical membrane potentials of about 50–100 mV, one-tenth maximal growth polarization should be induced by a potential drop across the target cell of 5–10 mV.

The same gradient in extracellular potential given by equation 4 will exert forces along the outside of the target cell membrane as well as across it. Many molecules and particles are freer to move along the outer membrane of cells than

out of this membrane (93, 94). Since many of these components are charged, it follows that application of tangential electrical forces to cells should redistribute them within the plane of the plasma membrane. This process may be called surface, lateral, or perhaps perimembrane electrophoresis (95).

The degree of redistribution effected in this way should ultimately be limited by back diffusion. Eventually an equilibrium, or more accurately a steady state between electrophoresis and back diffusion, may be reached. The time needed to reach a steady state will be the order of L^2/D, where L is the characteristic cell length and D is the affected components diffusion constant. Such constants have been measured in a number of cases and generally are in the range from 5×10^{-11} to 5×10^{-9} cm^2/sec (93, 94). Most relatively isodiametric target cells will have diameters of the orders of 10^{-3}–10^{-2} cm, so steady-state times will generally be of the order of 10^2–10^6 sec. Endogenous developmental fields are likely to be applied to target cells for the order of 10^4–10^6 sec, whereas most experiments have employed treatments with small fields for the order of 10^4–10^5 sec. It can be concluded that steady-state equations may be relevant in many though certainly not all real cases. In any case, such steady-state equations involve the ratio of the target component's electrophoretic mobility (m) to its diffusion constant (D), rather than D alone. Since this mobility-to-diffusion ratio, m/D, can be better estimated than can D itself, the steady-state equations seem far more useful at this stage of investigation. The following discussion is condensed from Jaffe (95).

Consider a component in the steady state on the surface of a spherical cell subjected to a uniform field. It can be shown that the surface concentration, C, is given by

$$C = \overline{C}\,[\epsilon \cdot \operatorname{csch}(\epsilon)] \cdot \exp(\epsilon \cos\theta), \qquad 7.$$

where $\overline{C}$ = the target particles' average concentration and $\epsilon = (m/D) \cdot V/2$.

For comparison with cell galvanotropic responses (or perhaps other directed responses) it is helpful to characterize the degree of polarization of such steady-state distributions by some parameter, ϕ. Since the tropic responses of cell populations are commonly characterized by the average cosine of the distribution of outgrowth angles (96), we define in an analogous way:

$$\phi = \mathrm{C} \cdot \cos\theta\ \mathrm{ds}/\ \mathrm{Cds}. \qquad 8.$$

Integration of equation 7 yields[3]

$$\phi = \operatorname{ctnh}(\epsilon) - \epsilon^{-1}, \qquad 8a.$$

and for $\epsilon < 1$ is usefully approximated by

$$\phi = (m/D) \cdot (V/6) \qquad 8b.$$

[3] Dr. William Hagins has pointed out an interesting fact. This expression is formally identical with the so-called "Langevin function, $L(x)$," which indicates the degree of polarization of a population of electrical dipoles at equilibrium in a static electrical field (97).

To apply this equation, we must estimate (m/D) for the target particles. It can be shown that this ratio is independent of the hydrodynamic drag they encounter (95). Hence we can use the values known for particles immersed in the usual one centipoise, 0.05 M ionic strength, aqueous media. Somewhat, arbitrarily we assume a mobility value of 3×10^{-4} cm^2/sec • volt, which is towards the high end of known mobilities, and a particle diameter of 10 nm since the larger particles seen in freeze-fracture electron micrographs of plasma membranes are of about that size.[4] Particles of that size, etc, have a diffusion constant of about 4×10^{-7} cm^2/sec. So, together these assumptions yield a D/m value for the growth particles of 1.3 mV. Then applying equation 8b with $\phi = 0.1$, we infer that 1/10 maximal polarization should be induced by a voltage drop of only 1 mV/cell. Note that this characteristic value (for a field effect mediated by perimembrane electrophoresis) is an order of magnitude smaller than that expected for one mediated by a gradient in membrane potential.

A simple but important point to note is that both cross-membrane and perimembrane effects should usually depend upon the voltage difference across a target cell rather than the field, i.e. the voltage difference per unit length. In other words one generally expects larger cells to respond to correspondingly smaller fields.

Strictly speaking, the above theory holds only for spherical cells, and gross deviations are expected for the responses to fields directed along extremely elongated cells (such as neurons) in which the cytoplasmic resistance can become large relative to the membrane resistance. Consider an idealized neurite of radius, a, exposed to a uniform longitudinal field. The internal field E_i must approach the external one, E_o, as the neurite length approaches infinity. For if it did not, the imposed voltage drop across the membrane would approach infinity and that is obviously false. A more detailed analysis shows that

$$E_i = E_o\ (1 - \exp\ (-x/\lambda)), \qquad 9.$$

where λ is the length constant of this cable equal to $\sqrt{a\sigma/2\rho}$, and x is the distance from its closed end. In reality, developing neurites have a highly dilated and convoluted growth cone at their tips. The large surface area of this structure should make a real neurite even more penetrable by an external field than equation 9 indicates. Altogether, external fields could well effect lateral transport of charged particles protruding from the inner side of the plasma membrane of growing neurites; indeed external fields might even move charged components suspended in the cytoplasm of such cells.

Finally, in field effects upon tissues (as opposed to single cells) one must consider extracellular targets. It has been suggested that bioelectric fields may sometimes act by orienting extracellular, growth-guiding filaments (98). However, studies on electrically induced birefringence indicate that fields of the order of 300–3000 V/cm are

[4] Since mobilities are independent of a particle's radius while diffusion constants vary inversely with this radius, r, the expected D/m values would be multiplied by 5 nm/r were r in fact to differ from 5 nm.

generally needed to half-orient populations of various highly elongated biological particles, e.g. 0.9-μm-long collagen molecules require 4000 V/cm, and 0.3-μm DNA require 500 V/cm (99–101). One exceptionally low value of less than 50 V/cm has been reported for aggregates of 1- to 10-μm (?) actin filaments (102). Nevertheless these fields are the order of 100 times or more greater than those either known or theoretically expected to affect cells more directly, and they similarly exceed the natural fields that may be expected in the extracellular regions of tissues. So this is one mechanism that can be generally considered to be highly unlikely. On the other hand, there is no reason why significant electrophoresis may not sometimes occur within extracellular regions.

Electrical Activation of Eggs

According to Yamamoto (103), unfertilized eggs of the medaka (a fresh water fish, *Oryzias latipes*) can be artificially activated by a field of 2 V/cm or more applied for 20–60 sec. Activation occurs at both the anodal (i.e. positive) and cathodal poles but the reaction time is half as great at the anodal pole as at the cathodal one. A field of 2 V/cm should produce a voltage drop of 360 mV across these 1.2-mm-diameter eggs. Initially at least this should hyperpolarize the membrane potential at the anodal pole by 180 mV and depolarize it at the cathodal one by 180 mV. An explosive increase in free cytoplasmic calcium is known to be a central event in the activation of these eggs (104), so it is reasonable to guess that anodal activation occurs by driving calcium into the egg; but how is cathodal activation mediated? Sea urchin eggs can also be anodally activated (at least locally) by a field that should hyperpolarize the membrane at the positive pole by about 200 mV; however, the field only seems to require a few seconds to act in this case (105). Finally, we may note that hamster ova can also be activated by an electrical field, albeit a poorly defined and apparently extreme one (106).

Direct Evidence of Lateral Electrophoresis

Before reviewing low- and moderate-field effects on cell development, we summarize what seems to be direct evidence of the predicted phenomenon of lateral or peri-membrane electrophoresis (107). Cultured embryonic *Xenopus* muscle cells were exposed to small fields for periods of 0.5 hr to 1 day. Then they were labeled with fluorescein-conjugated concanavalin A and examined with a fluorescent microscope. A striking redistribution of concanavalin A receptors can be seen after sufficient time in a sufficient field. Thus exposure of the 30-μm-wide cells to a voltage difference across them of 12 mV for 2 hr suffices to produce a gross accumulation of concanavalin A receptors on the negative side of the cell. A tenth this voltage difference (thus 1.2 mV) or a quarter of this time (thus 0.5 hr) gives detectible redistributions in the same direction. These would seem to imply that the receptors have a positive charge, which is quite surprising. Nevertheless, the facts leave relatively little doubt that the observed redistribution is brought about directly, by lateral electrophoresis, rather than some more complex and active mechanism. For one thing, the process goes at about the rate to be expected of lateral electrophoresis, namely the order of 10^{-7} cm/sec per V/cm, which is several orders of magnitude slower than the rates

known for the process of active redistribution called *capping* (108). For another, a voltage drop of less than a millivolt per cell gives detectible redistribution. As discussed above, this easily fits the expectation for lateral electrophoresis but not for one mediated by a gradient of membrane potential. For yet another, it is not affected by either high concentrations (5 μM) of colchicine or by reduction of external calcium from 2 mM to contamination levels of perhaps 1 to 10 μM. Most important, it is unaffected by treatment with a combination of metabolic inhibitors (10 mM arsenate, plus 1 mM dinitrophenol, plus 1 mM NaF applied for 2–5 hr) that would be expected to reduce cytoplasmic ATP to very low levels and is in fact observed to block the contractile response of these cells to the acetylcholine analogue, carbachol.

Field and Ion Gradient Effects on Plant Cells

Table 6 [modified from (109)] summarizes the sparse literature on single plant cell responses to prolonged, steady electrical fields. It shows the recorded responses falling into two distinct classes: a low-voltage and a medium-voltage group.

The low-voltage responses require about 0.2–0.4 mV/cell (to yield a tenth maximal response) and involve three different cells each no more than 10 μm in diameter. The medium-voltage ones require about ten times this voltage, namely about 3–6 mV/cell, and involve six much larger cells, actually cells from 70–10,000 μm in diameter (or length). The low-voltage responses may well be mediated by perimem-

Table 6 Voltage giving tenth maximal response in known cases of single plant cell galvanotropism[a]

Case[b]	Cell diameter (μm)	Genus	Cell type	Exposure time (hr)	Response	Direction	Millivolts/cell
1	~10,000[c]	*Acetabularia*	stalk segment	45	cap regenerates	+	a few
2	~1,000	*Griffithsia*	shoot	70	rhizoid starts	+	~4
3a	100	*Pelvetia*	egg	12	rhizoid starts	+	6
3b	100	*Pelvetia*	egg	12	rhizoid starts	–	3
4	70	*Fucus*	egg	10	rhizoid starts	–	4
5	65	*Equisetum*	spore	10	rhizoid starts	+	5
6	~100	*Narcissus*	pollen grain	~5	tube starts	–	~5
7	10	*Fucus*	rhizoid	3	curved growth	+	~0.3
8	9	*Ulva*	egg	12	rhizoid starts	+	0.4
9	6	*Funaria*	chloronema	3	curved growth	–	~0.2

[a] In cases 2, 3, 4, and 7 the tenth maximal response is taken as one where the average cosine of the outgrowth directions is 0.1; in cases 6 and 8, where the outgrowth curved by 10°; and in cases 1 and 5, where the response is just detectible.

[b] Case 1 is from Novak & Bentrup (30); 2 is from Schechter (110); 3 from Peng & Jaffe (109); 4, 5, 7, and 9 from Bentrup (111), Figures 5, 6 (*E. limosum*), and 12, and Table 4, respectively; 6 from Zeijlemaker (112); 8 from Sand (113) (wild type).

[c] Cell length, not diameter, in this case.

brane electrophoresis, as discussed above. That is, the tangential component of the electric field, along the periphery of the cell, could move certain mobile, growth-controlling plasma membrane components that 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 medium-voltage responses, on the other hand, involve voltage differences large enough to act across the plasma membrane, i.e. large enough to introduce a significant gradient in the membrane potential around each cell. Furthermore, according to Novak & Bentrup (114), 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 directions 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 tentatively interpret all medium-voltage responses as mediated by gradients in membrane potential rather than perimembrane electrophoresis.

When placed in a Ca^{2+} gradient, eggs of the fucoid alga, *Pelvetia,* tend to grow towards the end with the highest Ca^{2+} influx (115). Furthermore, when these eggs are cultured in a steady-voltage gradient, most batches tend to grow towards the positive pole (109), and increases in membrane potential (produced by reductions in external potassium) are found to yield proportionate increases in the rates of $^{45}Ca^{2+}$ influx (T. Chen and L. F. Jaffe, unpublished data). As discussed above, there is good experimental evidence for an early endogenous calcium current through the polarizing fucoid egg, with calcium entering the future outgrowth pole. Moreover, there is a substantial theoretical basis for expecting a calcium current to generate a significant field across the cytoplasm. Put together, these findings indicate that local calcium entry is part of a natural postive feedback loop that acts to localize growth in fucoid eggs, and they suggest that calcium entry may act, in part at least, by way of a cytoplasmic field effect.

Two other curious observations on fucoid eggs seem harder to interpret. First, a few anomalous batches of *Pelvetia* eggs responded to steady fields by growing towards the negative pole (109). Perhaps calcium entry is actually faster at the negative pole in these anomalous batches because depolarization excites the membrane and opens enough extra calcium gates to effect faster entry despite the lower driving force there. There is some support for this somewhat ad hoc suggestion. About 20% of the separate egg batches studied by Chen & Jaffe (unpublished data) 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%. Second, *Fucus* zygotes have been observed to show a fairly strong orientation towards the high potassium ion side in a potassium ion gradient provided the average potassium is abnormally high (116). This potassium gradient effect was originally interpreted as a direct effect of the overall cytoplasmic field produced by the resulting potassium current. However, this interpretation no longer seems plausible in view of the evidence for an endogenous calcium current and hence a relatively large endogenous field in the bulk

of the cytoplasm. Another explanation might lie in the local modification of the field within or near the channels that normally carry potassium ions (cf 117).

Reduction of the K^+ concentration around growing lily pollen tubes effects a rapid and parallel reduction in both the transcellular current and the tube growth rate (27). As indicated above, such responses (together with those to other ion changes) are taken to indicate that most of the current is carried inwards (and indeed through the interior) by K^+. Nevertheless, the field produced by this K^+ current is calculated to be far too small for its stoppage to quickly have much consequence. Perhaps K^+ reduction acts by hyperpolarizing the membrane (which it is known to do), which in turn closes calcium channels, and it is the resultant blockage of a calcium current that more directly blocks growth. A growth controlling (and growth localizing) role for Ca^{2+} is further supported by the observed curvature of pollen tubes towards a Ca^{2+} source in three other species (118). Since curvature of a tip-growing system like a pollen tube is mediated by bulging rather than bowing, this observation means that local application of high Ca^{2+} favors the initiation of local expansion. However any direct action of high Ca^{2+} on cell walls would be expected to increase their rigidity (by cross-linking acidic polysaccharides) and thus inhibit local expansion. Hence, local application of high Ca^{2+} may well act to favor local expansion of these cells by crossing the plasma membrane to refocus entry of an endogenous current.

Finally, we may consider *Acetabularia*. A cap tends to regenerate on that end of a stalk segment that faces the positive pole of an imposed field (30) or the hyperpolarized end of an imposed K^+ gradient (119), i.e. it regenerates at the end into which current is forced. The endogenous current pulses that long precede and predict the cap regenerating end in fact enter this end (30), and together these facts suggest that endogenous inward current pulses act to favor cap regeneration. Some further support for this possibility is provided by a report that insulation of one end of a stalk segment from the other markedly inhibits cap regeneration (120).

Field Effects on Animal Cells

Table 7 summarizes field effects on animal cells. In none of these cases is the target a single isolated cell. However, all five are relatively simple in cellular terms and it seems reasonable to interpret their responses at the cell level.

Case 1 refers to Lund's report that regenerating stem sections of a coelenterate (*Obelia*) respond to small, steady fields across them by growing towards the positive pole. A reconsideration of his data indicates that detectible galvanotropic responses occurred at current densities no higher than 300 $\mu A/cm^2$. The sea water used had a conductivity of 45 Ωcm (26) and drawings in this series indicate the stem section to have a diameter of about 250 μm. It follows that a detectible response was elicited by voltage drops as small as $3 \cdot 10^{-4}$ $A/cm^2 \times 45$ Ωcm $\times$ 1.5 $\times$ 0.025 cm = 0.5 mV. [Even this quite low figure is conservative since a replotting of the data (see 121, Table 2) seems to show substantial responses at less than 70 $\mu A/cm^2$ (rather than 300); however, small responses to the flow used to keep out electrode products somewhat confuse interpretation.]

Table 7 Effects of steady fields on animal cells

Case (Ref.)	Year	Organism	Cell type	Exposure time (hr)	Response	Field (mV/cm)	Milli-volts/cell
1 (121)	1924	*Obelia*	stem section (tube with two cell thick wall)	40	grows towards +	≥20	~0.1–0.3
2 (14, 121)	1970	*Perophora*	stolon (tube with one cell thick wall)	240	grows towards +	100	≤ 1.6[a]
3 (19, 41)	1973–74	*Cecropia moth*	oocyte-nurse cell complex	0.2–0.5	reverse bridge transport	~1,000?	≤0.3[a,b]
4a (123)	1946	chick embryo	medullary neurites	3–29	fibers suppressed towards +	≥500	?
4b "	"	chick embryo	medullary neurites	3–29	curve towards –	≥ 500	~0.6[c]
4c[d]	1977	chick embryo	dorsal root neurites	24	fiber speeded towards –; slowed towards +	≥ 500	?

[a] Smaller fields not tested.

[b] Obtained by multiplying applied current of 5×10^{-8} A (19), times a measured resistance of 6 kΩ (41).

[c] Across a 10-μm-wide growth cone.

[d] M. M. Poo and L. F. Jaffe, unpublished data.

The just-effective 0.5 mV or less was exerted across a stem section consisting essentially of a hollow tube with a wall, two cell layers thick. Thus the voltage was applied across four cells in series. The conductance of each wall may have been limited by one of the two layers. It is also possible that pairs of cells in the inner and outer layers are sufficiently coupled to effectively act as a unit. However, in any case, the effective voltage drop per responding unit is likely to have been no more than 0.25 mV, a figure that falls in the low-voltage group and suggests mediation by perimembrane electrophoresis.

Case 2 refers to a similar galvanotropic response by stolons of a tunicate (*Perophora*). In this case, however, only a single field strength was tested and the smallest one required to elicit a detectible response remains unknown.

Case 3 refers to a report [by Woodruff & Telfer (19)] that injection of current into an insect oocyte (in an effort to reverse the 10-mV endogenous potential between it and the nurse cell) is observed to reverse the otherwise unidirectional movement of fluorescein-conjugated protein across the cytoplasmic bridge from nurse to oocyte cell. Their actual statement is that "a current of 5×10^{-8} A was then passed in such a direction as to reverse the normal polarity." However, the electrical arrangements needed to demonstrate reversal of the voltage across the cytoplasmic bridge were not employed. Moreover, in a second report, direct measurement of the bridge resistances seemed to give values of 3–9 kΩ per bridge, implying that the voltage drop produced by 5×10^{-8} A was only 0.15–0.45 mV (41). One possible explanation is that the 10-mV difference was measured between points towards the middle of the nurse and oocyte cells, not directly across the bridge, whereas the calculated

back voltage was presumably developed almost entirely across this bridge. If most of the endogenous potential was not across the bridge—a condition that could occur if much of it arose from a fixed charge gradient rather than a conventional voltage drop—then the observed effectiveness of 5×10^{-8} A might be better understood.

Case 4 concerns field effects on nerve growth. The first convincing report of such effects was that of Marsh & Beams in 1946 (123). They reported that nerve outgrowths from explants of embryonic chick medulla were grossly affected by steady fields of 500 mV/cm or more: the initiation of outgrowths towards the positive electrode or anode were suppressed while other outgrowths grew in a curved path towards the cathode.

A careful reinvestigation of field effects on nerve growth has been recently initiated by M.m. Poo & L. F. Jaffe (unpublished data). When a steady electrical field is applied to explants of embryonic chick dorsal root ganglia, neurites that face the negative electrode or cathode grow faster; anode-facing ones grow slower or even retract, but no curvature is induced. Altogether, the outgrowths behave as if they were responding to the longitudinal component of the imposed field but not to the component across them. The remarkably asymmetric growth pattern that results can be reversed by reversing the field. This latter study agrees with Marsh & Beams (123) in finding complete suppression of growth towards the postive pole at about 500 mV/cm. It disagrees in finding no curvature induced in the outgrowths, but many differences in the experiments might account for this: different source of neurites, different media, different substrata, as well as the relative absence of glial cells in the latter study.

In addition to these two clear and positive results, the literature shows a dozen other attempts to study such field effects, most recently that of Sisken & Smith (124); however, their meaning is obscured by possible electrode effects, failure to determine the size of the fields applied, and/or failure to clearly describe the responses. In particular, objective interpretation of Weiss' negative report is blocked by the last two lacunae (98).

Field Effects on Multicellular Systems

Table 8 lists what seem to be the more significant developmental effects of imposing steady fields on multicellular systems. For comparison it also lists measurements of the corresponding endogenous fields or currents.

Case 1 concerns so-called electrotropic responses of oat (*Avena*) coleoptiles. Numerous studies of this phenomenon have been reported (reviewed in 13). However, the Wilkes & Lund article (125) is the only one employing relatively low fields for long periods and thus seems most likely to modulate or simulate a natural field. (Other studies used currents and hence fields at least a hundred times greater.) Schrank measured the transverse resistance of the coleoptile (between what appears to have been similar contacts under similar conditions) as about 0.3 MΩ (134). Therefore, one can estimate (or perhaps we should say guess) that the just effective (?) voltage drop employed by Wilkes & Lund (125) was about 30 mV. From Avery & Burkholder's study of the coleoptile's cellular structure (135), we learn that this voltage drop was expended upon about 100 cells in series, going around the periph-

Table 8 Effects of steady fields on the development of multicellular systems

Case	System	Field or current direction	Time	Size	Response	Year (Ref.)	Natural external potential gradient[a]	Ref.
1	Oat coleoptile	across axis	1 hr	0.1 μA= 30 mV ?[b,c] ≅0.3 mV/cell[d]	grows faster on negative side	1947 (125)	faster growing side[e] up to 80 mV positive	62
2	Oat coleoptile	base positive	20 min	0.3 μA[f]	growth rate doubles transiently	1937 (126)	base ≥ 40 mV positive	125, 133
		base negative	20 min	0.3 μA	15% slower transiently			
2a		base negative	1 hr	0.1 μA[b]	rate halved transiently	1947 (125)		
3	Onion root	base positive	50 min	380 mV/cm[b]	none	1947 (127)	base 30–60 mV negative	73–76
		base negative	50 min	380 mV/cm[b]	growth rate halved	1947 (127)		
3a		base negative	3 days	90 mV/cm[b]	lateral roots formed	1947 (128)		

Table 8 (*Continued*)

Case	System	Field or current direction	Time	Size	Response	Year (Ref.)	Natural external potential gradient[a]	Ref.
4	2-mm-long *Obelia* stem section	along axis (either way)	3 days	90 mV/cm[b]= 32 mV/section	stolons at - end and hydranths at + end	1921 (5)	hydranth positive	81
5	10-mm-long *Tubularia* stem sections	along axis (either way)	1 day	10 mV/cm = 15 mV/section	hydranths at + end	1934 (129)	variable	82
6	Stump of frog arm	base to apex	3 wks	0.1-0.2 μA[f]	regeneration	1967-77 (130-132)	base to apex	—[g]
6a		apex to base	3 wks	0.2 μA	destruction	1977 (130)		

[a] Or internal current direction in case 6.

[b] Minimum current giving detectable response.

[c] Schrank (134) gives a value of about 0.3 MΩ for the transverse resistance.

[d] Avery & Burkholder (135) give the cell structure of the coleoptile.

[e] Note that the coleoptile grows by longitudinal extension behind its tip. So it bows away from its faster growing side in response to unilateral light or gravity.

[f] Large response. Minimal stimulus not explored.

[g] Borgens et al, unpublished data.

ery of the tissue, and on a minimum of 40 going by the shortest path through the interior. So about 0.3–0.8 mV/cell was applied. It seems likely that the target of such a small field was in the cell walls or on the outer surface of the cell membranes, not within the coleoptile cells. Since the geoelectric and photoelectric potentials can be two to three times larger than 30 mV and have the opposite direction in the extracellular part of the circuit, this suggests the possibility of negative feedback (i.e. in the tropistic responses auxin is redistributed, which in turn both induces unequal growth and a transverse potential gradient; the field then acts back to reduce the asymmetry in the distribution of auxin and of growth). Such a mechanism could serve to prevent or limit over-response to tropistic stimuli.

Case 2, and particularly 2a, is opposite in its implications to case 1. Again, a substantial growth effect is exerted by as little as 0.1 μA for 1 hr. Again, the potential gradient produced by this current along the outer surface of the tissue is likely to be comparable to the natural field (although the data suggesting this are very crude). However, in this case the directions of the effects suggest that extracellular potential gradients act back in a positive way. Such a mechanism could help establish and maintain the longitudinal polarity of plant shoots.

Cases 3 and 3a are harder to assess, for roots do not normally grow in air (though they can) and certainly can grow in media conductive enough to largely eliminate any potential gradients along their outer surfaces. So if natural field controls are present, they are very likely to be along inner surfaces of the root or even inside chains of root cells, and the degree to which imposed fields get inside the root seems impossible to even guess at now.

Cases 4 and 5 concern the responses of regenerating stem sections of two closely related marine coelenterates. These sections consist essentially of a hollow tube, closed at both ends, whose wall is two epithelial cell layers thick. In both cases, hydranth regeneration is favored at the positive pole, but whether these imposed fields modify endogenous ones is obscure. It should be noted that observations of potential differences along regenerating stem sections placed in moist air for measurement purposes (Table 4) need not indicate that significant fields naturally exist along the outside of such sections. Indeed, the exterior region, as well as the internal cavity, of such a section are presumably so conductive as to be effectively isopotential under natural conditions; hence, too, the radial potentials (across the animal from inside to outside) would likewise be effectively equal at all points. However, these considerations do not preclude a significant natural field along the third extracellular space, namely the mesogleal space between the ectodermal and endodermal layers. Viewed in this way, Lund's observations might suggest that the mesogleal space of *Obelia* is normally electropositive at its distal, hydranth-regenerating end and that an imposed field acts by modifying this natural mesogleal one.

Case 6, the electrical stimulation of frog limb regeneration, is particularly interesting. In Smith's pioneering 1967 study (131), forelimbs of adult frogs were amputated and the stumps were implanted with silver-platinum bimetallic rods until the animals were sacrificed 3 months later. In amphibian Ringers, this primitive battery generated 200 mV (across a 3-mm gap). In most cases, conical outgrowths were

induced. These showed a variable degree of internal structure indicative of regeneration, including a thickened terminal epidermis with little dermis beneath it and isolated columns of lobulated cartilage reminiscent of wrist or hand bones. The response was somewhat greater when the electronegative silver component was distal rather than proximal. In his second investigation (132) Smith stimulated amputated frog forelimbs with a commercial battery implanted in the back that drew about 0.1 μA from the stump through a steel electrode for several weeks. The regenerative response was greatest when the dorsal postaxial part of the stump was stimulated. Moreover, eight stimulated animals attained "formation of at least one well-formed movable digit," and in one remarkable case "the regenerated limb is absolutely indistinguishable from a normal one . . . and the animal used the hand with what appeared to be normal coordination and range of motion. There was neither external nor internal indication of the original level of section."

Two criticisms must be made about these remarkable reports. First, it is not clear from them alone whether regeneration is stimulated by the field imposed upon the tissue or chemically by electrode products. However, a later study by Borgens et al shows regeneration to be stimulated by a comparable current provided by means that clearly avoided electrode products (130). Second, and more serious, is the question of the degree of regeneration claimed. It must be plainly stated that no significant evidence at all was presented to support the more extreme claims quoted above: no significant histological sections, no photographs or sketches, and no report on the course of regeneration. The one section that is shown is of the one perfect arm, and no evidence is available to show that this perfect arm was in fact regenerated rather than developing like other frog arms.

Nevertheless, Smith's main claim has been well confirmed by Borgens et al (130). Cathodal (i.e. distally negative) application of 0.2 μA of current to the postaxial part of the frog's forelimb stump for several weeks regularly induces partial regeneration. Such regeneration is indicated externally by substantial growth and internally by organized extension of: the severed radio-ulna, newly formed muscle; ligament; isolated, partially segmented cartilage rods; and a thickened terminal epidermis without an underlying dermis.

Moreover, extraordinarily large amounts of nerve developed in the regenerated tissue: about 20% of the volume of the distal portions of cathodally stimulated animals consisted of regenerated nerve trunks, whereas less than 1% of sham-treated or anodally stimulated animals consisted of nerve. On the other hand, anode-stimulated animals showed extensive and characteristic degeneration, particularly of the bone and muscle.

Together with the evidence of comparable endogenous currents in naturally regenerating newts (reviewed above), these findings suggest that these endogenous currents, and the fields they generate, are normally needed for regeneration. Some confirmation of this important inference is provided by recent evidence that regeneration of various Urodeles can be prevented by blocking the skin-driven regeneration current, either by depleting the medium of sodium ions or by treating the stump's surface with amiloride (Borgens et al, unpublished data).

Together with evidence that nerve growth is accelerated towards the negative pole in relatively small fields, these findings suggest that the targets of either endogenous or imposed fields are the nerve processes within the stump. Moreover, Bodemer attained a comparable degree of forelimb regeneration in adult frogs by a radically different kind of electrical stimulation (136). He simply stimulated the major forelimb nerve in the scapular region with about 1000, 0.1-msec-long, 300-mV pulses delivered during 1- or 2-min periods. The similarity of the regeneration responses obtained in this way to those obtained by prolonged, very-low-intensity fields may again suggest the limb nerves as the target of the latter; however, we are definitely not suggesting that the low-intensity fields act by stimulating action potentials. Rather, we are led to wonder if action potentials can also stimulate nerve growth; an interesting question that is logically beyond the stated scope of this review. Nevertheless, we note that the only evidence bearing directly on this question seems to be a report of Hoffman (137) that rather violent electrical stimulation of the spinal cord or the lumbosacral plexus of the rat substantially accelerates the re-innervation of denervated fibers, by the sprouting of intact axons, in partially denervated muscles.

Finally, we must note a truly astonishing recent report of electrically stimulated "partial limb regeneration in rats" (138). We find these claims very difficult to reconcile with the established information about regeneration. The forelegs of 21-day-old rats were amputated through the humerus proximal to the epiphyseal plate. The absolutely astonishing claim is made that 3 days later, in response 3–6 nA of current, the humerus has been almost perfectly regenerated. Furthermore, unlike other regenerative events, this did not progress with time. According to the data, no significant change in the degree of regeneration was attained between 3 and 28 days after amputation. Another astonishing feature of this report is the claim that a complete humerus ("supernumerary humerus is well formed") was formed only 7 days after amputation in one case.

Clinical Applications

Brighton et al (144) have recently reviewed the literature on electrically stimulated healing of bone fractures as well as congenital abnormal bone gaps. It is clear that clinically significant healing can be induced by implantation of cathodes delivering direct current of the order of 10 μA. It is also clear that the new bone forms in the immediate vicinity of the cathode. These facts suggest that bone healing is mediated by electrode products rather than by field effects. Other recent papers of Brighton et al (139, 140) show that comparable cathodes in vitro substantially reduce oxygen and moderately raise the pH in their vicinity, and these papers provide further evidence indicating that it is these changes, particularly the local reduction in oxygen, that in fact mediate electrically induced bone formation. No one has investigated the effects on fracture healing of direct currents delivered by techniques that avoid the introduction of electrode products into the tissue. So for the moment there is little or no reason to believe that direct current electrical fracture healing acts by directly modifying some endogenous electrical fields. The same may be said for electrically accelerated healing of wound and skin ulcers (141).

On the other hand, we would like to suggest a genuinely electrical explanation for two recent and well-documented reports that amputated finger tips in children may regenerate if surgical closure of the skin is avoided (142, 143). By analogy with the regeneration of amphibian limbs, we would suggest that skin closure blocks regeneration, in part at least, by blocking the flow of a skin-driven current through the stump.

SUMMARY

Developing systems generally drive steady ion currents through themselves and thus produce substantial fields within themselves. Established examples include currents that enter the prospective and continuing growth point of several tip growing plant cells, a voltage across the cytoplasmic bridge between an insect oocyte and its nurse cell, current traversing a recently fertilized (and segregating) fish egg from animal to vegetal pole, early potentials across several embryonic epithelia including the mammalian blastocyst wall and the chick chorioallantoic membrane, potentials along the surface of several growing plant organs (particularly the sheath of the young shoot), and a large current leaving the stump of a regenerating amphibian limb.

The imposition of small steady fields, or the modification of endogenous ones, often has profound effects on developing systems. Established examples include the control of growth direction in various tip growing plant cells, the control of outgrowth extension rate in explanted neurons, the control of growth distribution in several plant organs, and the control of limb regeneration in amphibians.

One important mechanism that may mediate these phenomena is electrophoresis along cell membranes of charged components that float in these membranes. At the equilibrium between such lateral electrophoresis and back diffusion, voltage drops of as little as 1 mV per cell should produce significant redistributions.

Literature Cited

1. Faraday, M. 1839. *Experimental Researches in Electricity.* London: Bernard Quaritch
2. Jaffe, L. A. 1976. *Nature* 261:68–71
3. Roux, W. 1892. *Sitzungsber. Acad. Wiss. Wien Math. Naturwiss. Kl.* 101:27–228
4. Mathews, A. P. 1903. *Am. J. Physiol.* 8:244–99
5. Lund, E. J. 1921. *J. Exp. Zool.* 34: 471–93
6. Lund, E. J. 1947. *Bioelectric Fields and Growth.* Austin: Univ. Texas
7. Spek, J. 1930. *Protoplasma* 9:370–425
8. Went, F. W. 1932. *Jahrb. Wiss. Bot.* 76:528–54
9. Burr, H. S. 1941. *Proc. Natl. Acad. Sci. USA* 27:276–81
10. Costello, D. P. 1945. *J. Elisha Mitchell Sci. Soc.* 61:277–89
11. Liboff, A. R., Rinaldi, R. A. 1974. *Ann. NY Acad. Sci.* 238:5–593
12. Crane, E. E. 1950. *Prog. Biophys. Biophys. Chem.* 1:85–136
13. Schrank, A. R. 1959. *Encyclopedia of Plant Physiology XVII/1,* ed. W. Ruhland, E. Bunning, pp. 148–67. Berlin: Springer-Verlag
14. Smith, S. D. 1970. *Am. Zool.* 10: 133–40
15. Lukiewicz, S. 1962. *Folia Biol. Krakow* 10:5–35
16. Jaffe, L. F., Robinson, K. R., Nuccitelli, R. 1974a. *Ann. NY Acad. Sci.* 238: 372–89
17. Cole, K. S., Curtis, H. J. 1950. *Bioelectricity: Electric Physiology in Medical Physics,* ed. O. Glasser, 2:82–90. Chicago: Yearbook Publ.

18. Slayman, C. L., Slayman, C. W. 1962. *Science* 136:876–77
19. Woodruff, R. I., Telfer, W. H. 1973. *J. Cell Biol.* 58:172–88
20. Grahm, L., Hertz, C. H. 1962. *Physiol. Plant.* 15:96–114
21. Grahm, L., Hertz, C. H. 1964. *Physiol. Plant.* 17:186–201
22. Scott, B. I. H., McAulay, A. L., Jeyes, P. 1955. *Aust. J. Biol. Sci.* 8: 36–46
23. Jaffe, L. F. 1966. *Proc. Natl. Acad. Sci. USA* 56:1102–9
24. Jaffe, L. F., Nuccitelli, R. 1974. *J. Cell Biol.* 63:614–28
25. Weisenseel, M. H., Nuccitelli, R., Jaffe, L. F. 1975. *J. Cell Biol.* 66:556–67
26. Lund, E. J. 1925. *J. Exp. Zool.* 41:155–90
27. Weisenseel, M. H., Jaffe, L. F. 1976. *Planta* 133:1–7
28. Jaffe, L. A., Weisenseel, M. H., Jaffe, L. F. 1975 *J. Cell. Biol.* 67:488–92
29. Robinson, K. R., Jaffe, L. F. 1975. *Science* 187:70–72
30. Novak, B., Bentrup, F. W. 1972. *Planta* 108:227–44
31. Novak, B., Sironval, C. 1976. *Plant Sci. Lett.* 6:273–83
32. Nuccitelli, R., Jaffe, L. F. 1974. *Proc. Natl. Acad. Sci. USA* 71:4855–59
33. Nuccitelli, R. 1975. *An investigation of the natural electrical currents generated by the Pelvetia embryo during early development.* PhD thesis. Purdue Univ. Lafayette, Ind.
34. Nuccitelli, R., Jaffe, L. F. 1976b. *J. Cell. Biol.* 70:50a
35. Nuccitelli, R., Jaffe, L. F. 1975. *J. Cell. Biol.* 64:636–43
36. Jaffe, L. F., Robinson, K. R., Picologlou, B. F. 1974. *J. Theor. Biol.* 45:593–95
37. Jaffe, L. F., Robinson, K. R., Nuccitelli, R. 1974b *ICN-UCLA Symp. Mol. Cell. Biol.* 2:135–47
38. Nuccitelli, R., Jaffe, L. F. 1976. *Dev. Biol.* 49:518–31
39. Hyde, I. H. 1905. *Am. J. Physiol.* 12:241–75
40. Hasama, B. I. 1935. *Protoplasma* 22: 597–606
41. Woodruff, R. I., Telfer, W. H. 1974. *Ann. NY Acad. Sci.* 238:408–19
42. Hagins, W. A., Penn, R. D., Yoshikami, S. 1970. *Biophys. J.* 10:380–412
43. Burr, H. S., Bullock, T. H. 1941. *Yale J. Biol. Med.* 14:51–57
44. Ramonoff, A. L. 1944. *Biodynamica* 4:329–58
45. Flickenger, R. A., Blount, R. W. 1957. *J. Cell Comp. Physiol.* 50:403–22
46. Peters, J. J. 1962. *Am. Zool.* 2:437
47. Dorfman, W. A. 1933. *Protoplasma* 21:245–57
48. Woodward, D. J. 1968. *J. Gen. Physiol.* 52:509–31
49. DeLaat, S. W., Wouters, W., Marques daSilva Pimenta Guarda, M. M., daSilva Guarda, M. A. 1975. *Exp. Cell Res.* 91:15–30
50. Young, R. W. 1967. *J. Cell Biol.* 33:61–72
51. Ussing, H. H., Thorn, N. A., eds. 1973. *Transport Mechanisms in Epithelia.* Copenhagen: Munksgaard
52. Bennett, M. V. L., Trinkaus, J. P. 1970. *J. Cell. Biol.* 44:592–610
53. Stewart, M. E., Terepka, A. R. 1969. *Exp. Cell. Res.* 58:93–106
54. Kyriakides, C. P. M., Simkiss, K. 1975. *Comp. Biochem. Physiol.* 51A: 875–79
55. Cross, M. H., Cross, P. C., Brinster, R. L. 1973. *Dev. Biol.* 33:412–16
56. Cross, M. H., Brinster, R. L. 1969. *Exp. Cell Res.* 58:125–27
57. Morrill, G. A., Kostellow, A. B., Watson, D. E. 1966. *Life Sci.* 5:705–9
58. Slack, C., Warner, A. E. 1973. *J. Physiol.* 232:313–30
59. Coleman, J. R., Terepka, A. R. 1972. *J. Mem. Biol.* 7:111–27
60. Weiss, P., Taylor, A. C. 1960. *Proc. Natl. Acad. Sci. USA* 46:1177–81
61. Scott, B. I. H. 1963. *Proc. Soc. Exp. Biol. Med.* 113:337–39
62. Grahm, L. 1964. *Physiol. Plant.* 17:231–61
63. Woodcock, A. E. R., Hertz, C. H. 1972. *J. Exp. Bot.* 77:953–57
64. Johnsson, A. 1965. *Physiol. Plant.* 18: 574–76
65. Johnsson, A. 1967. *Physiol. Plant.* 20: 562–79
66. Jenkinson, I. S. 1962. *Aust. J. Biol. Sci.* 15:101–14
67. Etherton, B. 1970. *Plant Physiol.* 45: 527–28
68. Marré, E., Lado, P., Ferroni, A., Ballarin-Denti, A. 1974. *Plant Sci. Lett.* 2:257–65
69. Cleland, R. 1973. *Proc. Natl. Acad. Sci. USA* 70:3092–93
70. Cocucci, M., Marré, E., Ballarin-Denti, A., Scacchi, A. 1976. *Plant Sci. Lett.* 6:143–56
71. Spanswick, R. M. 1974. *Symp. Soc. Exp. Biol.* 28:127–37
72. Goswami, K. K. A., Audus, L. J. 1976. *Ann. Bot.* 40:49–64

73. Lund, E. J., Kenyon, W. A. 1927. *J. Exp. Zool.* 48:333–57
74. Berry, L. J., Lund, E. J. 1947. *Bioelectric Fields and Growth,* ed. E. J. Lund, pp. 123–51. Austin: Univ. Texas
75. Ramshorn, K. 1934. *Planta* 22:737–66
76. McAulay, A. L., Ford, J. M., Hope, A. B. 1951. *J. Exp. Biol.* 28:320–31
77. Scott, B. I. H., Martin, D. W. 1962. *Aust. J. Biol. Sci.* 15:83–100
78. Scott, B. I. H. 1967. *Ann. Rev. Plant Physiol.* 18:409–18
79. Moment, G. B. 1949. *J. Exp. Zool.* 112:1–12
80. Kurtz, I., Schrank, A. R. 1955. *Physiol. Zool.* 28:322–30
81. Lund, E. J. 1922. *J. Exp. Zool.* 36:477–94
82. Barth, L. G. 1934b. *Physiol. Zool.* 7:365–99
83. Monroy, A. 1941. *Publ. Stn. Zool. Napoli* 18:265–81
84. Becker, R. O. 1961. *J. Bone J. Surg.* 43A:643–56
85. Rose, S. M., Rose, F. C. 1974. *Growth* 38:363–80
86. Barnes, T. C. 1952. *Fed. Proc.* 11:320
87. Stucky, G. L., Meyers, W. M. 1972. *Lancet* 1:1293
88. Friedenberg, Z. B., Brighton, C. T. 1966. *J. Bone J. Surg.* 48A:915–23
89. Becker, R. O., Murray, D. G. 1970. *Clin. Orthop.* 73:169–98
90. Cole, K. S. 1969. *Membranes, Ions, and Impulses,* pp. 15–17. Univ. Calif., Berkeley
91. Weisenseel, M. H., Jaffe, L. F. 1974. *Exp. Cell Res.* 89:55–62
92. Jaffe, L. F. 1969. *Dev. Biol.* 3:Suppl., pp. 83–111
93. Poo, M.-m., Cone, R. A. 1974. *Nature* 247:438–41
94. Edidin, M. 1974. *Ann. Rev. Biophys. Bioeng.* 3:179–201
95. Jaffe, L. F. 1976. *Nature* 265:600–2
96. Jaffe, L. F. 1958. *Exp. Cell Res.* 15:282–99
97. Kittel, C. 1966. *Introduction to Solid State Physics,* pp. 388–89. New York: Wiley. 3rd ed.
98. Weiss, P. 1934. *J. Exp. Zool.* 68:393–448
99. Gerber, B. R., Minakata, A., Kahn, L. D. 1975. *J. Mol. Biol.* 92:507–28
100. Kahn, L. D. 1951. *J. Agric. Food Chem.* 19:679–88
101. Colson, P., Houssier, C., Fredericq, E. 1975. *Polymer* 15:396–97
102. Kobayashi, S., Asai, H., Oosawa, F. 1964. *Biochim. Biophys. Acta* 88: 528–40
103. Yamamoto, T. 1947. *Cytologia* 14: 219–25
104. Ridgway, E. B., Gilkey, J. C., Jaffe, L. F. 1977. *Proc. Natl. Acad. Sci. USA.* In press
105. Millonig, G. 1969. *J. Submicrosc. Cytol.* 1:69–84
106. Gwatkin, R. B. L., Williams, D. T., Hartmann, J. F., Kniazuk, M. 1973. *J. Reprod. Fertil.* 32:259–65
107. Poo, M.-m., Robinson, K. R. 1976. *Nature* 265:602–5
108. Bretscher, M. S. 1976. *Nature* 260: 21–23
109. Peng, H. B., Jaffe, L. F. 1976. *Dev. Biol.* 53:277–84
110. Schechter, V. 1934. *J. Gen. Physiol.* 18:1–21
111. Bentrup, F. W. 1968. *Z. Pflanzenphysiol.* 59:309–39
112. Zeijlemaker, F. C. J. 1956. *Acta Bot. Neerl.* 5:179–86
113. Sand, O. 1973. *Exp. Cell Res.* 76: 444–46
114. Novak, B., Bentrup, F. W. 1973. *Biophysik* 9:253–60
115. Robinson, K. R., Jaffe, L. F. 1976. *J. Cell Biol.* 70:37a
116. Bentrup, F., Sandan, T., Jaffe, L. 1967. *Protoplasma* 64:254–66
117. Jaffe, L. F. 1971. *Biophys. Soc. Abstr.* 11:273a
118. Mascarenhas, J. P., Machlis, L. 1964. *Plant Physiol.* 39:70–77
119. Christ-Adler, M., Bentrup, F. W. 1976. *Planta* 129:91–93
120. Novak, B., Sironval, C. 1975. *Plant Sci. Lett.* 5:183–88
121. Lund, E. J. 1924. *J. Exp. Zool.* 39: 357–80
122. Freeman, G. 1964. *J. Exp. Zool.* 156: 157–84
123. Marsh, G., Beams, H. W. 1946. *J. Cell. Comp. Physiol.* 27:139–57
124. Sisken, B. F., Smith, S. D. 1975. *J. Embryol. Exp. Morphol.* 33:29–41
125. Wilkes, S. S., Lund, E. J. 1947. *Bioelectric Fields and Growth,* ed. E. J. Lund, pp. 24–75. Austin: Univ. Texas
126. Cholodny, N. G., Sankewitsch, E. C. 1937. *Plant Physiol.* 12:385–408
127. Lund, E. J., Mahan, R. I., Hanszen, A. H. 1947. *Bioelectric Fields and Growth,* ed. E. J. Lund, pp. 191–93. Austin: Univ. Texas
128. Berry, L. J., Gardiner, M. S., Gilmartin, R. T. 1947. *Growth* 11:155–76
129. Barth, L. G. 1934a. *Physiol. Zool.* 7:340–64
130. Borgens, R. B., Vanable, J. W. Jr., Jaffe, L. F. 1977. *J. Exp. Zool.* In press

131. Smith, S. D. 1967. *Anat. Rec.* 158: 89–97
132. Smith, S. D. 1974. *Ann. NY Acad. Sci.* 238:500–7
133. Newman, I. A. 1963. *Aust. J. Biol. Sci.* 16:629–46
134. Schrank, A. R. 1948. *Plant Physiol.* 23:188–200
135. Avery, G. S., Burkholder, P. R. 1936. *Bull. Torrey Bot. Club* 63:1–5
136. Bodemer, C. W. 1964. *Anat. Rec.* 148:441–57
137. Hoffman, H. 1952. *Aust. J. Exp. Biol. Med. Sci.* 30:541–66
138. Becker, R. O., 1972. *Nature* 235:109–11
139. Brighton, C. T., Friedenberg, Z. B. 1974. *Ann. NY Acad. Sci.* 238:314–20
140. Brighton, C. T., Adler, S., Black, J., Itada, N., Friedenberg, Z. B. 1975. *Clin. Orthop. Relat. Res.* 107:277–82
141. Wheeler, P. C., Wolcott, L. E., Morris, J. L., Spangler, M. R. 1971. *Neuroelectric Research.* ed., D. V. Reynolds, A. E. Sjoberg, pp. 83–99. Springfield: C. C. Thomas
142. Douglas, B. S. 1972. *Aust. Paediatr. J.* 8:86–89
143. Illingsworth, C. M. 1974. *J. Pediatr. Surg.* 9:853–58
144. Brighton, C. T., Friedenberg, Z. B., Zemsky, L. M., Pollis, P. R. 1975. *J. Bone J. Surg.* 57(A):368–77