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The Role of Ionic Currents in Establishing Developmental Pattern

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The role of ionic currents in establishing developmental pattern

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This paper reviews a theory of pattern establishment and pattern restoration by endogenous ionic currents. These currents are supposed to be generated by a certain separation of ion leaks and ion pumps in cell membranes. In so far as these currents act back to further this separation, they would be part of a regenerative process that initially establishes positional values. Later in development, particularly in epimorphic regeneration, when positional values are restored or extended, these currents are supposed to leak through sites of discontinuity in such values and thus trigger growth. This paper also reviews the factual evidence for this view: evidence that developmental currents are, indeed, very widespread; evidence in a few cases, particularly in *Cecropia* follicles and in wounded cavy skin, that they can generate substantial voltage differences or gradients; evidence that comparable artificial fields can move charged macromolecules along cell membranes and polarize cell growth; and direct evidence in a few cases, particularly fucoid eggs, *Cecropia* follicles and regenerating amphibian limbs, that ion currents do, in fact, act back to direct or further development. The paper also presents a particular theory, based upon ionic currents, of the reversal of thyroid cell polarity by serum.

Introduction

Developmentalists know that epigenesis is real but gradual. That is to say, eggs or oocytes go through a highly regulative state that involves little pattern or pre-pattern. Then their regulative ability slowly falls. As development proceeds, it requires more and more radical intervention to produce the same rearrangement of the final pattern and the same intervention produces less and less final response. (For a good recent example of this point, see Eyal-Giladi & Fabian (1980).) One of the clearest and most striking of such regulative responses is the formation of bipolar or twin forms from the whole system. Figure 1 illustrates this point.

1. The nearly patternless or apolar eggs of the fucoid alga *Pelvetia* can be induced to generate bipolar forms – with rhizoids at any (?) two opposite poles – by treatment with weak, plane-polarized blue light.†

2. Except for their germ plasm, eggs of the fly *Smittia* are not visibly stratified along their anterior–posterior axes during intervitelline cleavage. However, their anterior, and future, head ends are clearly indicated by their shape, while sufficient ultraviolet irradiation of these ends induces a large proportion of these eggs to develop into bipolar forms with an abdomen at both ends and no head.

3. Adult rat thyroid follicles can be prepared in suspension culture in such a way that their

† To be more exact, no more than half of a population of *Pelvetia* eggs do so. It is possible that the unresponsive half happened to have a region, perhaps induced by the point of sperm entry as in *Cystoseira*, that bore a weak predisposition to generate a rhizoid relatively close to one of the two antipodes along the axis of the electric vector of the light, while the responsive half had this region closer to the equator of these antipodes.

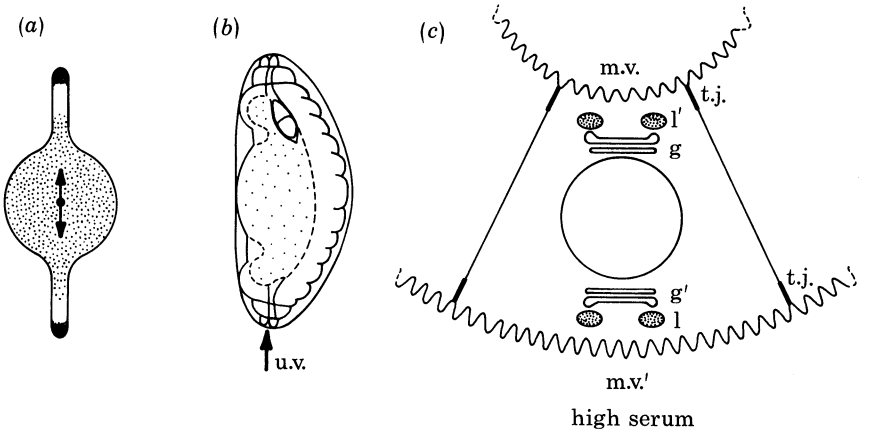

FIGURE 1. Bipolar forms. (*a*) *Pelvetia* embryo with two rhizoidal or basal poles. Induced by vertically directed plane polarized blue light vibrating in the axis of arrow. (From Jaffe (1956).) (*b*) Double-abdomened *Smittia* eggs induced by unilateral ultraviolet light. (From Kalthoff (1979).) (*c*) Biapical thyroid cells induced by raising the basal serum level. (From Nitsch & Wollman (1980*b*).)

cells exhibit their normal, gross morphological stratification and physiological polarity, except for the absence of a basement lamina (Nitsch & Wollman 1980*a*). However, if the concentration of serum in the medium (which bathes their outer faces) is raised from 0.5 to 5%, their cells undergo a remarkable reversal of polarity; one mediated by *biapical* forms with tight junctions, microvilli and Golgi complexes at both their normal luminal faces and their outer, serosal faces (figure 1*c*). It may be added that a reinversion of such inverted follicles, or cysts, can be induced by embedding the now outer apical faces in a collagen gel, although the expected bibasal intermediates of this process have not yet been seen (Mauchamp *et al.* 1980).

What broad mechanisms are involved in establishing (or reversing) pattern? As opposed to its repair – as seen in epimorphic regeneration, a process which may well involve the essentially local mechanisms seemingly implicit in the well known polar coordinate model (French *et al.* 1976) – pattern must surely be established, at least in important part, by some global mechanism(s). The theory of reaction–diffusion mechanisms has been emphasized and developed by a number of contributors to this symposium – particularly Gierer, Wolpert, Murray and Kauffman. Here I shall discuss a rather different mechanism based upon ionic currents and involving self-electrophoresis.

This ionic current hypothesis of pattern formation may be introduced via the frog skin paradigm (figure 2). It is now well known that the epidermis of frog skin actively takes up sodium ions from the pond water by localizing its sodium ion *leaks* in its cells' outer regions and its sodium ion *pumps* in its lateral and basal regions, the two regions being separated by so-called tight junctions. This view, while initially developed and established on the basis of physiological data, has been further confirmed in frog skin and many other epithelia by biochemical means. In particular, various cytochemical reagents, e.g. tritiated ouabain, have been used to localize sodium pumps within the lateral and basal surfaces of a remarkable variety of adult epithelia (DiBona & Mills 1979). Three general points may be emphasized about this paradigm. First, it will be noted that local current loops will be set up in such epithelia to the extent that sodium ions flow back through the more or less permeable tight junctions via the so-called paracellular route. Secondly, it will be noted that the leaky region is relatively small, presumably because leaks are simpler and smaller than pumps. Thirdly, the modern extensions of this paradigm to the secondary, i.e. Na^+-pump dependent, transport of a remarkable variety of other substances

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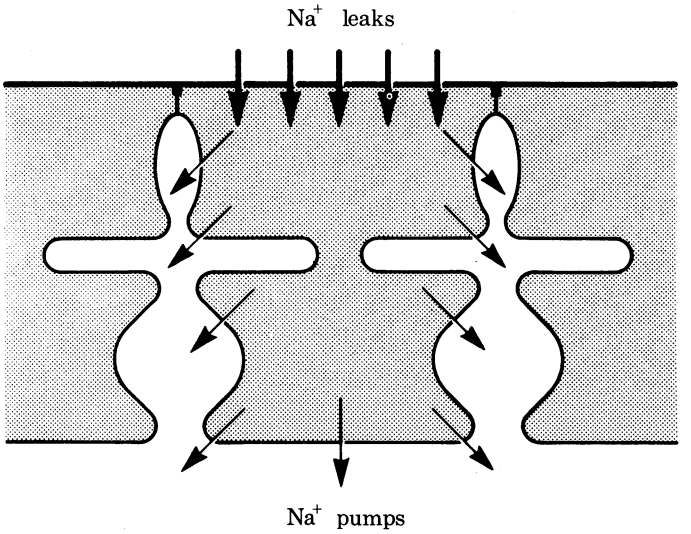

FIGURE 2. Diagrammatic view of the electrical structure of frog skin.

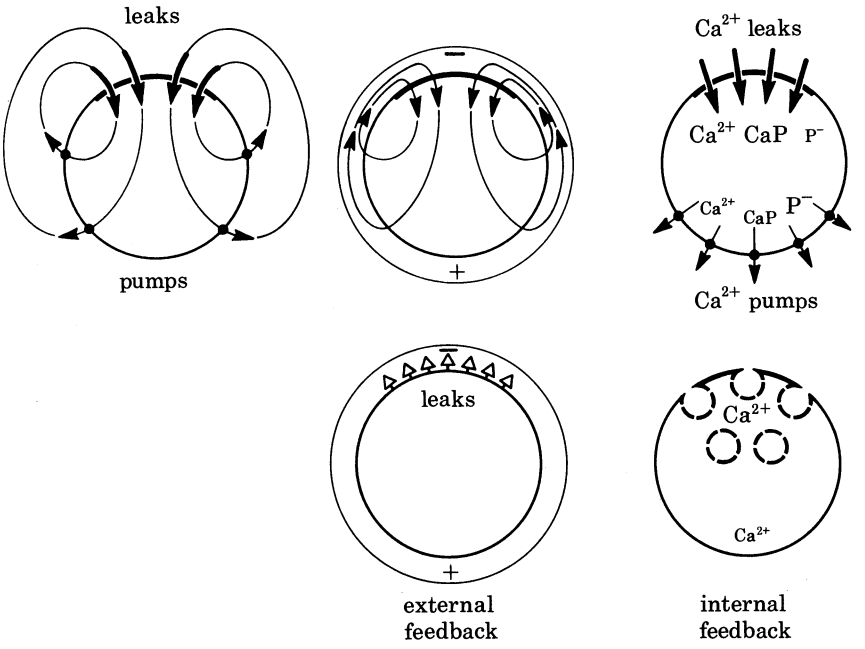

FIGURE 3. Some possible modes of self-enhancement by transcellular currents.

through adult epithelia is closely related to Mitchell's successful chemiosmotic hypothesis of energy generation in cells (Raven & Smith 1980).

Extension of this paradigm to pattern formation involves the basic assumptions that ion leaks and pumps are segregated during patterning; that this segregation generates ionic currents, which act back to segregate these components further; and that these currents also act to segregate other (morphogenetic) components and thus ultimately generate so-called pre-patterns. To the extent that relatively isolated cells, e.g. eggs, are involved, two main routes of positive feedback are imagined (figure 3). If the extracellular space is sufficiently restricted, then the *external* sections of the current loops should encounter enough resistance to generate substantial extracellular voltage gradients or fields; these in turn might further segregate the

leaks (and pumps) by electrophoresis *along* the cell membrane, i.e. by so-called lateral electrophoresis. A good example of a system where such phenomena might well be expected is the insect egg, where the conductive extracellular space consists of the exceedingly thin perivitelline space situated between the plasma and vitelline membranes. On the other hand, to the extent that the transcellular current consists of calcium ions, positive *internal* feedback might occur via transcytoplasmic calcium ion gradients. Since calcium ions are well known to be greatly immobilized in cytoplasm, both by sequestration within organelles and by binding to components of the continuous cytoplasmic phase, substantial steady calcium currents should almost certainly generate substantial transcytoplasmic calcium ion gradients. These, in turn, might generate a significant internal field by means of fixed charge gradients (Jaffe 1979, p. 225). Moreover, they might induce the exocytosis of vesicles bearing calcium leaks at the calcium leaky end – thus once again further segregating leaks from pumps by an internal mechanism.

To the extent that epithelia are involved, corresponding external feedback via 'paracellular' voltage gradients, and internal feedback via transcellular calcium currents may be imagined. Finally, it should not be forgotten that metazoan oocytes develop within epithelia, so compound mechanisms may well be expected. An interesting recent observation of oocyte–epithelium interaction is furnished by work on *Xenopus* oocytes (Wylie *et al.* 1979).

Developmental currents

A wide variety of developing systems have been found to drive relatively steady currents of the order of 1–100 $\mu A/cm^2$ through themselves (figure 4). Most of these measurements were made with an ultrasensitive vibrating probe for measuring extracellular currents (Jaffe & Nuccitelli 1974). The sensitive tip of this probe consists of a small (10–30 μm) platinum black ball, which is vibrated at several hundred hertz between two points in the aqueous medium near a living cell or organism. It reliably registers the minute voltage differences, typically nanovolts, maintained between these points by current traversing the living system. From this voltage and the resistivity of the medium, the local current density is inferred. A good example of the application of this new tool can be found in a study by Weisenseel *et al.* (1975) of the currents through germinating lily pollen: a steady current with a density of several microamps per square centimetre is found to traverse each grain several hours before it begins to grow out or germinate; moreover, the point of most intense current entry rather accurately predicts the point of subsequent growth.

Developing systems, which are known to drive substantial current through themselves, range from ones as simple as the fucoid egg (figure 4*a*) to more complex ones such as silkworm (*Cecropia*) follicles, chick embryos in the primitive streak stage, regenerating salamander limbs (Borgens *et al.* 1977*b*), and even regenerating human fingers (Illingworth & Barker 1980). In general, it is found that these currents tend to be concentrated at the leak rather than the pump, and that local growth (or other obvious developmental action) likewise occurs at the leak. Concentration, action, and perhaps control at the leak may be connected with the fact, discussed by Raven & Smith (1980), that ion leaks are relatively small and simple components capable of handling far higher current densities than ion pumps. The major ionic component of the leak current is known to vary greatly. It may be Na^+ as in regenerating limbs, K^+ as in growing pollen tubes, Cl^- as in *Xenopus* oocytes, H^+ as in growing barley roots (Weisenseel *et al.* 1979), or perhaps even Ca^{2+} as in early, and still visibly apolar, fucoid eggs (Robinson & Jaffe 1975). However, the *controlling* component is apparently the calcium ion in at least four cases,

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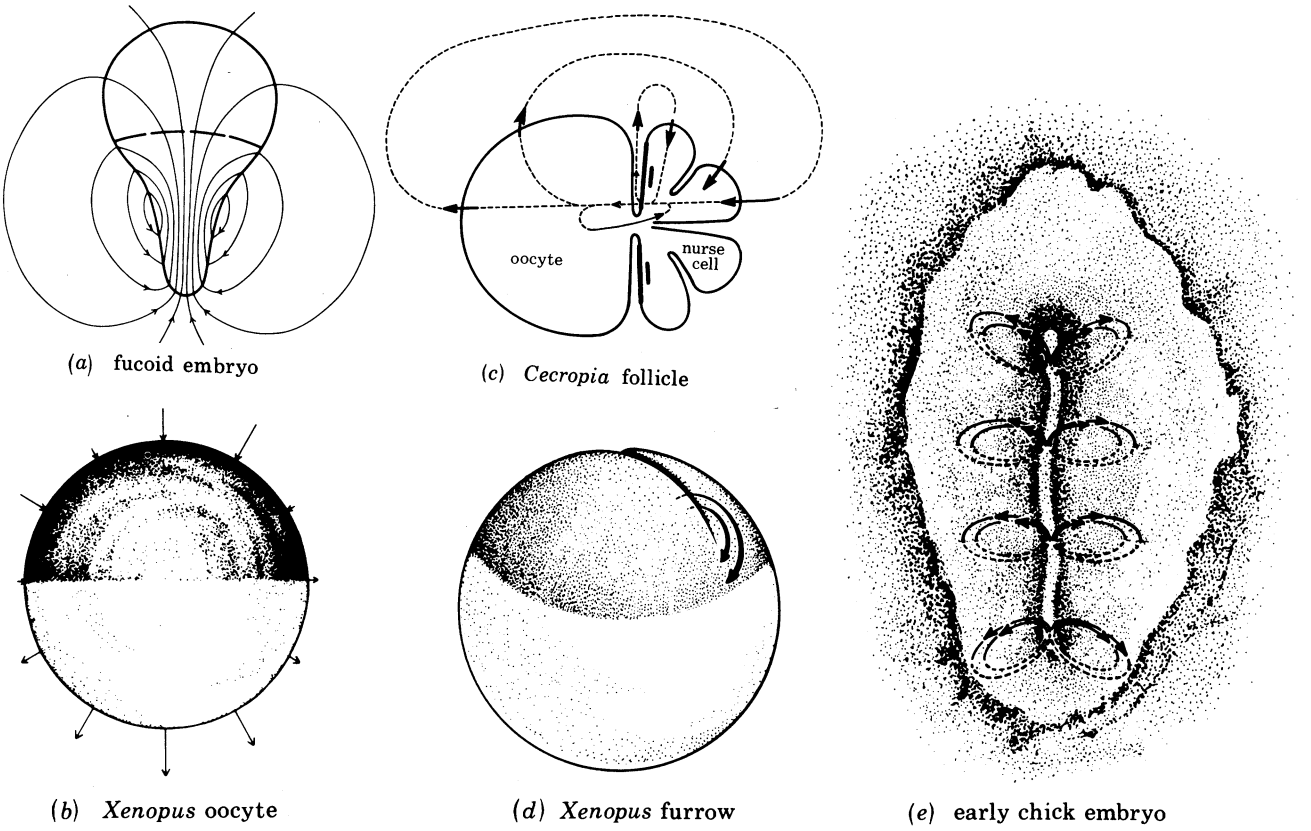

FIGURE 4. Some current patterns measured during early development. (*a*) From Nuccitelli & Jaffe (1975); (*b*) from Robinson (1975); (*c*) from Jaffe & Woodruff (1979); (*d*) from D. W. Kline, R. Nuccitelli & K. R. Robinson (unpublished); (*e*) from Jaffe & Stern (1979).

namely fucoid embryos, growing pollen tubes, *Blastocladiella* plantlets during papilla initiation (Stump *et al.* 1980) and *Xenopus* oocytes.

The *Cecropia* follicle is a particularly interesting case: external field measurements indicate a main, transfollicular current loop that enters, and presumably leaks into, the nurse cell end; they also indicate a less intense *furrow current*, which leaves the nurse cell – oocyte furrow; while internal measurements (with conventional microelectrodes) indicate an extremely intense, internal counter-loop. All these currents may be driven by a 'furrow battery' established by the apposition of the pumping, basal face of the nurse cells to the leaking apical face of the sibling oocyte. Recent, as yet unpublished, work of D. W. Kline & R. Nuccitelli also shows a small (not more than 1 $\mu A/cm^2$) current leaving the furrow of the cleaving *Xenopus* egg. However, this furrow current enters the pre-furrow region just ahead of the growing furrow. That current leaves this furrow might have been predicted from older observations that the new, furrow membrane has a relatively high transmembrane potential, apparently due to its high potassium ion permeability (DeLaat & Bluemink 1974). That current enters the pre-furrow region suggests that a transient cation leak is associated with, and perhaps even induces, contractile ring formation.

Another remarkable and as yet unpublished finding is that of Kenneth Robinson on early limb bud (stage 49) *Xenopus* embryos. Exploration of the field around such embryos indicates a strong (1–10 $\mu A/cm^2$) current leaking out of the hindlimb bud and returning under the gill flap or operculum (figure 5). This limb-bud–gill current is the only one detected at this stage;

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among the other areas explored and found to show no currents were the tentacles, eyes, mouth and anus. It is interesting that the normally developing amphibian limb bud exhibits strong outward current comparable with those found to leave limb stumps before regeneration (Borgens *et al.* 1977*b*). Furthermore, two preliminary observations suggest that this limb-bud current precedes and predicts the site of bud formation, and that it is closely correlated with bud growth. First, exploration of the general presumptive limb-bud area at stage 45 reveals a small (of the order of 100 μm diameter) spot with comparable currents leaving it. Secondly, treatment of the early (stage 49) bud with 20 μM retinoic acid causes it to regress and entirely disappear within a day. At this time the outward limb-bud current has likewise disappeared.

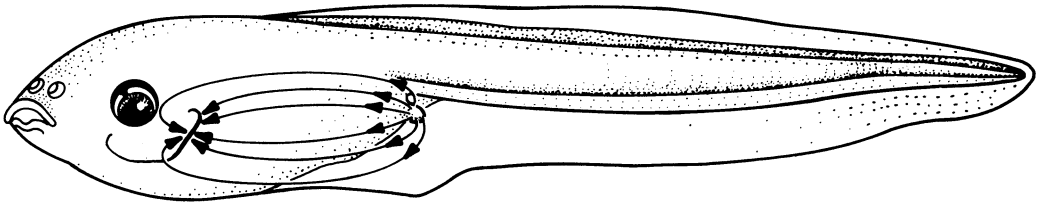

FIGURE 5. Electrical current pattern through stage 49 *Xenopus* embryos. Current is pumped into the gills through the opercular opening and leaks out of the hind-limb bud. (From K. R. Robinson, unpublished.)

VOLTAGE AND ION GRADIENTS IN DEVELOPING SYSTEMS

Developmental currents are surely widespread. However, living systems have no galvanometers that respond to the magnetic fields associated with currents. For steady currents to act back on the cells and tissues that drive them, they must encounter enough resistance to generate physiologically significant voltage gradients or specific ion gradients, or both. What evidence exists for such gradients?

1. *Paracellular and extracellular voltage gradients*

Good evidence for small (3–6 mV) voltage drops across a number of developing epithelia (e.g. the early mammalian blastocyst wall) has been published. Moreover, large (80 mV) voltage drops have long been known to appear along the surface of certain plant shoots in response to unilateral light or gravity (reviewed in Jaffe & Nuccitelli (1977), pp. 454–455; see also Powers *et al.* (1977)). Recently, large voltage gradients have been measured along the outside of another normally aerial creature, namely the guinea pig. When an incision is made through the general, i.e. hair and gland-free, epidermis of the cavy, about 10 μA is found to flow through each linear centimetre of incision. This current is pumped into the body by the cavy's powerful skin battery and returns through the incision and then along or near the thin layer of extracellular fluid between the living and dead layers of the epidermis (figure 6). On the way, it generates the remarkably large voltage gradient of 100–200 mV/mm. This voltage gradient can be considered to be developmental since it precedes (and perhaps accompanies and guides) the epidermal cell migration that closes such wounds.

2. *Intercellular and intracellular voltage gradients*

Woodruff & Telfer (1973; see also Telfer *et al.* 1981) have demonstrated a substantial (5–10 mV) standing voltage difference in one important *intercellular* case, namely across the coarse cytoplasmic bridge that joins the oocyte and nurse cells of the developing *Cecropia* follicle

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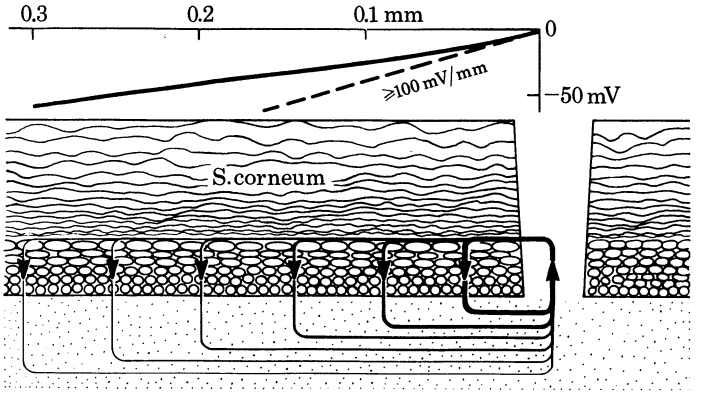

FIGURE 6. Developmental voltage gradients in wounded cavy skin.
(From A. T. Barker, L. F. Jaffe & J. W. Vanable, Jr, unpublished.)

(figure 4*c*). To my knowledge, no one has yet convincingly demonstrated a significant, steady *intracellular* voltage gradient in any developing system. However, some interesting reports of such voltage gradients within a number of non-developing cells (e.g. active melanophores) should be noted. Reports in the older literature are reviewed in Jaffe (1969), p. 105; more recently Zeuthen (1978) has published relatively sophisticated evidence for such gradients within gall bladder and intestinal epithelia.

3. *Intracellular ion gradients*

A striking accumulation of sequestered or bound calcium, or both, in the tips of growing pollen tubes and other tip-growing plant cells is indicated both by ^{45}Ca radioautography (Jaffe (L. A.) *et al.* 1975; Jaffe (L. F.) *et al.* 1975) and by chlorotetracycline fluorescence (Reiss & Herth 1979). With the aid of the photoprotein aequorin, large transcytoplasmic gradients o *free* calcium ions have been demonstrated in certain activating fish eggs (Gilkey *et al.* 1978).

I predict that these few cases of demonstrated voltage and ion gradients in developing systems will prove to be but first glimpses of very widespread phenomena indeed.

ARTIFICIAL FIELD EFFECTS

1. *Lateral or* in situ *electrophoresis*

Charged particles floating in the cell membrane should be an important target of field effects on cells and tissues. A simple theoretical analysis provides equations that indicate that the order of 1 mV per cell diameter should suffice to substantially redistribute many such membrane components at the steady state, with back-diffusion (Jaffe 1977).† The existence of such 'lateral', or '*in situ*' electrophoresis has been well confirmed by observations of the electrical redistribution of various lectin receptors, etc.; moreover, the field strengths and times needed

† It might easily be thought that the Nernst equation could be applied to the relation between voltage difference and concentration ratio in this steady state. Therefore, it should be noted that the Nernst equation is *not* applicable here, and can give radically erroneous results in this instance. The Nernst equation applies to the true equilibrium across a membrane solely permeable to the charged species considered. It would be applicable to the electrophoretic redistribution of a multiply charged particle such as a protein only if it were being redistributed across a membrane permeable to this charged particle and not to its counter-ion. In the degenerate case, where the 'particle' is a small, singly charged ion, it can be shown that the two situations are governed by the same equation. However, for a given distribution ratio of a 10 nm, multiply charged particle, the Nernst equation predicts a voltage an order of magnitude smaller than does equation (2) of Jaffe (1977).

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are compatible with the original simple theory (Poo & Robinson 1977; Poo 1981). However, the field-driven components are usually found to move towards the negative pole and thus opposite to the expected direction! I have suggested earlier that the explanation of this important anomaly lay in electro-osmosis. That is to say, the lectin receptors, or other observed components, are hydrodynamically dragged towards the negative pole by water, which is in turn dragged by electrophoretically driven counter-ions such as Na^+. The latter balance various fixed negative charges, e.g. those of sialic acid on the cell's surface. This concept has now been mathematically expressed and to some degree experimentally confirmed by McLaughlin & Poo (1981).

2. *Galvanotropism* in vitro

As early as 1923, Lund showed that the developmental axis and polarity of fucoid eggs could be controlled by steady fields of 5 mV or more per cell diameter. Since then, a wide variety of simple, and normally isolated, plant cells (such as fucoid eggs and moss spores) have been shown to be polarizable by small steady fields. Detectable effects appear at about 0.3–5 mV per cell diameter, and large effects, sometimes approaching complete growth polarization, are induced by field strengths an order of magnitude higher (reviewed in Jaffe & Nuccitelli (1977), p. 463; see also Brower & McIntosh (1980)). It should be borne in mind that such normally isolated and aqueous plant eggs, spores, etc., are certainly *not* exposed to fields of the strengths found to polarize them under natural conditions. On the one hand, then, these data can offer only rather indirect insights into natural control mechanisms. On the other hand, they suggest that cells such as aerial eggs or cells within metazoan embryos, which may well be exposed *in vivo* to fields as strong as those, have evolved a capacity to use such fields, or even weaker ones, to organize or direct their development.

Indeed, a remarkable paper by Hinkle *et al.* (1981) reports the responses of dissociated embryonic *Xenopus* neuroblasts to small steady fields. The site of neurite initiation is not affected by the field, but those neurites that happen to grow towards the negative pole or cathode tend to grow faster than those growing towards the anode; neurites that happen to grow out across the field then tend gradually or abruptly to grow towards the cathode. Clear responses were found down to 7 mV/mm. This report may be compared with the one unambiguous previous report of a galvanotropic response of neurites *in vitro* (Jaffe & Poo 1979). Neurites growing out from chick dorsal root ganglia likewise grew faster towards the cathode than the anode in a steady field, although clear responses were only observed down to about 70 mV/mm. One imaginable mechanism of these responses would be the 'lateral' electrophoresis of receptors for nerve growth factor along neurite membranes towards the cathode – a possibility that receives some support from Gundersen & Barrett's recent demonstration (1980) that chick dorsal root neurites tend to grow up gradients of nerve growth factor *in vitro*. Furthermore, since these neurites likewise tend to grow up calcium ion gradients in the presence of the calcium ionophore A23187, one wonders if calcium currents naturally enter growing neurite tips just as they enter the tips of tip-growing plant cells.

Hinkle *et al.* (1981) also studied the responses of dissociated embryonic *Xenopus* myoblasts to small steady fields. They discovered that the initially spherical myoblasts tend to grow out *perpendicular* to an applied field so as to form muscle cells aligned across the field. This tendency is first detectable at about 35 mV/mm (or 0.7 mV per cell diameter) and becomes very strong and striking indeed at field strengths of about 150 mV/mm.

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Direct evidence that ion currents help control development

General aspects

Xenopus neuroblasts and myoblasts respond to steady fields as small as 7–35 mV/mm *in vitro*, while wounded cavy skin generates fields of 100–200 mV/mm *in vivo*. This comparison suggests that natural fields may well help direct development, but proofs will clearly depend upon direct demonstrations of electrophoresis *in vivo*; or, better still, upon direct demonstration that the modification of natural fields or ion currents affects development. However, before discussing the available evidence of this sort, I wish to put my expectations of the developmental role of ionic currents within the general conceptual framework of this symposium.

Early in normal development, or in morphallactic regeneration, when pattern is first being foreshadowed, I should expect ion currents to help establish so-called positional values. However, later in normal development, or in epimorphic regeneration, the evidence – particularly that discussed in this symposium by French – suggests that positional values are established or re-established by highly localized mechanisms akin to crystallization or self-assembly. However, these later processes do require growth. I wish to propose that *this growth is initiated by ion leaks through sites of discontinuity in positional value*. Two specific cases come to mind. One is limb formation or regeneration in vertebrates as well as invertebrates. From this point of view, so-called complete circles would induce growth because they create positional discontinuities through which ions leak. The other is mouth formation in Protozoa. Tartar has shown that new mouths arise in *Stentor* at visible loci of stripe contrast (see Tartar 1962). I would suggest (1) that these 'contrast' loci are closely akin to the invisible discontinuities in positional value postulated in the well known French, Bryant & Bryant model, and (2) that they will prove to be sites of ion leakage which then somehow induce mouth formation. Perhaps the closely related phenomena of mouth induction in *Tetrahymena* – discussed elsewhere in this symposium by Frankel – can likewise be effectively considered from this viewpoint.

Polarization of fucoid eggs

As noted above, direct $^{45}Ca^{2+}$ experiments have shown that calcium ions are an important, and perhaps even major, component of the currents that enter the future growth region of photopolarizing fucoid eggs (Robinson & Jaffe 1975). More recently, Robinson & Cone (1980) found that local application of the Lily ionophore A23187 tends to establish the growth region towards the side of drug application. Furthermore, they provide strong evidence that the drug acts by making membranes leaky to calcium ions there. Together, these two observations strongly suggest that local calcium ion entry is part of the amplification or positive feedback loop that establishes a growth point (and the rhizoid pole) in these cells.

Self-electrophoresis in Cecropia *follicles*

Some time ago, Woodruff & Telfer (1973) found that an injected electronegative protein, fluorescein-labelled serum globulin, moves across the intercellular bridges from the electronegative nurse cells to the electropositive oocyte, but does not move back from the oocyte to the nurse cells. More recently, they have found that an injected electropositive protein, fluorescein-labelled lysozyme (or 'Fly'), exhibits one-way movement the other way, that is from the oocyte to the nurse cells; that lysozyme converted by methyl carboxylation of its terminal

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amino groups into an electronegative protein ('McFly') will only move from the nurse cells to oocyte; and that two near-neutral proteins, fluorescein-labelled haemoglobin and myoglobin 'were able to move in both directions across the bridges'. Taken together, these data provide strong evidence for self-electrophoresis in the developing *Cecropia* follicle. It would not be surprising if the materials transported into the anterior end of the oocyte in this way are to some extent localized there and help establish positional values in the *Cecropia* egg. I should, perhaps, also bring the reader's attention to a (somewhat preliminary) extension of this important approach to another insect follicle, the telotrophic *Rhodnius* follicle (Telfer *et al.* 1981).

Initiation of amphibian limb regeneration

Substantial evidence exists for the proposition that amphibian stump currents help initiate limb regeneration. This has been recently reviewed by Borgens *et al.* (1979*c*). In brief, the main evidence is as follows. (1) Currents of the order of 10–100 $\mu A/cm^2$ leave the stumps of amputated newt (and other amphibian) limbs for about a week after amputation (Borgens *et al.* 1977*b*). (2) Inhibition of these (skin-driven) stump currents in naturally regenerating species (by means of sodium-deficient media or amiloride) markedly inhibits regeneration (Borgens *et al.* 1979*a*). (3) Artificial supplementation of these stump currents (by means of implanted batteries) in normally non-regenerating or poorly regenerating species substantially stimulates regeneration (Borgens *et al.* 1977*a*, 1979*b*). (4) It has long been known that virtually any kind of chronic injury to salamander limbs – including insertion wounds, ligatures, fractures, degenerating implants, and ultraviolet irradiation – will induce the formation of a supernumerary limb or limbs (briefly reviewed in Ruben & Frothingham (1958)). Since such injuries would be expected to damage the skin and make it leaky to ions, the electrical hypothesis offers a reasonable explanation for such limb induction.

A MODEL OF SERUM-INDUCED POLARITY REVERSAL IN THE THYROID

Figure 7 shows the essentials of this model. (1) The undisturbed follicle (figure 7*a*) is supposed to maintain small transcellular current loops driven by basolateral sodium ion pumps. Charges, mainly Na^+ ions, leak back through the tight junctions and the apical membrane to complete the loops. (2) A rise in basal serum is supposed to rapidly open sodium ion, and perhaps calcium ion, channels in the basal membranes. These leaky basal membranes, together with the persisting pumps in the lateral membranes, rapidly establish relatively large, new, basolateral current loops, which flow out of the lateral spaces and back through the basal membranes, as shown in figure 7*b*. (3) These basolateral loops become self-maintaining by keeping membrane-active serum components out of the lateral spaces. Initially, the new loops exclude these components by squeezing fluid out of the lateral spaces. They generate the needed intracellular pressure by rapidly depolarizing the cell membranes; this in turn allows chloride to follow sodium into the cells and then water to follow the sodium chloride. After the lateral spaces collapse (figure 7*c*), their electrical resistance rises and the new loops continue to exclude these serum components by electro-osmotically driving water out these spaces. Since these serum components are likely to be positively charged, the new current loops may also keep them out by direct electrophoresis. (4) Electro-osmotic flow then slowly drags prejunctional components along the lateral membranes to the mouths of the lateral spaces where they aggregate to form new, basal tight junctions and thus the first visible components of a second apical pole. (5) The

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new current loops also change the ionic composition of the basal cytoplasm, particularly by raising Na^+ and Ca^{2+} and lowering H^+ there. These changes induce microvilli to form basally and thus the second visible component of the second apical pole. (6) Further changes may be partly mediated by reduction of the residual apicolateral current loops via closure of sodium leaks in the original apical membrane.

Some of the evidence that suggests and supports this model is as follows. (1) Reconstructed hog thyroid follicles in culture show transepithelial potentials of about 30–40 mV, inside negative (Mauchamp *et al.* 1979). So thyroid follicles maintain voltages of the magnitude and in the direction typical of many other tight epithelia. Moreover, there is evidence that the tight junctions in certain leaky epithelia (where their character is more evident) are indeed permeable primarily to Na^+ rather than Cl^- (Frizzell *et al.* 1979). (2) In 1971, Hülser & Frank reported that 10% calf serum depolarizes embryonic rat fibroblasts by 30 mV within minutes; more recently, Moolenaar & de Laat (1979) report that 30% calf serum depolarizes mouse neuroblastoma cells by 30 mV within seconds. The latter evidence further indicates that most of the

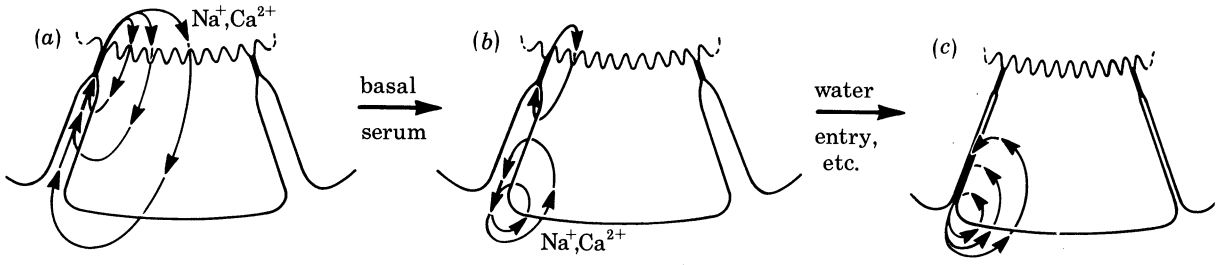

FIGURE 7. A model of the reversal of thyroid cell polarity by basal serum.

observed depolarization is due to the opening of Na^+ channels, but suggests that Ca^{2+} channels may also be opened. Furthermore there is a good deal of tracer evidence (reviewed by Rozengurt & Mendoza 1980) that increases in the level of serum speed the entry of Na^+ into various tissue culture cells of fibroblastic origin. These data provide some precedent for the proposed effects of serum on thyroid cells in culture. (3*a*) If the proposed depolarization by serum holds, then cell swelling is almost a theoretical necessity. Experimentally, rapid swelling of muscle cells in response to potassium depolarization is well established (reviewed by Davson (1970), p. 567ff), as is the swelling of a variety of other vertebrate cells in response to high external potassium, inhibition of metabolism, ultraviolet or viral permeabilization of the cell membrane, and other changes that should depolarize the membrane (reviewed by MacKnight & Leaf 1977). Furthermore, the substantial increases in total potassium found in certain serum-treated tissue culture cells indicate and involve cell swelling (Rozengurt & Heppel 1975; Tupper *et al.* 1977). (3*b*) An interesting precedent for the collapse of the lateral or intercellular space in an epithelium is furnished by the rapid collapse of these spaces in *Necturus* gall bladder in response to replacement of mucosal sodium chloride with sucrose or tetramethylammonium chloride (Spring & Hope 1979). (3*c*) Hints that membrane-depolarizing serum factors are basic proteins are furnished by the facts that so-called fibroblast-derived growth factor and fibroblast growth factor are both basic proteins (Bourne & Rozengurt 1976; Gospodarowicz *et al.* 1978). (4) Evidence that serum factors raise cell sodium in certain other cells has been noted above. Evidence that serum factors speed Na^+/H^+ exchange (and should therefore tend to decrease cytoplasmic H^+) in neublastoma cells has been reported by Moolenaar *et al.* (1981). Evidence

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that increases in cytosolic sodium in turn trigger calcium release is available for a number of cells, e.g. Langerhans islets (Lowe *et al.* 1976), while evidence that serum factors raise net calcium within embryonic rat fibroblasts has been reported by Frank (1973) Evidence that a comparable constellation of ionic changes – namely increases in Ca^{2+} and Na^{+} and a decrease in H^{+} – accompany the induction of microvillar formation in various fertilized eggs (particularly sea urchin eggs) has been reviewed by Jaffe (1980); evidence that it is the alkalinization in particular that induces the polymerization of actin in these forming microvilli is reported by Begg & Rebbun (1979). (5) Taylor & Windhager (1979) review evidence from various epithelia that a rise in cytosolic calcium at the basal pole somehow inhibits sodium entry at the apical pole.

Finally, I would point out that this theory makes a number of more or less easily testable predictions: that serum sharply depolarizes thyroid cells; that it swells them and shrinks the spaces between them; that it induces a flow of current out of these spaces; that it inhibits the entry of macromolecules, particularly electropositive ones, into these spaces; that it raises cytosolic Na^{+}, Ca^{2+}, pH, etc.

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