

Chapter 1

BIOELECTRICAL CONTROL OF GROWTH — A RETROSPECTIVE LOOK

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AT THE PRESENT TIME, there is a great deal of interest in and enthusiasm for biogenetic electrical phenomena. Experiments dealing with the origin, measurement, control, biological "meaning," and simulation of such fields are proceeding apace in many laboratories around the world. In addition, the rapidly growing agnate field of bioelectrochemistry is beginning to excite the chemical world as well. Societies are being founded and journals published, all devoted to electrical correlates of the life processes. Considerable speculation and debate, some of it premature and irresponsible, have even spilled over into the public press and media, arousing public interest and the inevitable governmental studies.

To many, the whole field seems to be one of the wonders of the space age, and a creature of modern scientific technology. Since such is most definitely not the case, it might be well to explore the antecedents of our present knowledge, to arrive at a somewhat more balanced view of our rate of progress and sophistication.

I admit in advance that this will not be an attempt at an exhaustive or detailed review — only a brief look at some of the highlights along the way, and a highly personal look at that. However, it may lead the reader into the older literature, which is remarkably rich.

The discovery of bioelectricity is lost in the mists of antiquity. No doubt some primitive shellfish gatherer who trod upon a torpedo ray or some Amazonian savage who jabbed an electric eel with a wet spear received the first hair-raising demonstration of its existence. However, the first truly methodical investigations of the relationship between electricity and life seem to be those of Galvani (1791), who demonstrated that muscle can be made to contract by the application of an electrical stimulus, and that muscle contraction is accompanied by direct electrical currents. The nineteenth century saw an array of scientists and charlatans studying and trying to capitalize on bioelectric effects. Unfortunately, the charlatans seem to have gained ascendance as well as notoriety. Anton Mesmer's famous theory of "animal magnetism" is one outstanding example (interestingly, as is often the case in otherwise outlandish theories, his contained some germs of truth). One need only peruse the advertisements of

the popular magazines of the day to find an amazing variety of devices offered that purported to cure everything from micromastia to melanoma by the application of unspecified "electrical principles." Needless to say, the reputation of electrobiology dwindled to an abysmal nadir in the face of such quackery. The spark was maintained by the neurophysiologists and myologists, who had solid reason to continue their studies.

Invertebrates

The reawakening of interest in this area seems to have come in the early 1900s (excepting the neurophysiologists and electromyologists, whose efforts lie outside the scope of this meander) with the studies of Matthews (1903) on hydroids. He used rather crude methods by today's standards but arrived at the correct conclusions nonetheless — marine hydroids are electrically polarized. E. J. Lund followed up on this observation with an elegant series of papers in the early 1920s (e.g., 1921, 1925). He confirmed Matthew's findings and discovered that the direction and polarity (hydranth versus holdfast) of growth and regeneration in *Obelia commissuralis* could be controlled by the application of small direct currents. In addition, he determined that the effects only occur within narrow, parametric limits — a finding that has been confirmed by every investigator in the field. His papers are true classics and should be consulted by anyone interested in this field — indeed by anyone interested in seeing how much can be learned using simple techniques and an original mind. Lund's work has been repeated many times, by a number of investigators, including Barth (1934) and Levin (1961). Their results confirm Lund's observations.

All of this activity led Burr (1932) and Burr and Northrop (1935) to develop the earliest generalized theories of the electrical correlates of development in particular (1932) and life in general (1935). Lund came out with his own version in 1947. All three are of considerable historical interest.

Experiments with invertebrates have continued, shedding much light on the general mechanisms of electrical control of development. Studies by Marsh and Beams (e.g., 1952) demonstrated that Platyhelminthes are quite susceptible to electrical control of not only their regeneration, but even of their entire morphological organization. Suitable fields can cause the complete reversal of head and tail ends in an intact animal! I showed (1970) that control can be exercised over the outgrowth of stolons in the colonial protochordate, *Perophora viridis*. The question arises, of course, as to the mechanisms of this control. The most obvious explanation lies in the induced movement of morphogenetically determinant molecules from place to place. Rose (1957, 1962) has postulated perhaps the most elegant of these theories, involving control loops of information-bearing molecules that can be passed from tissue to tissue and thus determine spatial

and temporal organization. Recent theoretical papers using *Hydra* as an example have provided mathematical models (Gierer, 1977). Experimental confirmation of this control method is not lacking. Rose (1963) has shown that molecules controlling hydranth formation in *Tubularia crocea* can be preferentially passed or blocked across an agar bridge by an electrical field. I showed (1963) that specific inhibitors of tail regeneration in the polychaete worm *Clymenella torquata* can be directed into or away from a wound surface electrically. There seems to be little doubt that morphogenetic molecules are moved by electrical fields and can control differentiation when they arrive at a reactive cell. However, as we shall see, other mechanisms may also be at work.

Tissue Culture

While all this activity with invertebrates was taking place, the tissue-culture people were also beginning to study the effects of electrical fields. Ingvar (1920) discovered that cell processes of nerves in culture orient along the field lines of a galvanic current. This observation was confirmed by Peterfi and Williams and by Karssen and Sager in the same year (1934). However, Weiss (1934) disputed their findings and stated that their effects were due to orientation of material in the plasma clots then in use, and that the effect was due to contact guidance. In effect, his report stopped everything for twelve years — a perfect example of how the progress of science is often impeded by its own leading lights. Marsh and Beams (1946) convincingly refuted Weiss, and progress could again commence. Recent work in our laboratory (Sisken and Smith, 1975) indicates that electrical fields not only can direct the outgrowth of neurites, but can also partially substitute for Nerve Growth Factor in maintaining cultured trigeminal ganglion neurons. Jaffe and Poo (1979), despite a rather caustic denigration of all previous experiments, have succeeded in adding little but confirmation and minor extension of the results. Work continues apace in several laboratories, including ours, and electrical control of the growth of nervous tissue continues to be a source of intense interest.

In another area, Becker and his colleagues (1964) discovered what appeared to be an amazing phenomenon. Adult frog erythrocytes are apparently transformed into callus cells at bone fracture sites in response to injury potentials. The dedifferentiation phase of this transformation was reproduced in culture by means of a small D.C. field (1967) in what seemed to be an impossibly short time (less than one hour). This paper generated a good deal of controversy, and the debate continues. However, the results have been independently confirmed in our laboratory (1975), and by Pilla (1974). In addition, Pilla showed that the effect seemed to be dependent upon Ca^{++} ions and could be simulated by generating an advancing "front" of Ca^{++} ions in the culture dish. We confirmed the role

of Ca^{++} ions at the membrane (1978) by showing that the effect could be blocked competitively with LaCl_3 , then reversed or simulated with calcium ionophore (A23187). Some important confirmation of the role of ion concentration differences or currents in development has been provided by Jaffe's group (e.g., 1974), working with a vibrating probe electrometer; their work continues to be of considerable interest. This area of investigation is currently very active, and the reader need only consult the recent literature to see that much effort is being expended to discover the role of various ions and molecules in the electrical effect. One of the more exciting possibilities seems to lie in the area of the calcium-binding proteins (CBP), Calmodulin being a typical example. It may well be that electrical control of Ca^{++} at the cell membrane may be the key to control of CBP activity and thus of the cell's synthetic machinery. Cheung (1980) reviews the CBP story very clearly and pleasantly, and the reader is directed to his article for an exposition. In any case, mechanistic explanations of observed electrical effects are appearing, and the "black box" nature of experiments is receding, if not into oblivion, at least somewhat.

Vertebrate Regeneration

This area seems to be the one that has stimulated the greatest interest in recent years, for the obvious reason that regenerated parts have considerable theoretical and practical advantages over transplanted or prosthetic bits and pieces. There are a few papers that can be considered as seminal in this area. Perhaps the first was that of Monroy in 1941. He followed the electrical behavior of regenerating urodele limbs and tails and discovered that there is a pattern to the electrical events. Shortly thereafter (1944) Rose showed that partial limb regeneration could be induced in adult anurans (*Rana pipiens*) by repeatedly dipping the stump into strongly hypertonic saline, presumably thereby producing irritation and an increase or prolongation of the "wound potential" generally seen at sites of injury. These two papers, together with Singer's demonstration of the influence of nerves on regeneration (see Singer, 1960, for an early discussion) are surely the foundations upon which practically all the later work is based, for we then knew that induced regeneration is possible, that there are electrical correlates of regeneration, and that nerves somehow are very important in the process.

Becker provided the next leaps forward in 1960 and 1961. In 1960, he repeated some of Monroy's measurements and constructed an ingenious electronic analogue of the potential maps. He correlated this with axial current flow in peripheral nerves (1961a) and with the differences between regenerating and non-regenerating forms of amphibians (1961b). Moreover, he showed (1961b) that the rate of regeneration could be influenced in regenerators by altering the strength of the field. Charles Bodemer (1964) added another link in the logical chain by demonstrating

that partial regeneration in adult frogs could be obtained without irritation of the stump or augmentation of the nerve supply to the limb. He did so by simply electrically stimulating the nerves of the brachial plexus.

The logical extension of all of this seemed to be simple — if the electrical/nervous behavior of a non-regenerator's limb could be made to simulate that of a regenerator, regeneration should ensue. The question was, how to do it? The answer turned out to be simplicity itself. Just put a battery in the animal, and run the leads to the wound surface. It seemed to me that the simplest way to do this would be to let the animal be its own battery. Implanted in a limb, a pair of dissimilar metal wires, if the junction were insulated, should generate a current in the saline tissue environment. Knowing absolutely nothing about the required voltages, current, etc. that would be effective (except that they would be small), I picked a bimetallic pair (silver and platinum) that seemed likely to do little harm, at least, if metal ions were released, as would be expected from electrochemical considerations.

By sheer luck, the Ag-Pt couple produced current that fell within the effect's thresholds, and I obtained considerable regeneration in adult frog limbs (1967). It would be nice to be able to say that I had worked out all the parameters in advance, and knew exactly what I was doing, but such was not the case. As so often has happened in the history of science, I stumbled onto the right procedure. The approach was logical enough, perhaps, but the details were worked out on the "wrong" basis (at least in retrospect).

The end result was the same, however. Since then, Becker (1972) has used a modified but similar device to stimulate partial regeneration in juvenile rat limbs.

More recent experiments have been performed using implantable battery/resistor packs that are much more reliable and predictable. In 1974, I showed that regenerates of remarkable completeness were obtained in adult frogs if the stimulating electrode were placed in approximately the position occupied by the apical ectodermal ridge during ontogeny. It seems that in addition to the stimulatory effect of the current, its polarizing effect on the regenerate is extremely important. It may well prove to be true that to obtain the best possible regeneration, a combination of a D.C. polarizing stimulus with the sort of general excitation provided by pulsed stimuli (*see* Chapter 8) may be required. It seems probable that regeneration is a several-step process:

- A. The animal must "recognize" that a piece is missing;
- B. A blastema must be formed by dedifferentiation and/or cell replication;
- C. The cells within the blastema must "know" where they are and what to become; and
- D. Differentiation must then ensue.

Though this hypothesis seems at least minimally logical to me, and is based on more evidence than is appropriate to review in this brief introduction, the 1974 paper on which it is partly based has aroused a great deal of controversy. Apparently there are some who believe that it states that perfect regeneration will surely ensue if one follows the techniques outlined. Careful reading of the text reveals no such claim. *One* animal out of many regrew a perfect hand. It is a certainty that we must know a great deal more about regeneration before such a result is predictable statistically. However, I do not think it unreasonable to state that if we do learn enough about the controlling factors, perfect limb regeneration ought to be obtainable. There seems to be little doubt that the cells contain the information necessary to build a new arm, hand, leg, or liver. We just do not know at present how to make them use it in an entirely predictable fashion.

In any case, work on limb regeneration is continuing in many laboratories, including ours, with promising and informative results. The trend, at least, is to more and better understanding of the processes involved.

Even in the best of all possible worlds, routine induction of limb and organ regeneration seems a fairly distant prospect. Of much more immediate scientific and clinical interest is the induction of tissue regeneration/repair. The tissue whose properties are best understood, and which is being more or less routinely repaired by electrical means in clinical settings, is bone. It would require the rest of this volume to review the literature on bone, but it may be of interest to look at some of the highlights. Some of the earliest work on the electrical properties of bone was done by Fukada and Yasuda in 1957. Fukada had investigated the piezoelectric properties of wood in 1955, and the work was extended to bone in cooperation with Yasuda in 1957. In 1962, Bassett and Becker reported that stressed bone produces steady state potentials as well. Noting that bone is deposited in areas of negative charge when stressed, Bassett, Pawluk, and Becker (1974) implanted electrodes into the medullary canals of dog femurs. Indeed, bone formed in response to the stimulus, nearly filling the canal near the cathode.

Becker presented a theory for the electrical stimulation of bone formation in 1967, based on its known electrical properties. Mascarenhas demonstrated that bone may also act as a stable electret (1974), and Eriksson added the possibility of streaming potentials (1974) to the mix of possible electrical effects in stressed bone.

Prior to these last two papers, however, intervention in fracture healing had already been undertaken in a number of laboratories using a variety of direct-current techniques. Inductively coupled currents derived by pulsing electromagnetic fields were added by Bassett, Pawluk, and Pilla in 1974. Since then, the use of electrical currents to heal fractures has become widespread, and the reader might well refer to one of the numerous

modern volumes (e.g., Brighton et al., 1979) for a comprehensive look. As a general statement, three types of treatment techniques seem to be in use at the present:

- A. Low-level (ca. $1\mu\text{A}/\text{mm}^2$) D.C., as exemplified by Becker and his colleagues;
- B. High-level ($> 1\mu\text{A}/\text{mm}^2$) D.C., as exemplified by Brighton's colleagues; and
- C. Inductively coupled pulsed fields, as exemplified by Pilla and Bassett's studies.

It seems probable that the low-level D.C. and inductively coupled methods operate by producing stimulation at the cell or its surfaces. The inductively coupled method apparently works by a different mechanism than the low-level D.C., producing little callus as opposed to much. In contrast, the high-level D.C. method probably operates electrochemically by releasing quantities of electrode products (O_2 molecules, Cl_2 , etc.) that either stimulate or irritate the tissues into a response. The net effect of all treatment seems similar, however: the bones heal.

Widespread use of electrical tissue stimulation seems to be imminent, in any case, and is beginning to include many tissues other than bone (see Brighton et al., 1979, for some examples). One of the more interesting is peripheral nerve. If the regeneration of severed axons could be accelerated across a gap, and could be directed preferentially to their original site of termination, a knotty clinical problem would be solved. Some progress in this direction is being made in many laboratories, including ours, and I believe that the near future will prove to be very interesting in the area of induced peripheral nerve regeneration, as well as in many other fields relating to electrical effects in a variety of other biological systems.

Perhaps not the least of the questions arising from much of the modern work relates to environmental exposure to small fields. It is not inconceivable, given the large effects seen in response to very small currents and fields, that modern technology exposes us daily to biologically significant doses of electricity about which we know practically nothing at all. On this modestly sobering note, I will end this evidently personal reminiscence, and commend the symposium that follows to the reader's attention. It presents a plethora of information on a wide variety of subject systems and, as is usual for such volumes, raises far more questions than it answers.

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