SECTION III

BASIC SCIENCE AND PATHOLOGY

Fracture Healing in Rats Exposed to Extremely Low-Frequency Electric Fields

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Weak electric current has been shown capable of stimulating and altering bone growth and healing[8]; its clinical use in orthopedics holds considerable promise.[1]

There are 2 basic methods of applying electric current to bone (Fig. 1). In one, electrodes make direct physical contact with the test subject, usually both superficially and at the site of the osteogenic response[1] (Fig. 1A). In these instances the electric current flows between the electrodes through pathways within the subject. In the second method the electrical energy is coupled into the subject via an electric or magnetic field—physical contact is not required. An electric field from a pair of energized plates induces currents which depend on the exposed subject's electrical characteristics[9] (Fig. 1B). In magnetic field applications, electric fields are created which similarly result in induced currents[2] (Fig 1C). Regardless of the method used, but quantitatively depending on it, there is always some current flow in tissue surrounding the intended site of osteogenesis. Such ancillary currents arise essentially because fields and currents are inextricably linked—the presence in tissue of the latter insures the former—and because there is no practical way by which either may be focused on specific tissues or sites to the exclusion of any superficial or adjacent tissue. From a safety standpoint, therefore, one must consider whether the currents produce effects other than those intended—either adverse effects on bone, or unanticipated effects on other organs.

Beyond applications involving only bone, fields have given rise to a variety of biologic effects in many different animals.[5] Many of the observed effects indicate that the fields used were biologic stressors.[5,7] If the fields and currents used in electrical osteogenesis can also be stressors, one may expect a range of additional

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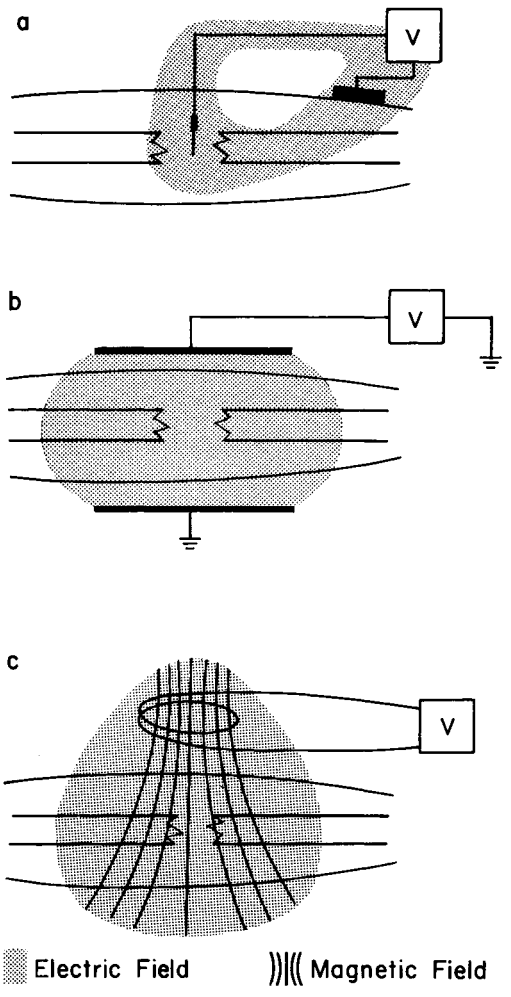

FIG. 1. Methods utilized in electrical osteogenesis. V = voltage.

effects which depend on field or current strength, and on individual subjects' predispositions. Any stress produced by ancillary currents has the potential to adversely affect bone healing because such stress is an adventitious physiologic burden.[7]

Because biologic stress is a contraindication to the therapeutic use of fields and currents in orthopedics,[7] it is clearly desirable to ascertain the range of fields—strength, frequency, and waveform—which can be stressors, particularly with regard to bone. To this end, we have developed a procedure to determine the effects of full-body exposure to various electrical environments on bone healing following a standardized fibular osteotomy (SFO) in rats. In our initial study we chose an electric field whose precise characteristics have not been utilized in bone stimulation studies.

MATERIALS AND METHODS

Our procedure consisted essentially of the histologic evaluation of the impact of a full-body electric field on the degree of fracture healing.

Male Sprague-Dawley rats, 21 days old, were obtained commercially and acclimatized to our animal care facility for 6 days, after which the SFOs were performed. Under anesthesia, the head of the fibula was palpated for orientation and a small incision was made on the lateral margin of the leg midway between the fibular head and the lateral malleolus. With the skin retracted, the fascial plane between the posterior compartment plantar flexor muscles and the lateral compartment peroneal muscles was bisected. Gently separating the 2 muscle groups exposed the shaft of the fibula, and a midshaft fracture was produced using sharp, fine-tipped scissors. Following an open reduction in which the exposed bone ends were manually aligned using forceps, the wound was closed with 4-0 silk sutures. Because the fracture was visualized during reduction, consistently good alignment was achieved. The rats were then divided into control and experimental groups, with an equal number of right and left SFOs in each, and exposure was commenced.

Each animal was housed individually in a nonmetallic cage; vibration, light, light-dark cycle and temperature were controlled. From 5 to 8 cages were situated on shelves of specially constructed exposure assemblies. Each shelf consisted of a sheet of metal, the plate, sandwiched between 2 layers of wood. Each of 2 assemblies, housing respectively the control and experimental rats, consisted of 3 pairs of vertically arranged shelves. When a voltage was applied to the plates, the region between them, which contained the cages, became filled with a uniform, homogeneous electric field. In the assembly which housed the control rats, the plates were electrically grounded. The lower plates in the second assembly were energized and the upper plates were grounded, thereby creating the electric field in each of the 3 regions. Except for the electric field, the environment of each rat was identical in all respects. We applied 1590 volts, at 60 Hz, which produced an electric field of 5000 volts/meter in the living space of each experimental rat; the 60-Hz field in the control assembly was essentially zero.

TABLE 1. General Characterization: Grading System

Histologic Evaluation	*Numeric Value*
Union	
Fibrous clot	1
Fibrous connective tissue and cartilage	2
Cartilage only	3
Cartilage and cancellous bone	4
Cancellous bone	5
Compact bone	6
Remodelled compact bone	7
Alignment	
Poor	0
Fair	1
Good	2
Callus size	
Minimal	1
Average	2
Extensive	3
Attenuated by remodelling	4

TABLE 2. Regional Characterization: Grading System

Anchoring callus
1. Cartilage
2. Bone

Bridging callus
1. Cartilage
2. Bone

Uniting callus
1. Cartilage
2. Bone

Sealing callus
1. Cartilage
2. Bone

Cartilage values: none = 0; healthy—small amount = 1; healthy—large amount = 2; hypertrophic—small amount = 3; hypertrophic—large amount = 4; extensive resorption = 5.

Bone values: none = 0; thin trabeculae—cartilage cores = 1; thin trabeculae without cartilage = 2; thick lamellar trabeculae = 3; compact bone = 4; remodelled compact bone = 5.

We studied extensively the time course of healing of the SFO.[3] At 14 days post-fracture, a multi-faceted histologic picture is presented and deviations from the normal healing rate are most easily seen and documented. For this reason, each animal was sacrificed 14 days after the SFO; the fracture site was dissected free of soft tissue, fixed, sectioned, and stained with hematoxylin and eosin. The extent of healing of each fracture was determined after study of the entire serial sequence, approximately 150 sections. To avoid subliminal prejudice, the slides were coded and grading was done without knowledge of the experimental treatment.

Histologic assessment of the degree of fracture healing was based upon the numeric grading system shown in Tables 1 and 2, using the regional distinctions in the fracture callus shown in Figure 2. This method provides a systematic means for analyzing the healing process, and produces a general healing profile in which union represents the most general description of the fracture repair process. Alignment and callus size are also included because of their relationship to the degree of union: poor alignment often causes unusually large callus. The regional categories relate cartilage and bone to particular areas of the callus; they are the most sensitive and specific measures of fracture healing. Summing over all 11 categories yields a healing index; a high index indicates advanced fracture repair.

The experiment was repeated and the results were identical; consequently no distinctions are made between the replicates. Every animal placed on study survived to sacrifice.

RESULTS

The fractures of rats exposed to 5000 volts/meter exhibited a distinctly altered histologic appearance compared with that of the control rats. The healing callus in the control group was dominated by new trabecular bone several lamellae thick. New periosteal compact bone was also found in the region of anchoring callus. Cartilage, if present, was limited to isolated regions of the bridging and uniting callus, and was sometimes observed in the central portion of the more delicate trabeculae. The cartilage was generally hypertropic; chondrocytes were swollen and had nuclei with varying degrees of pyknotic condensation. Often, chondroclasts were seen in necrotic areas of cartilage. Some fractures displayed such an advanced degree of healing that the fracture site was not readily apparent. In these cases, the necrotic fragment ends provided the only landmarks for identifying the region of the fracture because new com-

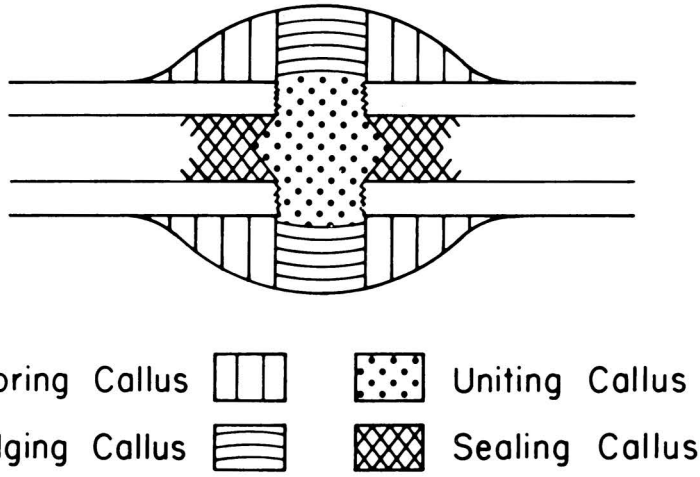

FIG. 2. Regional distinctions in the fracture callus following SFO.[10]

pact bone completely filled the fracture gap and no external callus was present.

In the field-exposed animals, the new bone had much thinner trabeculae—most with cartilaginous cores—than the control animals. Large blocks of cartilage were evident throughout the callus. In some instances, a cartilage plate was seen at the union site in the region of the uniting callus. This cartilage exhibited typical epiphyseal plate organization with zones of resting, proliferating, hypertropic, and calcifying cartilage, often at both the superior and inferior margins. New compact bone was strictly limited to the anchoring callus and was never extensively developed. After 2 weeks of field exposure, fractures exhibited less new bone formation, and correspondingly more cartilage, than did the control fractures. Characteristic fracture sites in experimental and control rats are shown in Figures 3 and 4 respectively.

Table 3 summarizes the numeric histologic scores for both experiments. In both, the mean healing index for the field-exposed animals was unquestionably less than that of the corresponding control animals ($P \ll 0.001$). Additionally, in all individual categories of comparison, with

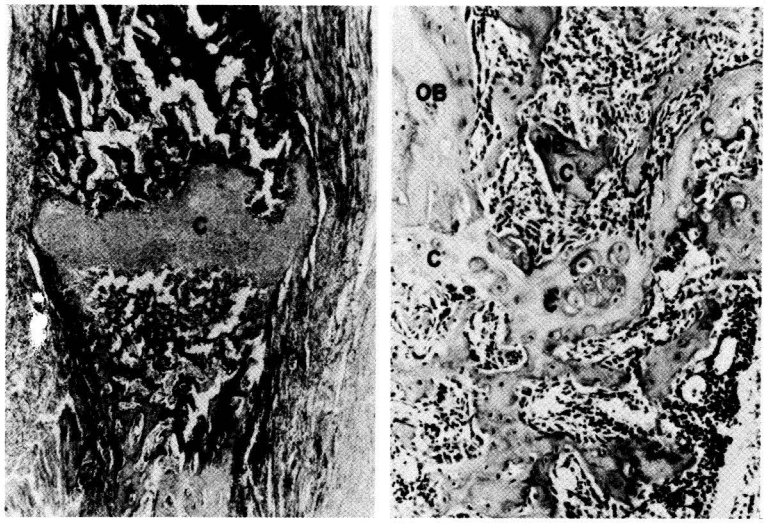

FIG. 3. Fracture site in field-exposed rat 14 days after osteotomy. Original magnification: left, ×25; right, ×100. C = cartilage; NB = new bone; OB = old bone.

FIG. 4. Fracture site in control rat 14 days after osteotomy. Original magnification: left, ×40; right, ×100. Bracket indicates injury site.

the exception of Alignment, the experimental fractures were significantly less advanced ($P < 0.01$).

The increased amounts of cartilage and the scarcity of new bone in the field-exposed fractures are indicative of a relatively immature stage of fracture repair. By comparing the observed healing in the experimental animals with the normal temporal sequence of repair of the SFO,[3] we estimate that the 14-day field-exposed fracture resembled the normal fracture site at perhaps 10 days after osteotomy.

At 5000 volts/meter, 60 Hz, bone healing was adversely affected, probably because of a systemic stress interaction. The metabolic alteration produced by the field resulted in a microenvironment at the fracture site ill-suited to osteogenesis. The early stress response is

TABLE 3. Comparison of Histologic Gradings of Experimental and Control Animals*

	Experiment 1		*Experiment 2*	
Criterion	*Control* $n = 17$	*Experimental* $n = 18$	*Control* $n = 20$	*Experimental* $n = 20$
Union	5.28 ± 0.89	4.24 ± 0.97	5.40 ± 0.75	4.45 ± 0.76
Alignment	1.89 ± 0.32	1.35 ± 0.70	1.60 ± 0.68	1.35 ± 0.75
Callus size	2.89 ± 0.83	1.94 ± 0.75	2.95 ± 1.05	1.95 ± 0.89
Cartilage-anchoring	3.94 ± 1.63	2.06 ± 1.39	4.10 ± 0.97	1.80 ± 1.64
Cartilage-bridging	3.83 ± 1.38	2.29 ± 1.21	3.65 ± 0.88	2.45 ± 0.89
Cartilage-uniting	3.89 ± 1.45	2.00 ± 1.32	3.35 ± 0.67	2.45 ± 0.89
Cartilage-sealing	3.78 ± 1.63	2.12 ± 1.36	3.30 ± 0.73	2.10 ± 1.45
Bone-anchoring	4.22 ± 0.55	3.18 ± 0.53	4.05 ± 0.76	2.80 ± 0.89
Bone-bridging	2.94 ± 0.87	1.71 ± 0.92	3.00 ± 0.97	1.45 ± 0.76
Bone-uniting	3.11 ± 1.23	1.82 ± 1.07	2.20 ± 0.83	1.20 ± 0.41
Bone-sealing	3.50 ± 0.79	2.53 ± 0.94	3.50 ± 0.61	2.05 ± 0.83
Healing Index	39.28 ± 7.70	25.24 ± 3.46	37.25 ± 6.14	23.95 ± 3.19

*The means and standard deviations are listed. The means in each category in both experiments are significantly different ($P < 0.01$), except for alignment. For Healing Index, $P \ll 0.001$ ($t = 6.889$ in Experiment 1 and $t = 8.601$ in Experiment 2).

catabolic in nature; it includes labile protein breakdown and concomitant negative nitrogen balance.[7] Under these circumstances bone-collagen synthesis may be impaired and healing time lengthened accordingly.

Although full-body, electrodeless exposure was used, the possibility that the field acted directly on the fracture site cannot be ignored. The amount of electrical power delivered to each rat was much less than that typically applied in electric osteogenesis.[4,6]

Present experimental and theoretical knowledge of the interaction of electricity with biologic systems is embryonic. Explicit assessment, therefore, of the relative healing efficiency of the various methodologies used for electrical osteogenesis is not possible at present. It is clear however, that each of these methods has attendant risks because, as we have shown, a very small portion of the electrical power normally dissipated during electric osteogenesis is capable of producing antagonistic changes in bone. Therapeutic evaluation of electricity-promoted bone healing must, therefore, be a balancing process involving considerations of local and systemic effects, and local manifestations of systemic effects. Utilizing the SFO, along with other test systems, it should prove possible to evaluate the relative impact of the various constant and time-varying electric and magnetic fields and currents presently used to stimulate bone. Pending such evaluation, it seems prudent to strive for the most localized fields.

SUMMARY

Fibular osteotomies in rats were exposed to an extremely low frequency field for 14 days. By histologic evaluation it was found that the healing rate was retarded by the field. The effect (which was replicated) occurred at much lower power levels than are presently employed in electrical osteogenesis.

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