Electric Currents May Guide Development

Naturally generated electric currents have been linked to changes in developing embryos and in regenerating limbs

Ever since Luigi Galvani found, some two centuries ago, that frog legs jumped when he hooked them up to what was essentially a primitive battery, research on the biological effects of electric currents has waxed and waned, even falling into disrepute at times. Lately it has begun to wax again, buoyed by reports that electric currents, generated within embryos themselves, guide the development of species as diverse as brown seaweeds, frogs, insects, and chicks. Lionel Jaffe of Purdue University says, "We knew from physiology that the electrical machinery was there; living cells have these powerful batteries in their membranes. If they were distributed unevenly, they would lead to electric currents that might affect cell processes."

Regeneration of body parts, which amphibians such as salamanders do very well and higher animals such as humans do very poorly, has also been linked to electric currents in the regenerating parts. Although the possibility of growing new human limbs is remote, to say the least, electric currents are already being used to promote healing of bedsores and bone fractures that have failed to respond to other treatments.

As an embryo develops from a single cell, the fertilized egg (zygote), to an organism with perhaps many millions of cells, its constituent parts are constantly undergoing rearrangements. "The problem," as Jaffe describes it, "is how to develop pattern out of no pattern."

Pattern formation in the egg may start very early, even before fertilization. Egg cytoplasm is often not uniform; it is organized into clearly distinguishable zones, which give the egg a polarity, a top and bottom, that is maintained when it starts to divide. Still unsolved is the question of what causes the polarity and preserves it through embryonic cell divisions and rearrangements.

Recent research suggests that embryos produce electric currents that are related to the establishment of polarity and to the unfolding of other developmental events. These currents—in contrast to the brief ones, with durations in the millisecond range, that flow in firing nerve and muscle cells—may last for hours or even days. The existence of the embryonic currents does not necessarily mean that they help to control development, but in at least one species there is strong evidence that they do.

The species is a lowly alga, the brown seaweed *Pelvetia fastigiata*. The origin of polarity in *Pelvetia* eggs is especially easy to study because, in contrast to many other types of eggs, they do not become polarized until a few hours after fertilization. Until then they are spherical and uniform in appearance. In addition, the orientation of the polar axis can be controlled by shining a light on the eggs from one direction or another.

The study of electric currents in embryos was facilitated by the invention, in 1974, of a vibrating microelectrode that can measure currents in a completely noninvasive way, without disturbing the embryo. The microelectrode, which was devised by Jaffe and Richard Nuccitelli, who is now at the University of California at Davis, consists of a probe terminating in a platinum black ball, about 10 to 30 micrometers in diameter. As the probe vibrates between two points in the aqueous fluid surrounding an embryo, it detects the voltage drop between those two points that is produced by an electric current from the embryo. Ohm's law can then be used to calculate the current density from the voltage drop and the resistance of the fluid, which is also easily measured.

Using the vibrating probe, Jaffe and Nuccitelli found that the fertilized *Pelvetia* egg, in Nuccitelli's words, "drives an ion current through itself as early as we can measure, which is about 30 minutes after fertilization. . . . The inward current region always predicts the germination site."

Germination in the *Pelvetia* egg begins with changes in the cytoplasm, including movement of vesicles that contain materials needed for germination to the site where it will occur. Then the site, which is on the side of the egg away from that illuminated by an incident beam of light, begins to bud outward. Jaffe and Nuccitelli find that the inward current is always located at the potential germination site and appears at least 6 hours before budding begins.

The site of germination in *Pelvetia* eggs can be changed by changing the orientation of the incident light beam, provided it is done early enough, after the vesicles have moved but before the bud forms on the egg. In that event the position of the inward current always changes first, moving to what will be the new germination site, another indication that the current is defining the site.

Eggs or early embryos of other species also generate ionic currents that have been correlated with developmental events. Unfertilized frog (*Xenopus laevis*) eggs are already polarized into a dark pigmented half, the animal pole, and a clear half, the vegetal pole, before they are released from the ovary. Kenneth Robinson of the University of Connecticut Health Center in Farmington has identified a current that flows out of the vegetal pole and into the animal pole of frog eggs taken while they are in a quiescent preovulatory stage. When these eggs are induced to mature by hormonal treatment, the current decreases to about one-tenth of its initial density. Robinson hypothesizes that the current somehow holds the eggs in the quiescent state.

Strong ionic currents reappear in frog eggs that have been fertilized and have started to divide, however. The cleavage furrow that forms to separate the two new daughter cells begins at the animal pole and moves down the egg to the vegetal pole. According to Nuccitelli, the dividing eggs generate a current that flows out of the furrow, where new

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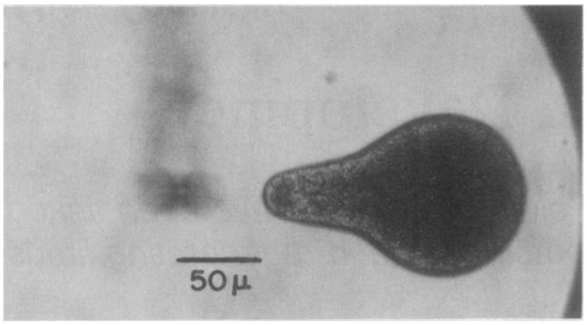


Germinating Peletia *egg, with a vibrating microprobe just to the left. [Source: Lionel Jaffe, Purdue University]*

membrane has been formed, and enters the region just ahead of the moving furrow. "The inward current is always strongest," Nuccitelli says, "in the region where the furrow will next appear as it moves down the egg." He has observed an analogous current in the dividing zygote of the zebra fish.

At present, investigators do not understand how these currents might drive developmental events, although there are a number of possibilities. The currents are generally carried by moving ions. In some cases, at least part of the current depends on the migration of calcium ions into cells. In a series of experiments, Robinson and Jaffe have shown that this happens in the fertilized *Pelvetia* egg. Conversely, part of the outward current is caused by an efflux of calcium ions from the other end of the zygote. As a result, a concentration gradient is established, with the highest concentration of ions at the germinating pole.

Finally, Robinson has shown that exposing the eggs to calcium ion gradients triggers germination in the expected direction; the side exposed to the highest concentration is the one to bud. Calcium ions are known to regulate what sometimes seems to be a vast array of cellular activities, and a local increase in their concentration might well trigger those needed for developmental changes.

Even without evoking any specific ion effects, electric currents might affect cellular activities by causing a redistribution of cell components, which usually carry net positive or negative charges. In other words, biochemists may not have been the first to employ electrophoresis, in which charged molecules are separated by subjecting them to electric fields. If electrophoresis does occur within the cell, it might be the cause of such changes as the migration of vesicles in the *Pelvetia* egg before it germinates.

Mu-Ming Poo of the University of California at Davis has shown that membrane components may redistribute themselves in response to electric currents. And electrophoresis can also occur in the cell cytoplasm, according to Richard Woodruff and William Telfer of the University of Pennsylvania, who have been studying the egg of the silk moth *Hyalophora cecropia*. This egg comes attached to seven nurse cells by narrow bridges of cytoplasm. The nurse cells produce RNA's and other material for the oocyte, and these substances must move through the bridges.

Woodruff and Telfer have shown that the equilibrium potential of nurse cells is several millivolts more negative than that of the oocyte. Moreover, proteins move through the bridges as predicted by the direction of the electric gradient. Woodruff says, "We think that the electric gradient sets up an electrophoresis situation." Because RNA's are usually negatively charged, this cellular form of electrophoresis may be what propels them into the oocyte.

Limb regeneration is a developmental problem not unlike embryogenesis. Both require that undifferentiated cells divide and form cells that are destined to produce bone, muscle, cartilage, and other tissues with specific functions. In amphibians, such as the salamander and newt, there is good evidence that electric currents might foster regeneration of amputated limbs.

That this might happen is not a new idea. Over the years several investigators have reported changes in voltage patterns at the surface of amputated limbs. For example, in the early 1960's, Robert Becker of the Upstate Medical Center of the State University of New York compared the voltage differences in the amputated forelimb stumps of salamanders with those in the frog, which is not capable of limb regeneration. The changes followed a specific pattern in regenerating salamander limbs that was quite different from that in the frog limb.

More recently, Richard Borgens of the Jackson Laboratory, Joseph Vanable of Purdue University, and Jaffe used the vibrating probe to measure the currents produced by newt limbs before and after amputation. Before amputation there was an inward current more or less evenly distributed over the limb surface. After it, the inward current was increased in the skin-covered surface of the stump and there was a dramatic efflux of current from the cut end. Borgens says, "We found that very large currents are driven out of an injured limb, up to 100 microamperes per square centimeter of tissue. At the time [1977], this was the largest current measured in any developing system." The question, according to Vanable, was "whether the currents that exist have a role in helping regeneration, or are just there."

Early evidence that they help regeneration came from Stephen Smith of the University of Kentucky, who showed in the late 1960's that applying a small direct current to the amputated forelimb stumps of frogs produced at least partial regeneration of the limb. Because adult frogs are not normally capable of any degree of limb regeneration, this was a striking result.

Borgens, Vanable, and Jaffe have confirmed Smith's report of partial regeneration of electrically stimulated frog limbs, but they have never seen an example of complete regeneration. In contrast, Smith reports that "extremely rarely, if conditions are exactly right, we may get a hand back." He does not yet know what conditions are exactly right, however, and other investigators are skeptical of the occurrence of complete regeneration.

The source of the electric currents in regenerating limbs is also a bone of contention. In his early papers Becker suggested that they originated in the nervous system, either in the neurons or in the cells associated with the neurons. He based this hypothesis partly on his maps of the electric field potential in the salamander. Areas with the most positive voltages corresponded to the locations of the three largest accumulations of central neurons in this species, whereas those with the most negative voltages coincided with the termination of the major peripheral nerves leading out of the central neuron concentrations. This suggested that the central neurons were the source of the salamander's bioelectric field. Becker also points out that Marcus Singer of Case Western Reserve University found that amphibian limbs would not regenerate unless the stump contained some critical percentage of nerve cells.

Borgens, Vanable, and Jaffe do not dispute the fact that nerves are needed for regeneration, but they do not think neurons are the source of the stump currents. They propose, as Borgens puts it, that "the skin surrounding the limb stump is a battery that drives the current." Other investigators, including

Bernard Lassalle of the Université des Sciences de Lille, France, have found that removing the nerves from salamander limbs does not affect their surface potential, but removing the skin virtually abolishes it. Moreover, when the nerves in a limb are cut before it is amputated, the resulting current out of the stump is, if anything, larger than usual.

To further test the hypothesis that the skin is the source of the stump currents, Borgens, Vanable, and Jaffe attempted to knock out the skin battery of newts to see what happens. Amphibian skin contains an active "pump" for moving sodium ions from the external medium to the inside, and the investigators hypothesized that this pump might be the source of the current. They added the drug Amiloride, which blocks sodium transport by the skin pump, to the newts' water. What they found, Vanable says, "is that it reduces the current to a considerable extent. And about half the animals regenerated poorly or not at all." But he continues, "The data are frankly not clear-cut; it is easy to be skeptical because half the animals were able to regenerate anyway."

In another experiment the investigators are attempting to block the stump current by applying an opposing current generated by a battery. "So far," Vanable says, "these results seem more clear-cut; the opposing current prevents or inhibits regeneration, and augmenting the current seems to accelerate it."

Smith, with Arthur Pilla of Columbia University's College of Physicians and Surgeons, has been testing the effects of low-level pulsating currents, rather than direct currents, on salamander limb regeneration. They find that such currents can produce anything from degeneration of the stump, to no change, to acceleration of regeneration, depending on the characteristics of the currents used.

Frogs have active sodium pumps in their skin, too, even though they do not regenerate at all unless the limb stumps are electrically stimulated. The difference between them and salamanders or newts may lie in the fact that frogs have lymph spaces immediately under the skin, which could shunt the current away from the underlying tissues and prevent it from reaching them. When Borgens, Vanable, and Jaffe measured the current flowing out of the stumps of amputated frog limbs, they found it to be concentrated just under the skin.

Although nerves may not be the source of the limb currents, they may be the targets. Borgens, Vanable, and Jaffe detect unusually large quantities of nerve tissue in electrically stimulated frog stumps. Moreover, Robinson recently found that embryonic frog nerves, when grown in culture under the influence of an electric field, grow toward the negative pole of the field, even turning through large angles to do so. In addition, the field stimulates the growth of greater numbers of nerve projections.

In contrast to the embryonic neurons, which line up parallel to an electric field, cultured embryonic muscle cells line up perpendicular to the applied field. Robinson points out that this resembles the situation in the living embryo, where the developing neurons connect with muscle cells at right angles. "It is tempting to speculate" he says, "that embryonic currents are guiding the nerve growth."

Mammals are very poor at regeneration, but even humans have some ability to restore lost tissue. Simple wound healing, although a meager talent compared to regrowing whole limbs, is one example.

Clinicians are using electric currents to promote the healing of bone fractures in human patients. In many cases, these fractures had proved resistant to conventional therapies, often remaining unhealed for years or decades. However, Becker, who is one of the pioneers of the technique, expresses concern that it is being used too often and inappropriately on people who might respond to other treatments. He maintains, "There are basic zones of uncertainty that are of considerable importance." In particular, he was referring to the possibility that the electric currents, while stimulating normal bone regrowth, might also convert a normal growth process to a malignant one.

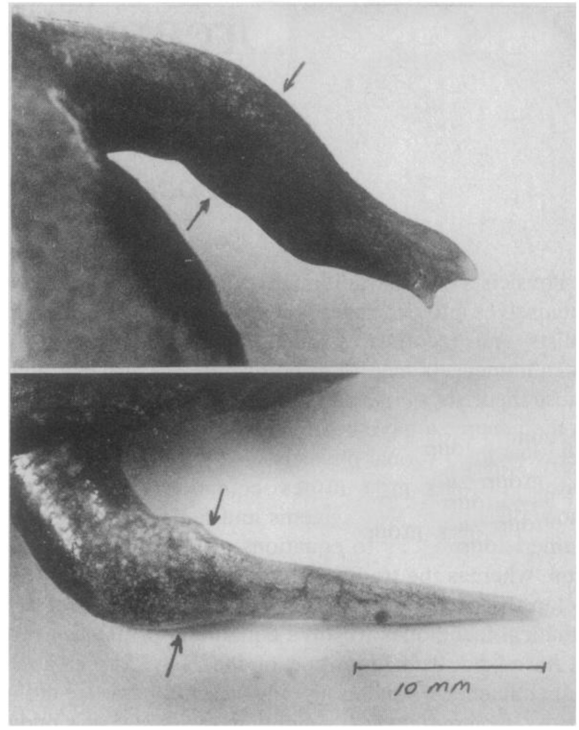
Children under the age of 11 can regrow lost fingertips, according to Cynthia Illingworth of Children's Hospital in Sheffield, England, and B. S. Douglas of Children's Hospital in Adelaide, Australia. To regenerate, the fingers must have been severed above the first joint.

No one knows yet whether electric currents have anything to do with this ability, although Illingworth and Anthony Barker have detected currents leaving the cut surfaces of fingertips. There is also an interesting resemblance between fingertip regeneration in children and limb regeneration in amphibians. A salamander will not regenerate its limb if the stump has been covered with skin, presumably because this blocks the strong outward current. For a child's fingertip to grow back, the stump must also be left uncovered. It is just cleaned and the finger splinted to protect it. In that case, the fingertip will grow back, complete with nail and fingerprints.

Investigators are currently experimenting with mammals, primarily rats and mice, to see if they can regenerate body parts. For example Borgens is trying to determine whether the amputated digits of mouse paws will grow back, and Smith is looking at the effects of electric currents on the regeneration of amputated rat limbs.

Few people expect that regeneration of complete human limbs will ever be possible. Nevertheless, lesser regeneration of specific tissues may be.

—JEAN L. MARX



Influence of electric current on frog limb regeneration. The upper limb, which received 0.1 microampere of current, regenerated in a more normal form than the sham-treated one below. The arrows indicate the plane through which the limbs were amputated. [Source: Joseph Vanable, Purdue University]