2

Endogenous Electric Fields in Animals

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CONTENTS

2.1 Introduction

In a volume presenting the biological effects of electromagnetic fields it is appropriate to review the information we have regarding endogenous electric fields in the body. After all, imposed electromagnetic fields may augment the naturally occurring ones, so a complete understanding of the possible effects of imposed fields requires consideration of those electric fields already present. Here, I will provide a brief overview of the direct current (DC) endogenous fields that have been best characterized in animals and will touch on the evidence that these electric fields are required for the function of various cellular and organ systems. Other well-known variable fields that are generated by various electrically excitable organs such as the heart (electrocardiogram), brain (electroencephalogram), and

eye (electrooculogram) will not be covered here. Another very comprehensive review of endogenous fields that will be of interest has appeared quite recently in *Physiological Reviews* by McCaig et al. (2005), and another review of the roles of such fields in development was presented by Levin (2003).

In order to put these endogenous fields in perspective for the reader, I would like to summarize here their main characteristics. Unlike much of the material considered in this volume, these endogenous fields are small and very slowly changing. Endogenous fields typically fall into the 10- to 100-V/m range and are generally very steady, DC fields generated by the flow of ionic currents through cells and embryos. This can be compared with the much higher fields required to electroporate cells (3 V/cell diameter or 3×10^5 V/m for a 10-μm-diameter cell), which are usually only applied for a short time on the order of a millisecond.

2.1.1 Sources of Endogenous Electric Fields

In order to generate an electric field in the body, a voltage generator or power source is required. There are two main sources of such a power source in living systems: (1) the plasma membrane surrounding every cell in the body and (2) the epithelium that surrounds every organ in the body as well as the entire body itself in the form of the skin. The plasma membrane forms the defining boundary for every cell and is a lipid bilayer with many embedded transporter proteins whose main function is to control the movement of molecules inside or outside of the cell. One of these transporter proteins is the Na^+/K^+-ATPase, which is responsible for maintaining two ion concentration gradients across the plasma membrane (high internal [K^+] and low internal [Na^+]). The K^+ concentration gradient, in combination with a large number of K^+ channels in the plasma membrane, results in the outward diffusion of K^+. This outward movement of K^+ leaves behind the anion that was associated with maintaining electroneutrality and thereby separates charge across the membrane. This separation of charge generates a voltage difference or membrane potential (inside negative). This membrane potential is used for a wide variety of cellular functions, from capturing nutrients to signaling the occurrence of important events, such as sperm–egg fusion in many egg types or light absorption in retinal rods. Indeed, we expend more than half of our energy in maintaining this voltage across all of the cells in our brain and kidney, where excitatory events and Na^+-dependent transport constitute major functions (Clausen et al., 1991).

Voltage differences are also found across all epithelial layers, and this is called the transepithelial potential (TEP). Both organs and embryos are surrounded by one or more monolayers of cells called an epithelium. The outer epithelium belongs to the organ while the plasma membrane to the cell, and epithelia pump ions across themselves to generate the TEP. One clear difference between these two voltage sources (plasma membrane and epithelial layer) is their opposite polarity. Whereas the plasma membrane potential is usually negative on the inside with respect to the outside, the TEP is usually positive on the inside or basal side of the epithelial monolayer with respect to the outside or apical side of the monolayer.

This polarity difference results from the opposite flow of charge across these layers. The plasma membrane of most animal cells generates a potential difference across itself that is negative on the interior. This results from the outward diffusion of K^+ that separates charge across the membrane. In contrast, most animal epithelia are composed of highly polarized cells in which Na^+ channels are localized to the apical end of the cell and both the K^+ channels and the Na^+/K^+ ATPases are localized at the basal end. This polarized distribution of these transport proteins leads to Na^+ influx at the apical end and both Na^+ and K^+ efflux at the basal end to drive positive charge to the interior of the epithelial

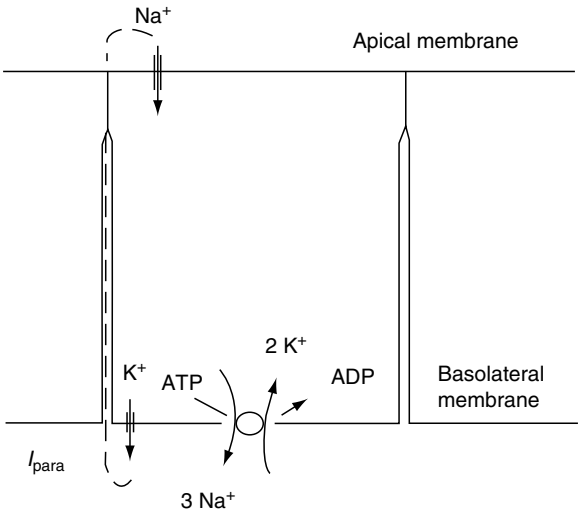

FIGURE 2.1
Diagram of a typical epithelial cell in a monolayer with Na^+ channels localized on the apical plasma membrane and K^+ channels localized on the basolateral membranes along with the Na^+/K^+-ATPase. This asymmetric distribution of ion channels generates a transcellular flow of positive current that must flow back between the cells through the paracellular pathway (I_{para}). This current flow generates a TEP that is positive on the basolateral side of the monolayer.

layer. However, this current cannot flow freely in the extracellular medium because these polarized epithelial cells are attached to each other with both tight and adherent junctions. As the apical–basal transcellular current flows back extracellularly, it must follow a pathway between the cells called the paracellular pathway (Figure 2.1). Current flow through this pathway encounters a high-resistance region at the tight junctions near the apical end, and the current flow through this resistance leads to a TEP that is positive on the basal side of the monolayer with respect to the apical side. The TEP will be proportional to the resistance of this paracellular pathway, and typical values for this TEP range from 15 to 60 mV, basal side positive.

It is this TEP that is the driving force for most endogenous ionic currents in embryos and adults. This voltage across epithelia will drive current out of regions of low resistance where there has been a break in the epithelium (wounds) or where tight junction resistance is low, such as along the primitive streak (Jaffe and Stern, 1979; Winkel and Nuccitelli, 1989), the posterior intestinal portal (Hotary and Robinson, 1990) in chick or mouse embryos, or at the forming limb bud in amphibian, chick, and mouse embryos (Borgens et al., 1983; Robinson, 1983; Altizer et al., 2001). This ''leakage current'' will in-turn generate a lateral electric field along its path that will be proportional to the resistivity in that region. This electric field results from Ohm's law in a conductive medium,

$$E = \rho J$$

where J is the current density and ρ is the local resistivity.

The earliest measurements of the leakage current associated with wounds were made more than a century ago. DuBois-Reymond (1843) used a unique galvanometer that he built himself with more than two miles of wire and measured about 1 μA flowing out of a cut in one of his fingers. This was confirmed in 1849 and 1910 by other investigators, and the history of these measurements is presented in a scholarly review by Vanable (1991). More modern techniques have also been used to study this wound current as discussed. Direct measurements of electric fields *in situ* have been made, and I will discuss them next.

2.1.2 Methods for Measuring Endogenous Electric Fields

2.1.2.1 Self-Referencing Probe

Lionel Jaffe and I developed a technique for exploring transcellular ionic currents, called the vibrating or self-referencing probe (Jaffe and Nuccitelli, 1974). This instrument vibrates a small platinum sphere between two points about 10 μm apart at about 300 Hz and measures the voltage between those points using signal averaging to improve the signal-to-noise ratio. In a conducting medium where most cells find themselves, a voltage difference can exist only where there is a current flowing through the medium. Ionic currents entering or leaving cells can be readily detected by measuring the voltage they generate as they flow through the extracellular medium, and the past 30 years of research on more than 30 cell types has revealed that most cells have an asymmetrical distribution of ion channels that naturally leads to a transcellular current density on the order of 1–10 $\mu A/cm^2$ (Nuccitelli, 1988, 1990). Most epithelia that have been studied exhibit extracellular current densities on the order of 10–100 $\mu A/cm^2$ flowing through the organ or embryo with which they are associated. This technique detects the current that is flowing outside the cell or tissue, and the exact electric field that is generated by this current when it flows inside the cell or tissue can only be estimated based on tissue resistivity. It is more accurate to directly measure the electric fields in the tissue or cells as described here.

2.1.2.2 Microelectrode Techniques for Measuring Endogenous Electric Fields

The classic approach to these measurements is to use KCl-filled glass microelectrodes to penetrate the outer epithelium and measure the voltage just beneath it in several positions. Such electrodes are typically connected via a Ag–AgCl junction to a very high-input impedance preamplifier, so that they do not drain current from the system under study, and their tips are small (on the order of 0.1–1 μm) to minimize tissue damage (Wallis, 1993). If ionic currents are flowing within a tissue, these currents will generate an electric field that can be detected as the difference in potential at various sites along the current path. We will see below that this is the most commonly used approach to measure intraembryonic electric fields.

2.1.2.3 Voltage-Sensitive Fluorescent Dyes for Measuring Endogenous Electric Fields

Another popular technique for measuring the voltage across lipid membranes uses lipophilic fluorescent dyes whose fluorescence is voltage sensitive (Loew, 1992; Loew et al., 2002). For some of these dyes, their fluorescence intensity is dependent on their position within the lipid bilayer, which is in turn influenced by the membrane potential drop across this region. Membrane potential changes can be monitored by measuring the fluorescence intensity of these dyes, and differences in the membrane potential of cells making up an organ or embryo can also be detected. One example of the use of this approach, which will be discussed below, is to provide information about electric fields within sheets of cells that are electrically coupled via gap junctions in the chick embryo.

2.2 Measurements of Endogenous Extracellular Electric Fields

2.2.1 Amputated Limbs

Among the earliest direct measurements of endogenous extracellular electric fields were those made in the regenerating amphibian limb (McGinnis and Vanable, 1986). Upon

amputation, the skin battery of the amphibian limb drives 10–100 $\mu A/cm^2$ out of the cut end of the limb stump. This current flow generates an electric field within the limb tissue that is 60 mV/mm near the lesion during the first hours after amputation, and this field drops to about 25 mV/mm within 6 h as the healing process leads to an increase in the resistance of the wound.

Electric fields of this magnitude have been found to stimulate the growth of neurons into the limb via galvanotropism, and the presence of enhanced nerve in limbs has been correlated with enhanced regeneration (Borgens et al., 1979). The study of nerve galvanotropism has a long and fascinating history that is thoroughly reviewed by McCaig et al. (2005). Briefly, most neurons exhibit sensitivity to imposed electric fields by either bending their outgrowth direction toward the negative pole of the field or in the case of a neural ganglion, exhibiting a higher density of outgrowths on the side of the ganglion facing the negative pole. This galvanotropism may play a role in the guidance of neurons to their targets during development and has also been found to play an important role in regeneration.

2.2.2 Embryonic Electric Fields Beneath the Skin

Direct measurements of electric fields have been made in both avian and amphibian embryos during normal development. Hotary and Robinson (1990) used both the self-referencing probe and microelectrodes to first detect the transembryonic current in the 2- to 4-day-old chick embryo and then measure the electric field that the transembryonic current generates beneath the epidermis. They measured current entering much of the epidermis during stage 14 of development with the outward current focused mainly at the posterior intestinal portal, where up to 105 $\mu A/cm^2$ was measured (Figure 2.2). One would expect that this large anterior–posterior current would generate an internal electric field, so they then used microelectrodes to measure the TEP along this axis. Here, they measured electric fields of 5–20 mV/mm.

They then proceeded to test the hypothesis that these fields are important for normal development by perturbing them (Figure 2.3). They implanted a glass capillary that was filled with either conductive saline agar or nonconducting glass, used as a control, through the ectoderm at the dorsal trunk of the embryo (Hotary and Robinson, 1992). The conductive capillary allowed large currents of about 5 $\mu A/cm^2$ to leak out of the embryo. While the control embryos developed quite normally, most embryos with the implanted conducting capillary exhibited abnormalities in posterior structures where the endogenous electric field is normally the largest but was reduced by the capillary shunt. Perturbing the normal voltage pattern within the embryo resulted in striking tail abnormalities; and an investigation of a genetic mutant, rumpless, that exhibits similar tail abnormalities led to a very interesting correlation. They found that most rumpless mutants exhibited a much lower transembryonic current density and lower electric field within the embryo and those mutants that exhibited a normal electric field pattern also exhibited normal development. Therefore, they found a good correlation between the internal electric field and the normal posterior development. These observations certainly support the hypothesis that the endogenous field is important for the development of posterior structures.

Studies of endogenous fields have also been carried out on the stage 14–21 developing axolotl embryo (Metcalf et al., 1994; Shi and Borgens, 1995). Current is driven out of the lateral walls of the neural folds and the blastopore and enters most of the rest of the embryo's body surface (Figure 2.4). Measurements of the TEP indicate an internal, caudally negative electrical field beneath the neural plate ectoderm. The magnitude of the endogenous field is on the order of 10–20 mV/mm (Figure 2.5). When these embryos

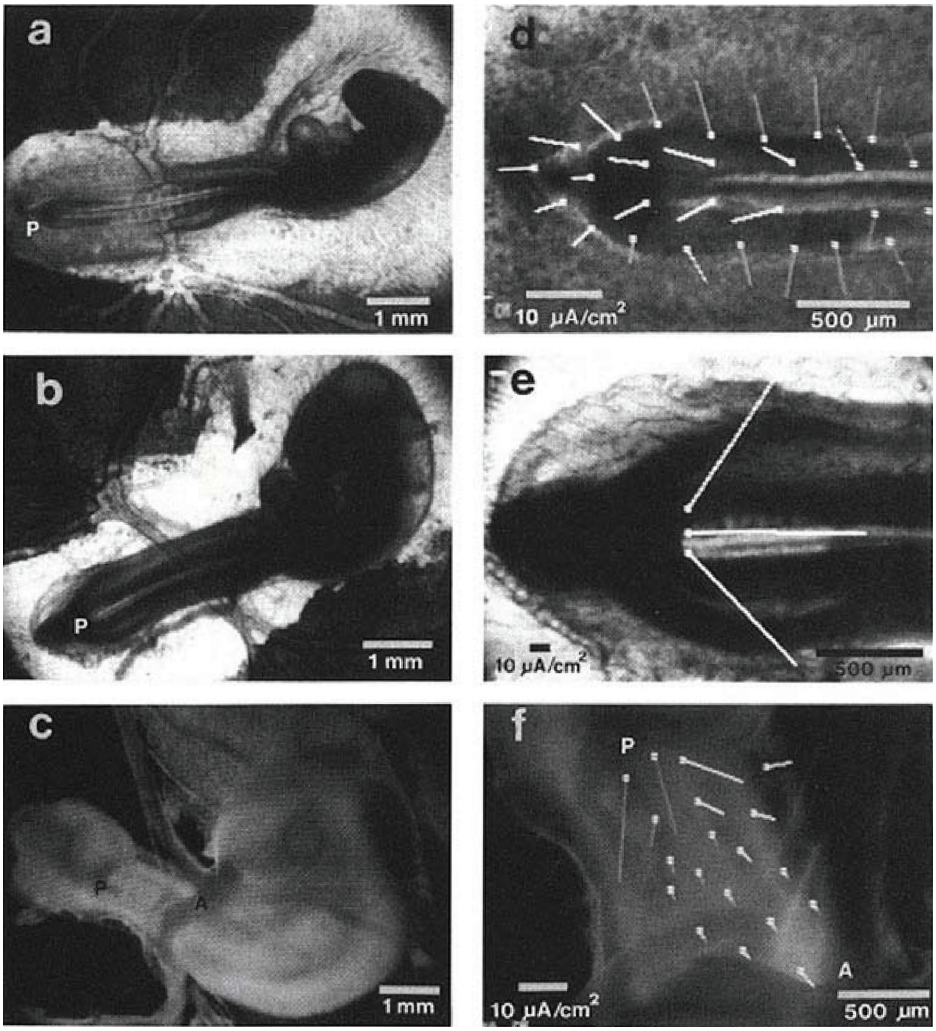

FIGURE 2.2
Ventral surface of three chick embryos at stages 14 (a and d), 17 (b and e), and 20 (c and f). Low-magnification views of the whole embryo are shown in (a)–(c), while (d)–(f) show the current pattern around the posterior intestinal portals of the embryos. Current vectors are represented by lines originating at a dot that indicates the position of the self-referencing probe when the measurement was made. The direction of the vector line away from the dot indicates the direction of current flow at that point, and the length of the line is proportional to the current density. At stage 14 (d), all vectors point toward the posterior intestinal portal or the lateral walls of the midgut. The three vectors shown at stage 17 (e) indicate large currents of about 100 $\mu A/cm^2$ leaving the posterior intestinal portal. At stage 20 (f), outward currents were also found at the posterior intestinal portal. Current densities were much lower by this stage. Note the inward current at the anterior intestinal portal (A). (From Hotary, K.B. and Robinson, K.R. (1990). *Dev. Biol. 140*, 149–160. With permission.)

were placed into an external electric field designed to modify the internal field, abnormalities were observed that depended on the developmental stage (Metcalf and Borgens, 1994). Gastrula-stage embryos exhibited normal development after exogenous field exposure, indicating that the imposed field does not harm the embryo in some nonspecific way. In contrast, neurula-stage embryos exhibited developmental abnormalities when exposed to similar electric fields of 25–75 mV/mm. These data support the hypothesis that the natural electric field within the embryo influences normal morphogenesis.

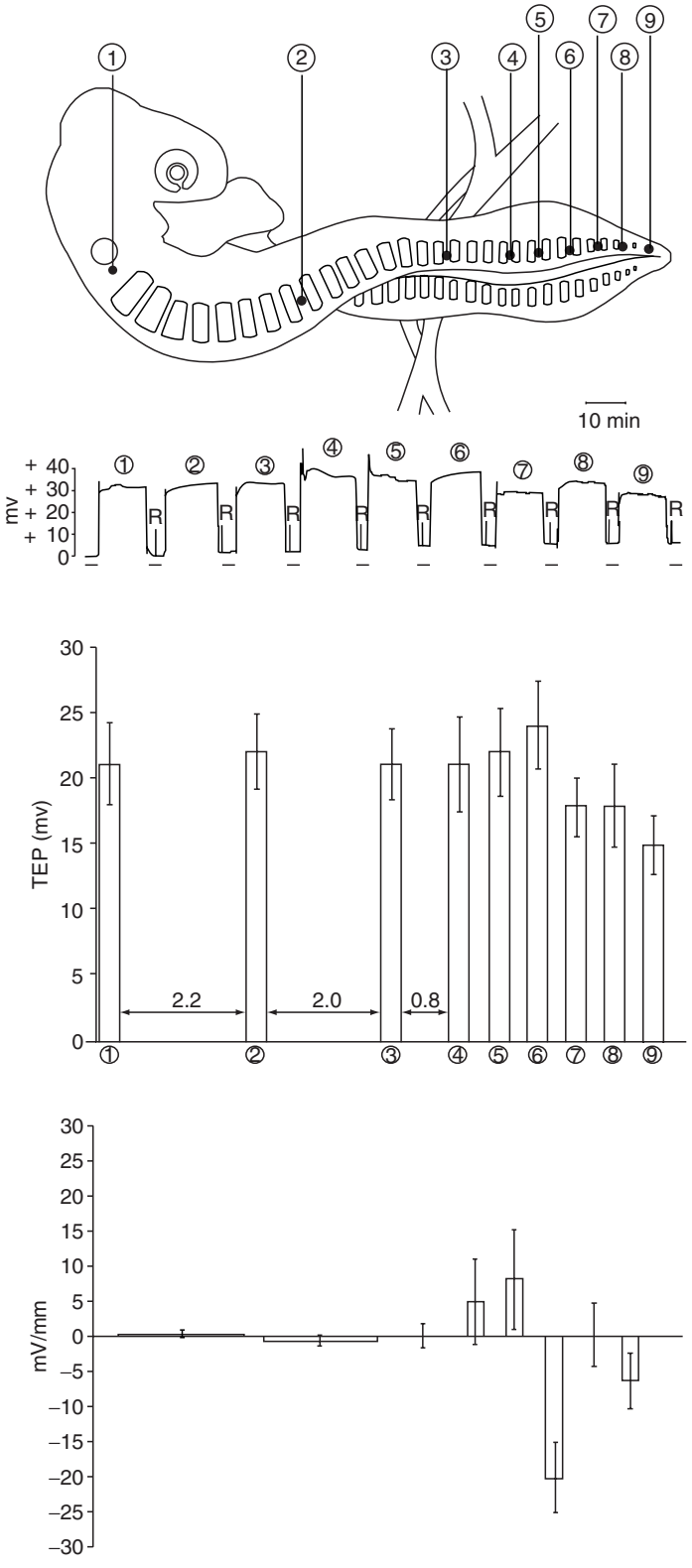

FIGURE 2.3
Stage 17 chick embryo TEP measured in nine rostral–caudal positions. Numbered measurement positions are shown in the drawing in the upper part of the figure. Below the drawing is a chart recording, tracing, and showing the TEP at the different measurement positions. At each numbered peak (corresponding to the positions shown in the drawing) the integument of the embryo was impaled and a stable positive potential was measured. Times at which the embryo was not impaled are indicated by a solid line below the recording. The upper bar chart shows the TEP at each position. The numbers below each bar correspond to the measurement positions indicated. The numbers between bars indicate the average distance (in mm) between each position. Where this is not indicated, the average distance is 0.3 mm. The lower chart shows the average voltage gradient between each consecutive position. Error bars indicate the standard error of the mean, $N = 6$. A steep voltage gradient was found between positions 6 and 7. (From Hotary, K.B. and Robinson, K.R. (1991). *Dev. Biol.* 140, 149. With permission.)

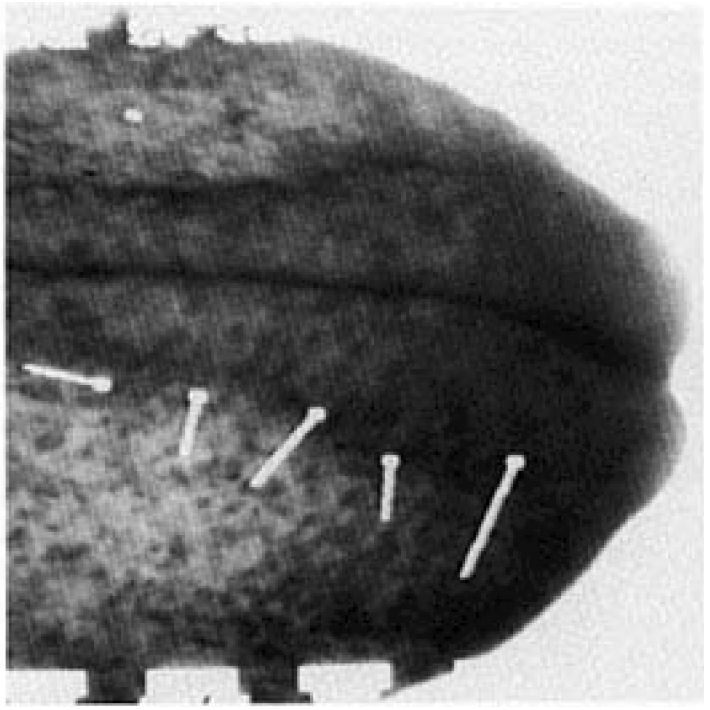

FIGURE 2.4
Neural fold currents in a stage 18 axolotl embryo measured with a two-dimensional self-referencing probe. Current vectors are displayed as a line originating at a dot that marks the measurement position. The direction of current flow from the dot is denoted by the line direction, and its length is proportional to the current density. Note the outwardly directed currents at the edge of the cranial neural folds. (From Metcalf, M.E.M., Shi, R.Y., and Borgens, R.B. (1994). *J. Exp. Zool. 268*, 307–322. With permission.)

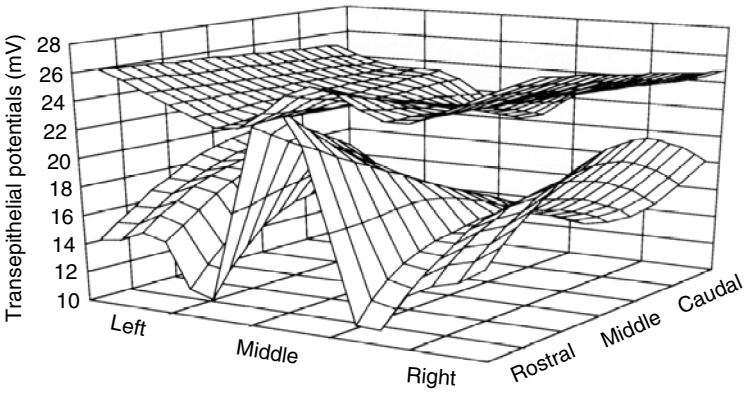

FIGURE 2.5
Summary of three-dimensional plot of TEPs at stage 15/16 (bottom) and stage 18/19 (top) axolotl embryos. The overall increase in the magnitude of TEPs at stage 18/19 is real, permitting these two views to be presented adjacent to each other. Note that only a hint of the characteristic voltage gradients beneath the ectoderm is evident at stage 18/19, and the potentials are not statistically different from one another. The embryo is essentially isopotential within the extracellular domain of the neural plate near the climax of neurulation. (Reproduced from Shi R.L and Borgens R.B. (1995) Dev. Dyn. *202*, 101–114, 1995. With permission.)

2.2.3 Fields Associated with the Neural Tube

The neural tube forms during development as a folding over of neural plate epithelium, inverting the normal polarity of this layer. The apical end of the neural plate becomes the inside of the tube so that transport of Na^+ occurs from the inside to the outside of the tube and the trans-tube potential is inside negative. This potential has been measured in both frog and axolotl to be as large as −90 mV (Hotary and Robinson, 1991; Shi and Borgens, 1994). Neuroblasts within the wall of the tube are therefore exposed to very large electric fields, since the wall is only about 50 μm thick and the 90 mV across this distance generates a field of roughly 1800 mV/mm. There is little doubt that such a large field will influence the migration and sprouting of these neuroblasts.

2.2.4 Fields Associated with Epithelial Wounds

As noted above, the earliest measurements of the electrical phenomena associated with wounds did not measure the electric field itself but rather the current flowing out of the wound. More modern techniques have also been used to study this wound current. The leakage current that is driven out of epithelia in low-resistance regions has been measured using the vibrating probe technique (Jaffe and Nuccitelli, 1974) in several systems. One of the earliest such measurements was a current as large as 100 $\mu A/cm^2$ leaving the stumps of regenerating newt limbs (Borgens et al., 1977). Similar measurements have also been made on fingertip amputation currents in humans (Illingworth and Barker, 1980), where up to 30 $\mu A/cm^2$ was detected leaving the accidentally amputated stump for about 3 wk. These currents will certainly generate electric fields just beneath the epidermis that will be proportional to the resistivity encountered in the tissue. The range of human tissue resistivity spans 200–1000 Ω cm (Faes et al., 1999), so these currents would be expected to generate an electric field within the tissue of about 10–100 mV/mm.

However, since this tissue resistivity can vary substantially as a function of cell density and tissue anatomy, it is always more reliable to measure these fields directly in the tissue rather than estimating them based on the transembryonic current density. This has been accomplished in four different wound types in skin and cornea. The classic approach to these measurements is to use KCl-filled glass microelectrodes to penetrate the outer epithelium and measure the voltage just beneath it in several positions along a line leading away from the wound. However, for skin measurements, another approach is more common. This method is to measure the potential gradient just beneath the stratum corneum on the surface of the epidermis, either with surface electrodes or by other means. The field generated by the current flowing between the upper surface of the epidermis and the stratum corneum is often larger than that generated below the epidermis because of the higher resistivity of that upper region. The range of field strengths measured in the four cases in the literature is surprisingly small, between 40 and 200 mV/mm (Table 2.1). The field direction is a function of position. Beneath and within the epidermis the field polarity has the negative pole at the wound center, and above the epidermis the wound current is flowing in the opposite direction so that the positive pole is at the wound (Figure 2.6).

These wound fields have some useful properties for signaling. First, they appear immediately upon wounding since the TEP is continuously present to drive current out of any low-resistance region as soon as it is formed. Second, the lateral electric field illustrated in Figure 2.6, that is generated by the wound current, will persist until the resistance increases as the wound heals. Thus, we have a signal that is immediate and persistent. These are ideal properties for a physiological signal to stimulate wound healing. If the epithelial cells forming the epidermis were able to detect such electric

TABLE 2.1

Endogenous Electric Fields Measured near Wounds

Species	Tissue	Wound Type	*E* Field (mV/mm)	Reference
Bovine	Cornea	Cut	42	Chiang et al., 1992; Sta Iglesia and Vanable, 1998
Notophthalmus viridescens	Digit	Digit tip amputation	40	McGinnis and Vanable, 1986; Chiang et al., 1989; Iglesia et al., 1996
N. viridescens	Limb stump	Amputation	7–50	McGinnis and Vanable, 1986
Guinea pig	Skin	Small cut	100–200	Barker et al., 1982

fields, they would be able to initiate wound healing immediately upon wounding. This is in fact the case, as discussed in the next section.

Measurements of endogenous electric fields near epithelial wounds have been made in three different systems. The first was a skin wound in the guinea pig (Barker et al., 1982). The transepidermal potential was measured in several locations lateral to a skin wound (Figure 2.7). At the wound itself, there is no epidermis, so the transcutaneous potential is zero, whereas about 1 mm away, the transcutaneous potential exhibited the normal value of 50–70 mV. The steepest voltage gradient was found immediately adjacent to the wound edge where values as high as 150 mV/mm were measured. The second direct measurement of the electric field near a wound was made in two regions of the newt limb. The electric field adjacent to an amputated digit was measured with microelectrodes and found to be about 40 mV/mm (McGinnis and Vanable, 1986; Chiang et al., 1989; Iglesia et al., 1996). This is very similar to the field near an amputated limb of the newt of 7–50 mV/mm (McGinnis and Vanable, 1986). The third direct measurement was made on the bovine cornea, and the magnitude of the electric field was 42 mV/mm (Chiang et al., 1992; Sta Iglesia and Vanable, 1998).

The fields in the cornea and the newt digit have been found to play a role in wound healing. When the field strength associated with the wound is modified, the rate of wound healing changes. The newt's wound healing rate can be optimized under normal

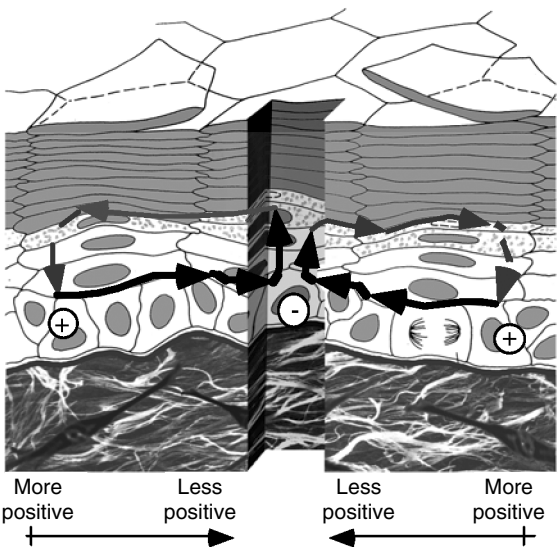

FIGURE 2.6

Generation of skin wound electric fields. Unbroken skin maintains a ''skin battery'' or TEP, generated by the apical influx of Na^+ and basolateral efflux of K^+. When there is a wound, the potential drives current flow through the newly formed low-resistance pathway, generating an electric field whose negative vector points toward the wound center at the lower portion of the epidermis and away from the wound on the upper portion below the stratum corneum.

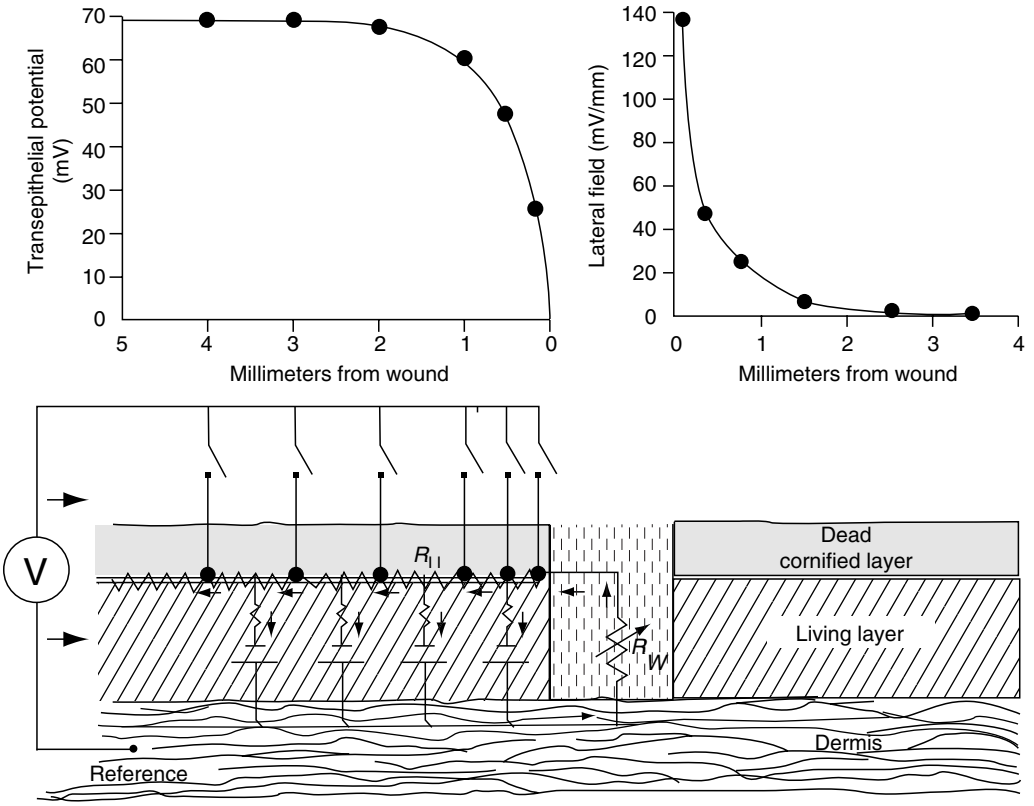

FIGURE 2.7
The electric field near a skin wound in a guinea pig. Bottom: diagram of the method used. A metal probe penetrated the stratum corneum to contact the epidermis at various distances from the wound site, and the surface voltage at each location was recorded with respect to the potential within the dermis. This voltage is the TEP and is plotted in the upper left of the figure. The voltage at the wound must be zero because the epidermal layer has been broken there, and far from that region, the TEP is 70 mV. The electric field at a given location is given by the voltage difference between sampling sites divided by the distance between those sites and is plotted on the upper right of the figure.

conditions but cannot be speeded up, removing the endogenous field does slow down the rate of wound healing by about 25%. However, the rate of bovine corneal wound healing can be enhanced by increasing the electric field strength. Epithelization was fastest in wounds with the field strength raised to 80 mV/mm, more than twice the normal field strength present in wounds maintained in Hanks' solution alone. Epithelization decreased, however, when the field strength was increased to 120 mV/mm. A similar pattern was also observed when the field's polarity was reversed. Decreasing the field strength by submersion of the lesions or by treating the lesions with the Na^+ channel blocker, benzamil, significantly retarded healing. In addition, an increase in the field strength of lesions treated with Na^+-depleted Hanks' solution, by the addition of DC, increased the rate of epithelization. These observations suggest that the endogenous electric field plays a role in the normal wound healing process.

More recent studies of rat cornea have reported a similar correlation between the wound healing rate and the electric field strength. The mammalian cornea exhibits an internally positive TEP of 30–40 mV. Na^+ and K^+ are transported out of the tear fluid and into the epithelium while Cl^- is transported in the opposite direction. A wound in this

epithelium exhibits the same current pattern as skin (Figure 2.6), and a laterally oriented electric field is generated by this wound current with the cathode at the wound site. Recent work from McCaig, Zhao, and colleagues has indicated a strong correlation between the rate of wound healing in the rat cornea and the electric field strength lateral to the wound (Zhao et al., 1996; Song et al., 2004). They have used drugs to either increase the trans-corneal potential or decrease it. Drugs that increased this voltage difference also increased the rate of wound healing, while drugs that reduced this voltage difference also slowed down the rate of wound healing. In addition, when current was injected to restore and amplify the endogenous electric field in bovine cornea, the wound healing rate increased (Sta Iglesia and Vanable, 1998). These data provide very strong support for the hypothesis that these electric fields near corneal wounds play an important role in influencing the healing of these wounds.

2.3 Measurements of Endogenous Intracellular Electric Fields

2.3.1 Nurse Cell Complex in Insects

In the silk moth oocyte-nurse cell complex or the ovarian follicle, the oocyte cytoplasm is about 10 mV more positive than that of the nurse cell cytoplasm despite their connection by a broad cytoplasmic bridge. Woodruff and Telfer have published several studies of this system in which they show that the polarized transport of fluorescently labeled proteins between nurse cells and oocyte depends on the charge of these proteins in a manner consistent with intercellular electrophoresis driven by the voltage gradient across the cytoplasmic bridge of the silk moth and other insects (Jaffe and Woodruff, 1979; Woodruff and Telfer, 1980; Woodruff and Cole, 1997; Cole and Woodruff, 2000). Since these nurse cell complexes arise from incomplete cell division they must be considered electrically as a single cell that is generating a significant voltage gradient across a small region.

2.3.2 Development of Left–Right Polarity in Chick and Frog

The most recent study of the role of electric fields in development utilized both frog and chick embryos. The generation of left–right asymmetry in these systems was found to depend on both functioning gap junctions and voltage difference between blastomeres in very early stages of development (Levin and Mercola, 1998, 1999; Levin et al., 2002). This work started with a pharmacological screen to identify drugs that interfered with the development of left–right patterning. The most effective drugs were the ones that interfered with K^+ and H^+ ion fluxes. Lansoprazole and chromanol 293B were the most effective and these block the H^+ pump and a specific K^+ channel (KvLQT-1), respectively. Next most effective was SCH28080 and omeprazole, which block the H^+/K^+-ATPase, followed by $BaCl_2$, which blocks all K^+ channels.

They further tested the hypothesis that these ion fluxes were important for left–right patterning by injecting mRNA for either the alpha or beta subunits of the H^+/K^+-ATPase or the K^+ channel into the frog egg. They found that heterotaxia was induced when either was injected but by far the largest increase, 37%, occurred when all three mRNAs were injected. This suggests that overexpression of these molecules, which can influence or perturb ion concentration gradients of K^+ or H^+, disrupted the normal left–right patterning of the embryo.

In order to learn more about the mechanism through which H^+/K^+-ATPase influenced patterning, they used *in situ* hybridization to show an asymmetrical distribution of the K^+/H^+-ATPase. It was concentrated in the right ventral blastomere of the 4-cell stage frog embryo. the addition of Both K^+ channels and K^+/H^+-ATPase should hyperpolarize cells making them more negative than their neighbors. This voltage difference between the blastomeres of the 4-cell stage embryo could be used to segregate low molecular weight determinants through gap junctions to achieve asymmetric gene expression. They further tested this hypothesis by measuring the membrane potential of cells near the primitive streak during early chick development. They used an anionic fluorescent dye, $DiBAC_4$, whose distribution depended on membrane potential to show that the early chick embryo exhibited a voltage gradient across the primitive streak. The left side of the primitive streak was 10–20 mV more positive than right side and this difference was inhibited by the K^+ channel blocker, $BaCl_2$, and the H^+/K^+-ATPase inhibitor, omeprazole. Since these drugs also partially inhibited the development of left–right patterning, the membrane potential difference appears to play a role in this aspect of development. This paper is the first to demonstrate a role for membrane potential differences between blastomeres in early vertebrate pattern formation.

2.4 Methods for Modifying Endogenous Electric Fields

Modifying endogenous fields within embryos or cells is really quite challenging. The only way to generate a relatively steady field in a conducting medium is to pass current through it. However, the outer membranes or epithelia surrounding the targets here make this very hard to accomplish. These structures have high resistance to current flow so that slowly varying fields placed outside the cell or embryo will not effectively penetrate to the inside but will mainly generate current flow around the outside of the cells or embryos. Because of this there have been precious few attempts to modify endogenous fields, but I will discuss some of them.

The only exception to this field penetration problem is the application of ultrashort pulses in the nanosecond domain that have risen times faster than the charging time of biological membranes (Schoenbach et al., 2004). These pulses penetrate beyond the plasma membrane into the interior of cells and tissues but are present only for very short times. It is conceivable that by combining millions of these short pulses per second, one could significantly modify the DC electric field within cells and tissues. This remains to be explored.

2.4.1 Passing Current between Electrodes

If two electrodes can be placed within a single cell or in different regions of an embryo, current can be passed between them and a well-defined electric field can be generated. However, this requires penetrating the outer membrane or epithelium and may damage this outer layer. The longer the field is applied, the more likely that some damage will occur due to vibrations or embryo movement. This approach has been used with some success in the regenerating tip of newt limbs (Chiang et al., 1991; Iglesia et al., 1996), where current was passed along the limb by inserting one electrode into a slit made at the knee and the current delivery electrode was placed distal to the wounded digit tip. The electric field at the newt limb could be manipulated in this way, and elimination of this field significantly slowed down the rate of wound healing.

2.4.2 Low-Resistance Shunts

One way to perturb the natural transembryonic current pattern is to place a low-resistance pathway or shunt into a resistive membrane or epithelium. The endogenous fields will drive current out through this shunt, and this new current flow will definitely perturb the endogenous field within the tissue. This technique was used with striking results by Robinson's group in the chick embryo as described above (Hotary and Robinson, 1994).

2.4.3 Placing Tissues in an External Electric Field

While DC electric fields cannot penetrate into the conducting cytoplasm of a cell or tissue, they have been found to perturb normal development at certain stages in amphibians (Metcalf and Borgens, 1994). In addition, there is a very extensive old literature in which the polarity of both development and regeneration could be strongly influenced by imposed DC electric fields (Jaffe and Nuccitelli, 1977; Levin, 2003). One interpretation of these observations is that the imposed field has its effective target along the exterior of the embryo via lateral electrophoresis of membrane glycoproteins.

The only external electric field that can penetrate into the interior of cells is the one that rises faster than the charges within the cell can redistribute (Schoenbach et al., 2004). Such a pulsed field could certainly modify internal electric fields for the extremely short duration of the pulse. Perhaps by using either a large field strength or multiple pulses, such brief pulsed fields might have a significant effect on endogenous fields. This relatively new area deserves future investigation.

2.5 Summary

All plasma membranes and epithelia generate voltage differences across themselves. These batteries are the power sources that drive ionic currents through cells, tissues, and organisms. These currents will generate internal electric fields as they traverse tissue, and such fields can do work through electrophoresis of charged molecules within and between cells as well as in the plane of the plasma membrane. Cases in which such fields have been measured include the following:

1. Two- to 4-day-old chick embryos generate a 20-mV/mm field near the posterior intestinal portal that is important for normal development of posterior structures.
2. Stage 14–21 amphibian embryos generate similar internal electric fields, and modifying these fields during neurulation but not gastrulation results in developmental abnormalities.
3. Neural tubes in amphibians generate an internally negative voltage difference of as much as 90 mV across the wall of the tube, and cells in this region are exposed to fields as large as 1800 mV/mm.
4. Mammalian skin wounds generate 150-mV/mm fields just below the stratum corneum, and corneal epidermal wounds exhibit fields of 40 mV/mm lateral to wounds.
5. The development of left–right asymmetry in frog and chick embryos utilizes an electric field between blastomeres that is generated by an asymmetrical distribution of the K^+/H^+-ATPase among the blastomeres.

In nearly all of these well-documented examples, the electric field plays a critical role in either the development of the organism or the wound healing and regeneration of adult structures. Because of this, imposed electric fields may be utilized to perturb or influence normal development. One intriguing possible perturbation is the use of imposed electric fields to enhance the rate of wound healing, particularly in cases where the normal healing process is slower than normal, as observed in chronic wounds.

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