Augmentation of Bone Repair by Inductively Coupled Electromagnetic Fields

Abstract. *Pulsing electromagnetic fields of low frequency and strength have been inductively coupled across skin, directly to bone, to enhance the repair of canine osteotomies. The induced voltage field in bone appears to increase the organization and strength of the repair process at 28 days after "fracture."*

Until recently, it generally was accepted that repair processes in biological systems could be retarded, but not accelerated. In the past 10 years, however, investigators have reported that osteogenesis can be stimulated electrically (*1, 2*). These observations, first put on a firm foundation in 1964 (*1*), provided both the rationale and technologic background for recent attempts by others to increase the tempo of fracture healing and to repair pseudarthroses in man (*3, 4*). Following initial investigations on dogs in these laboratories, however, clinical application of the effect was rejected because osteogenesis was spatially limited to discrete regions about the cathode and because of electrolysis effects. Furthermore, such an approach required surgical implantation of current-delivering electrodes. An invasive technique, it was felt, could not be justified in the majority of fractures which, normally, will heal satisfactorily with plaster or other means of external fixation. Accordingly, a search was initiated for surgically noninvasive means of altering the electrical environment of a fracture and, thereby, to increase the rate of repair.

Two external methods were considered to achieve these ends, namely, capacitive coupling of electrostatic and electrodynamic fields to biological systems and the use of inductively coupled, pulsed, electromagnetic fields. In 1968 the first of these demonstrated that capacitively coupled fields could increase DNA and collagen synthesis in fibroblasts grown in culture (*5*). Concomitantly, ultrastructural changes, consistent with increased synthetic activity, were observed (*6*). Further evidence of biologic activity by capacitively coupled fields has been reported in vivo (*7*) and in vitro (*8*).

On the basis of culture results, capacitively coupled 100 volt/cm electrostatic fields were found to increase the repair rate of fibular osteotomies in rabbits (*9*). Although these results seemed promising, direct application to man posed at least one major problem. Increasing interplate distance to accommodate bulkier human extremities would have required larger voltages which, on the basis of culture results, were already marginally low. Even a 100-volt source attached to a patient constitutes a possible hazard, and larger voltages, therefore, were unattractive. For this and other reasons, it was decided to investigate the effects of electromagnetic fields on bone repair in animals.

Pulsing, magnetic fields produced by air-gap, rectangular coils were used to induce dynamic, orthogonal, voltage fields in bone and soft tissue. A block diagram of the circuitry is shown in Fig. 1. The basic coil was about 2.5 by 3 cm, internal dimensions, allowing a homogeneous voltage field to be created along 1.5 to 2 cm of bone, placed in a plane parallel to the inside coil edge. Voltage drops were measured with Conel metal-shielded, Pt-Ir electrodes on and within the bone (Fig. 2). Forty-three adult beagles were used. Bilateral, transverse, fibular osteotomies were performed 3.5 to 4.5 cm distal to the proximal fibular head. Each of a pair of the spatially aligned coils was placed medially and laterally to each leg at the level of the osteotomy. Coils were mounted 8 cm apart and 0.5 cm from the skin on plastic carriers fixed to two-threaded Steinmann pins in the distal tibial metaphyses. The mounting pins were well outside the effective magnetic field and exhibited insignificant, induced voltages. X-ray control assured that the transverse central axis of a coil pair was posterior or anterior to the osteotomy, in order to eliminate an induced voltage field null in the region under study. Circuits and batteries were carried in pockets of canvas vests on the dogs' backs for 28 days. A coil pair on the contralateral limb was unpowered and served as a control. After 28 days, pairs of healing osteotomies were dissected, with precautions not to disturb the callus, x-rayed, and mounted in a mechanical testing device to assay stiffness and viscoelastic properties. Cantilever testing of specimens in four positions of rotation about the longitudinal axis determined load as a function of deformation (*10*). After this nondestructive mechanical testing, specimens were processed for longitudinal hematoxylin and eosin stained sections. Analysis of the histologic and radiographic material was carried out, initially, by observers with no knowledge of specimen origins.

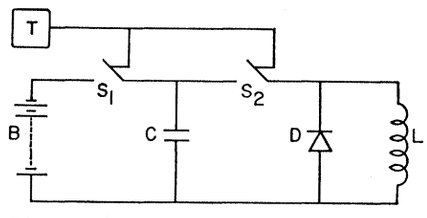

Fig. 1. Block circuit diagram. Capacitor, *C*, is charged from batteries *B* (24 volts), with S_1 closed, S_2 open. *C* then discharges into coil, *L*, S_2 open, S_1 closed. Diode, *D*, assures amplitude asymmetry of induced voltage field (see Fig. 2). Adjustable timer, *T*, provides basic charge-discharge repetition rate.

Two different, pulse-shaping, circuit designs were used in these experiments. In the first group of 22 animals, a pulse of 1 msec duration, repeating at 1 hz, produced a peak voltage field of 2 mv/cm in bone. Circuits of this design are referred to as 1 power, 1 hz (1P, 1hz). In the second group of 21 animals, a circuit was used with a pulse of 150 μsec duration, repeating at 65 hz and producing a peak of 20 mv/cm in bone. These circuits are referred to as 10 power, 65 hz (10P, 65 hz).

In the first series of animals, employing the 1P, 1hz circuits, 20 of 22 animals were available for study at 28 days. In ten animals, the stimulated leg produced larger load values than the contralateral control. In the remaining ten animals, all had greater load values associated with the unstimulated side. These results were obtained in the following manner: an average of the sums of the four load values in each specimen orientation (0°, 90°, 180°, and 270°), at 1 second, was derived for each deformation (0.025 inch, 0.050 inch, and 0.075 inch) (1 inch=2.54 cm) for both the stimulated and unstimulated control fibulae. These values were expressed for each pair of fibulae as a percentage, with the lower load value fixed at 100 percent. The average of the accumulative value for the percentages in the ten animals in which the stimulated leg was stiffer than its control was 260 percent. In the ten animals with control legs testing stiffer than the contralateral stimulated leg, the average of the accumulative values was 70 percent. The mean load values, however, revealed no statistically significant difference between stimulated (actives) and their controls.

Radiographic and histologic examinations demonstrated a wide range of healing patterns, from a small callus and evidence of early bridging of the osteotomy gap to a bulky callus with an appearance of an "incipient" pseudarthrosis. In general, specimens with maximum stiffness values were characterized by a small callus, whether experimental or control. Histologically, various stages of normal fracture healing were observed, and there was no evidence of cellular or architectural aberrations in injured or uninjured hard or soft tissue. Mitoses were uncommon in stimulated sections, and none of the sections suggested that neoplastic changes were present.

The second series contained a total of 21 animals bearing 10P, 65 hz circuits. Of these, 19 dogs were available for analysis 28 days after operation. Six animals in this group had circuits delivering wider and narrower pulses than the 150 μsec normally employed. Although results were similar, the waveform parameters were sufficiently different to warrant analysis elsewhere. Of the remaining 13 dogs, 10 demonstrated higher load values (greater stiffness) in the treated legs, with percentage increases in load far larger than those produced by the inactive legs in this or the first series of 1P, 1 hz animals. Three animals had a greater load value associated with the control leg. These findings are significant by the sign test, $P < .07$. The results were obtained on the basis of an analysis of 1-second load values as described above for the 1P, 1 hz animals. The average of the accumulated percentage values for the ten animals was 89 percent, and 40 percent for the three animals in which the control leg gave larger loads. Figure 3 presents the mean load values, together with the standard errors. It can be seen that, at each deformation, the active values were statistically significant, when compared with controls. As the larger deformations were reached, however, there was a greater range of variability in the results. In 90 percent of the 26 individual specimens in this series, mechanical behavior could be predicted from an analysis of the radiographs or histologic material. Seven of the ten active animals demonstrated a small callus, with evidence of partial to complete bridging of the osteotomy gap with fiber bone. When present, the fiber bundles of the tissues in the gap had a predominately parallel longitudinal orientation. Unlike the majority of controls in this and the previous series, very little cartilage or chondroid material was present, and when it was, an advanced pattern of "endochondral" ossification was observed. Again, there was no evidence suggesting neoplastic changes in normal or injured tissues.

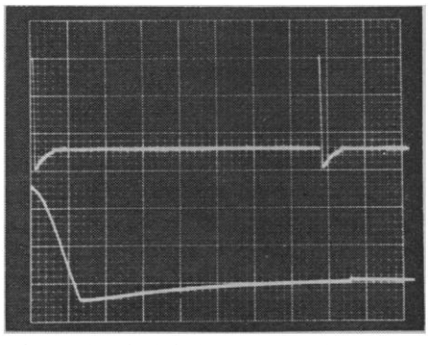

Fig. 2. Typical induced dynamic voltage field per centimeter of bone for a 10P, 65 hz system. Vertical scale: 10 mv per major division for both traces. Horizontal scale: 2000 nsec per major division for upper trace illustrating duty cycle. Lower trace (100 nsec per major division) demonstrates pulse shape in bone.

This study demonstrates for the first time, to our knowledge, that low-frequency, low-intensity, external pulsing electromagnetic fields can be inductively coupled to a tissue to achieve an increase in the tempo of a repair response. More recent investigations in these laboratories indicate that these fields also can increase the rate of neural regeneration and alter significantly the behavior of Meth A sarcoma in Balb/C mice (*11*). It appears, therefore, that inductively coupled electromagnetic fields may prove to have wide biological and medical importance as investigative tools and therapeutic modalities. The salient difference between the present method and that reported recently by Kraus and Lechner (*4*) lies in the present direct inductive couple between the field and the tissue, without an intermediary device. Although the fields used in this study were much weaker, the method and circuitry bear some similarity to that of Maass and Asa (*12*), who used pulsed electromagnetic fields for direct nerve stimulation.

The exact mechanisms behind the present results are not evident, although a wide variety of biological systems are known to be affected by electromagnetic radiation (*13*). It is highly unlikely, in view of the low power employed in the system (10^{-4} watt/cm), that joule heating was responsible, and therefore the effect probably is nonthermal in origin. For those who are interested, a review of possible interactions between nonexcitable cells and changes in their electrical environment is available elsewhere (*6*). Although the shape of the induced waveform bears some similarity to that observed in deformed moist bone (*14*), it was selected mainly on the basis of a theoretical analysis of time constants involved in charge distribution at interfaces, such as those at electrodes and cell surfaces (*15*). Despite the fact that the frequency of the 1P, 1 hz circuit was chosen to approximate that occurring in many dynamic biologic events (heart beat, walking, and so forth), it did not appear to be as effective in this application as the 10P, 65 hz circuit.

Although the 10P, 65 hz circuit augmented bone repair, it is anticipated that more efficient magnetic stimuli can be developed. The possibility that each different tissue may require signals of specific frequency, amplitude, and duration must be considered. Certainly, the promise for controlling regenerative

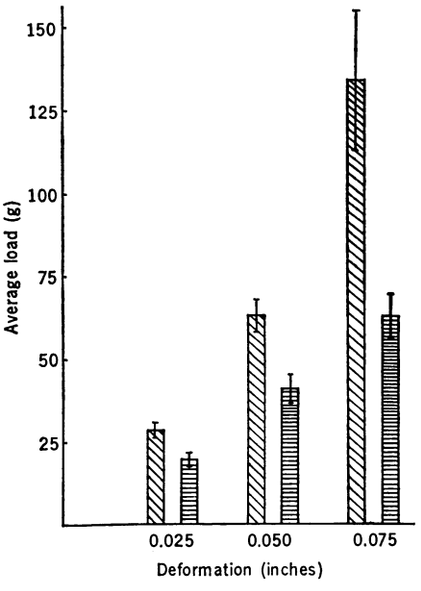

Fig. 3. Average load in grams at each of three deformations of 26 cantilever-loaded specimens treated with 10P, 65 hz circuits. Experimentals, obliquely slashed bars. Controls, transversely slashed bars. The standard error is included at the top of each bar. Note the significantly different slopes of control and experimental values produced by projecting a line through the origin and the tops of the bars. At a deformation of 0.025 or 0.050 inch (1 inch = 2.54 cm), the difference between experimentals and controls is significant at the $P < .05$ level; at 0.075 inch, $P < .10$.

phenomena by electrical means (*16*) dictates that considerable effort in these areas is more than justified, even if the present result in improving repair processes did not obtain.

The nature of the increased repair response deserves some comment. Although field-treated osteotomies generally were stronger than their contralateral unstimulated controls, the bulk of callus was less. This result stems most probably from the more highly organized nature of the "stimulated" callus, which may have served to stabilize the mobile fragments at an earlier time than the controls. Certainly, there was no evidence of increased cellularity or mitoses in the field-exposed fibulae. Furthermore, untraumatized soft tissues and bone exposed to the field appeared entirely normal.

Although, recently, this method has been applied successfully to treat a young girl with congenital pseudarthrosis of the tibia, its general use in fresh fractures remains to be defined. Should the present surgically noninvasive method prove to be effective in man, it may be possible to reduce fracture disability time significantly. In view of the relatively simple methodology, which would employ magnetic coils in plaster, such a reduction might well free hospital beds and alleviate a major economic drain.

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Oxygen Binding in Cyanmet Hybrid and Normal Hemoglobins: Applicability of Sequential and Two-State Concerted Models

Abstract. *The cyanmet hybrid hemoglobins* $\alpha_2\beta^{+CN}{}_2$ *and* $\alpha^{+CN}{}_2\beta_2$ *are widely held to be similar or equivalent in structure and subunit interactions to the partially oxygen-liganded species* $\alpha_2(\beta \cdot O_2)_2$ *and* $(\alpha \cdot O_2)_2\beta_2$, *respectively. An analysis of precise data on oxygen binding to the cyanmet hybrids and normal hemoglobin shows that if this is the case, then cooperative ligand binding in hemoglobin is more properly described by some model of the sequential type than by any two-state concerted model.*

For some years a controversy has existed over whether the cooperative ligand binding properties of hemoglobin may be more appropriately described by one of two classes of allosteric models. One class of models, called sequential, explicitly defines ligand-dependent pairwise-additive interactions between the four subunit chains (or hemes). According to this approach, the liganding of a subunit directly modifies the ligand affinity of other subunits with which it interacts. The first model of this type was proposed by Pauling (*1*) and the sequential concept has been subsequently broadened and extended by Coryell (*2*), Koshland *et al.* (*3*), Thompson (*4*), and Saroff (*5*). The other class of models, called concerted, treats the hemoglobin molecule as existing as an equilibrium mixture of distinct quaternary conformations, within each of which a subunit is defined as possessing an invariant ligand affinity. According to this approach, partial liganding of the hemoglobin shifts the equilibrium between quaternary conformations, thereby indirectly effecting an alteration in the (equilibrium average) oxygen affinity of remaining unliganded subunits. The concept of a concerted allosteric transition was first introduced by Monod, Wyman, and Changeux (*6*), who presented a formal two-state model for oligomers of identical subunits, referred to as the MWC model. Later this model was extended by Ogata and McConnell (*7*) to allow for possible nonequivalence of α and β chains in hemoglobin.

A number of investigators have gone to some lengths to show that a particular model is capable of rationalizing, or is compatible with, a variety of experimental data. However, in order to show that cooperative ligand binding in hemoglobin is more appropriately described by a sequential or concerted model, it is necessary to demonstrate that there exists some set of reliable experimental data which is compatible with only one of the two approaches. It is proposed that oxygen saturation data for the cyanmet hybrid hemoglobins $\alpha_2\beta^{+CN}{}_2$ and $\alpha^{+CN}{}_2\beta_2$, reported by Maeda *et al.* (*8*), together with oxygen saturation data for normal hemoglobin ($\alpha_2\beta_2$), reported by Tyuma *et al.* (*9*), constitute such a set. The data to be analyzed were obtained with the same apparatus under identical conditions for all three species, as specified in the legend to Fig. 1. These conditions are typical of those under which a large number of experiments on hemoglobin have been performed. Numerous experimental studies of the cyanmet hybrids have been undertaken with the objective of elucidating features of the mechanism of cooperative ligand binding in normal hemoglobin (*7, 10*). In all of these studies it has been either explicitly or implicitly assumed that the cyanmet hybrid $\alpha_2\beta^{+CN}{}_2$ is similar if not identical in structure and subunit interactions to the partially oxygen-liganded species $\alpha_2(\beta \cdot O_2)_2$, and that the com-