

# The Effect of Pulsing Electromagnetic Fields on Bone Metabolism in Experimental Disuse Osteoporosis

R. L. CRUESS, M.D., F.R.C.S.(C), K. KAN, PH.D., AND C. A. L. BASSETT, M.D.

Since the original report describing the electrical properties of bone,<sup>17</sup> it has been realized that a relation exists between mechanical forces, bioelectricity, and the behavior of cells in bone. It was specifically recognized that electrical forces are capable of stimulating bone growth.<sup>3</sup> Despite a considerable body of literature that has been accumulated in the field, the exact effect of electrical currents is still poorly understood, and no direct measurements of bone formation or resorption rates under the influence of exogenously applied currents have appeared in the literature. Bassett *et al.*<sup>3</sup> reported an effective model of disuse osteoporosis in the rat and demonstrated that the application of pulsing electromagnetic fields can largely influence the development of this condition. Biochemical measurements of bone formation and resorption, as well as compositional data utilizing this model, are reported. The model was selected because the histologic and biomechanical data have been reported<sup>3</sup> and therefore, did not require repetition.

From the Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada and Columbia Presbyterian Medical Center, New York, New York.

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Reprint requests to Richard L. Cruess, M.D., Dean, Faculty of Medicine, McGill University, 3655 Drummond Street, Montreal, Quebec H3G 1Y6, Canada.

## MATERIALS AND METHODS

Male rats, 250 g. were utilized in all experiments. The animals were assigned to three groups. The animals in Group I had bilateral removal under general anesthesia of the gastrocnemius and soleus muscles, together with the Achilles tendon. A neurectomy of the posterior tibial and peroneal nerves was carried out in the popliteal space. Immediately after operation, the animals were placed in a plaster cast from the thorax to the toes, a threaded Kirschner wire having previously been inserted into the tail to prevent the animals from escaping from the cast. A post was applied over the ventral surface of the cast and utilized to mount the animals on a frame so that they could be placed comfortably over food and water. The forelimbs were free, and a gravity-fed water supply permitted drinking. The animals in Group II underwent identical surgical and casting procedures, but were maintained between two vertically mounted Helmholtz-aiding "O" shaped coils 24 hours after operation. The experiments were carried out for 14 days, as Bassett's study had indicated significant osteoporosis by then. Group III consisted of freely roaming controls on-normal cage activity.

A single, quasi-rectangular primary (positive going) wave form, 325  $\mu$ SEC wide, including 1.0–1.5 mv/cm of bone was utilized 24 hours per day, with the pulse repeated at 65–72 Hz. (Bassett used a second pulse, characterized by a burst of 20 positive going quasi-rectangular pulses 200  $\mu$ SEC wide, each separated by a negative going pulse 30  $\mu$ SEC wide, with bursts repeated at 10 Hz. A pilot study indicated no biochemical differences between these two pulse characteristics, and consequently, it is only the first that is reported in this work.) At the termination of the experiment, final body weights were recorded. The animals were

killed by decapitation, and serum was collected for calcium and phosphorus determination in an autoanalyzer.

The tibiae were removed immediately, dissected free of soft tissues and periosteum, and weighed. The epiphyses were discarded, and the bone marrow was removed by flushing with ice cold saline. Both tibiae of a single rat were utilized for each set of determinations. Incubation studies were carried out according to the method of Deiss *et al.*<sup>9</sup> Minced fragments were incubated in buffered Krebs-Ringer bicarbonate medium, pH 7.4, in a Dubnoff incubator under 95% oxygen-5% CO<sub>2</sub>, at 37° for four hours. The incubation medium contained either 10  $\mu$ Ci L-(<sup>14</sup>C) proline (SA, 232 mCi/mmol) or 10  $\mu$ Ci D-(<sup>14</sup>C) glucose (SA, 4.06 mCi/mmol). After incubation, the bones were washed several times with saline and cold water and hydrolyzed at 100° for 17 hours with 6 N HCl for hydroxyproline according to the method of Stegemann,<sup>15</sup> or with 3 N HCl for hexosamine according to the method of Boas.<sup>5</sup> The (<sup>14</sup>C) hydroxyproline was separated on paper, and the specific activity of the hydroxyproline fraction was determined according to methods previously described.<sup>7</sup> To determine the specific activity of (<sup>14</sup>C) hexosamine, the hydrolysate was treated with ion exchange resin (Dowex 50 W) according to the method of Boas.<sup>5</sup> An aliquot was dissolved in 15 ml of Aquasol (New England Nuclear Corp., Boston, Massachusetts), and the radioactivity was determined in a liquid scintillation counter. The degree of quenching was estimated by internal standardization, and the data were corrected.

Collagenolytic activity was determined according to the method of Kaufman *et al.*<sup>14</sup> Minced bone fragments (50 mg) were placed in a tube containing 100  $\mu$ l of purified neutral soluble rat skin collagen labelled with (<sup>3</sup>H)-proline and (<sup>3</sup>H)-hydroxyproline (approximately 5000 cpm) with 400  $\mu$ l of 0.05 M Tris-HCl buffer, pH 7.5. The tubes were incubated at 35° for three days, and the collagenolytic activity of the bone was determined by counting the release of radioactivity into the medium. Blank values were obtained by parallel incubation of heat-inactivated bone (boiled at 100° for 3 minutes). To judge the uptake of mineral into bone, rats were injected intraperitoneally five days before they were killed with 100  $\mu$ Ci of <sup>45</sup>Ca. The bones were ashed in a furnace at 680° for 20 hours, and the bone was dissolved with 1 ml of 2 N HCl; 100  $\mu$ l of the solution was mixed with 100 ml of Aquasol and counted in a liquid scintillation counter. To judge the release of mineral from bone, a group of animals was injected five days prior to operation with identical amounts of <sup>45</sup>Ca, and the amount retained in bones was measured. Finally, the ash content of

aliquots of bone was determined by ashing in a furnace at 680° for 20 hours. In both groups of animals, the residual radioactivity in the serum was measured.

Statistics were compiled by performing an analysis of variance and applying Scheffe's test.<sup>1</sup>

## RESULTS

All animals started at approximately the same weight (Table I). The operated and casted animals of Groups I and II actually lost weight during the experimental period. Animals in Group III exhibited normal weight gain.

There was no statistically significant difference in the weights of the tibiae from animals of each group during the short period utilized.

Compositional studies of the bone showed no significant difference in the ash content of the bones from animals of the various groups, and the hydroxyproline contents were also equal. Both experimental groups (Groups I and II) demonstrated an increase in the hexosamine content, and the difference between the bones of animals treated with electromagnetic fields when compared with those roaming free showed that this value was statistically significant.

The indices of bone formation demonstrated significant differences. The incorporation of labelled proline into collagen as hydroxyproline showed that both experimental groups had higher rates of collagen formation than the free roaming controls. In addition, treatment of casted and denervated animals of Group II showed that there was a significantly higher rate of incorporation in the bones of these animals when compared with the casted and untreated animals of Group I. The synthesis of proteoglycans, as measured by the incorporation of (<sup>14</sup>C) glucose into hexosamine showed no difference between the casted untreated animals and the freely roaming controls. However, the treatment of casted animals with electromagnetic fields caused a statistically significant increase in this value, as compared with that of both controls (Group I) and freely roaming

TABLE I. Biochemical Data Derived from Experiment

	Group I (Control)	Group II (E.M.F.)	Group III (Roaming)	p	Groups Compared
Animal weight (g)	246 ± 3 (8)*	246 ± 3 (12)	246 ± 3 (8)		
At operation	259 ± 8 (11)	259 ± 16 (12)	306 ± 13 (8)	<0.05	I vs II, II vs III
At casing	244 ± 15 (11)	245 ± 16 (12)			
When killed					
Wet bone weight (mg)					
Left tibia	435 ± 37 (11)	449 ± 49 (10)	418 ± 18 (8)	NS	
Right tibia	430 ± 23 (11)	428 ± 46 (12)	423 ± 54 (7)	NS	
Percent ash	67.1 ± 4.4 (10)	66.4 ± 2.5 (10)	68.6 ± 0.7 (10)	NS	
Hydroxyproline (µg/10 mg bone)	206.9 ± 26.2 (23)	208.0 ± 28.2 (17)	205.6 ± 14.3 (25)	NS	
Hexosamine (µg/10 mg bone)	208.7 ± 26.6 (26)	218.0 ± 28.9 (21)	189.3 ± 22.8 (26)	<0.05	II vs III
Serum calcium (mg/100 ml)	9.68 ± 0.35 (29)	9.50 ± 0.68 (18)	10.54 ± 0.25 (10)	<0.05	I vs III, II vs III
Serum phosphorus (mg/100 ml)	7.91 ± 0.65 (29)	8.70 ± 0.96 (18)	8.37 ± 0.52 (10)	<0.05	I vs II
Incorporation of <sup>14</sup> C proline (dpm/µg hydroxyproline)	6.87 ± 2.88 (18)	9.03 ± 3.05 (18)	1.56 ± 0.92 (7)	<0.05	I vs II, II vs III, I vs III
Incorporation of <sup>14</sup> C glucose (dpm/µg hexosamine)	12.6 ± 4.2 (10)	19.9 ± 10.1 (12)	8.3 ± 3.8 (7)	<0.05	I vs II, II vs III
Uptake of <sup>45</sup> Ca (cpm/mg ash, cpm/ml serum)	1011 ± 156 (10)	2347 ± 746 (10)	1707 ± 165 ( )	<0.05	I vs II, II vs III, I vs III
Retention of prelabelled <sup>45</sup> Ca (cpm/mg ash, cpm/ml serum)	1156 ± 162 (10)	1115 ± 204 (9)	1313 ± 147 (9)	NS	
Collagenase dpm/hr/100 mg bone	3378 ± 343 (7)	4340 ± 47 (6)	4937 ± 338 (8)	<0.05	I vs II, I vs III
	1060 ± 162 (8)	1141 ± 322 (7)	965 ± 105 (8)	NS	
	1059 ± 452 (20)	169 ± 77 (12)	174 ± 156 (10)	<0.05	I vs II, I vs III

\* Numbers in parentheses refer to the number of animals studied per group.

\*\* NS = not significant.

animals (Group III). The incorporation of radioactive calcium into bones showed that the operated and caged animals had a significantly lower value as compared with freely roaming controls. The animals treated with electromagnetic fields had a significantly elevated incorporation rate as compared with either the freely roaming controls or the caged untreated animals. There was no difference in the residual radioactivity in the serum.

The indices of bone resorption showed that radioactive calcium levels in prelabelled bone were lower in the operated and caged animals than in either the treated or freely roaming control animals. Treatment with electromagnetic fields returned the value to normal. The collagenolytic activity of the bone fragments was significantly elevated in operated and caged animals. Treatment with pulsed electromagnetic fields returned this value to normal.

Both caged groups showed a lowered serum calcium concentration as compared with the freely roaming controls. The serum phosphorus concentration was significantly decreased in the animals from Group I, and treatment with electromagnetic fields returned it to the normal range.

## DISCUSSION

Bassett and co-workers<sup>3</sup> have previously reported that the experimental methods utilized could rapidly and predictably cause disuse osteoporosis. The previous report contained histologic and biomechanical data indicating that there was a measurable loss of bone mass and that this loss in mass caused mechanical weakness of the osseous structure. Treatment with pulsed electromagnetic fields identical to those utilized in these experiments prevented the histologic and biomechanical changes noted. However, the earlier report provided no information as to how the signal affected cellular behavior. Specifically, it was not possible to tell whether bone formation or resorption rates were changed,

either singly or together. It is also noteworthy that the literature relating to bioelectricity in the skeletal system contains no direct biochemical measurements that would indicate how electrical signals of either an endogenous or exogenous nature bring about their very real effects.

Immobilization leads to loss of both mineral and matrix, as indicated by increased excretion of calcium and phosphorus<sup>10</sup> and hydroxyproline.<sup>11</sup> Microscopically, disuse osteoporosis demonstrates increased porosity of the intracortical envelope<sup>16</sup> and, as shown in the data from these experiments, is the result of increased levels of resorption accompanied by apparently diminished levels of formation. Experimentally, the presence of parathyroid hormone is required, inasmuch as parathyroidectomized animals do not develop disuse osteoporosis.<sup>13</sup>

The data reported here suggest several conclusions. The compositional studies show no change in the percent of ash or in the content of collagen among the three groups and only a small difference in proteoglycan content. This indicates that the bones from animals in all three groups are normal in composition but vary in mass. The methods utilized to assess rates of bone formation have given data that are somewhat less consistent. Immobilization osteoporosis is accompanied by normal rates of proteoglycan synthesis and a normal uptake of radioactive calcium. There is an increased collagen synthesis in the immobilized and untreated animals, which is somewhat more difficult to explain. This fact, coupled with the diminished uptake of radioactive calcium, should lead to undermineralized bone, but this is not supported by the normal ash content. However, perhaps the short period did not allow this condition to develop. Treatment with pulsed electromagnetic fields increases collagen and proteoglycan synthesis, as well as the uptake of radioactive calcium in the bones of treated animals as compared with either freely roaming or untreated experimental animals. An indirect examination of bone resorption rates

finds that there is an increase in bone resorption in the untreated operated animals, as indicated by a significant decrease in the retention of prelabelled calcium and a large increase in the level of collagenolytic activity. Treatment of immobilized animals with pulsed electromagnetic fields returns these values to normal. Thus, pulsed electromagnetic fields diminish bone resorption, apparently to nearly normal ranges, and increase rates of bone formation to levels that appear to be above those of control freely roaming animals.

The data concerning serum calcium and phosphorus levels are more difficult to explain. The hypocalcemia demonstrated by both caged groups may well have been nutritional. The alteration in serum phosphorus levels, which was noted in the animals treated with pulsed electromagnetic fields, has not previously been reported. The coils were large enough that the posterior two-thirds of the animals were within the electromagnetic fields. It is therefore possible that a systemic, rather than a local, effect was responsible for some or all of the changes noted. There is no information available in the literature relative to systemic effects of pulsed electromagnetic fields on the parameters of normal physiology related to calcium and phosphorus metabolism. In particular, no influence on parathyroid activity or target organ receptivity can be found, nor are there any known effects on renal function or vitamin D metabolism. It certainly is conceivable that the alterations in serum phosphorus levels in the treated animals could be involved in the mechanism of action of the fields, but this does not detract from the measurements noted.

In addition to the possible systemic effects of pulsed electromagnetic fields, there are other potential sources of errors in the experiments noted. First, the actual weight loss exhibited by the experimental animals could well have affected the data. However, as there was no significant difference between Groups I and II, it is doubtful that this would influ-

ence the information relative to these two groups; when experimental osteoporosis with and without exposure to pulsed electromagnetic fields is compared. Epiphyseal growth would have continued in these animals, and therefore the influence of the fields on this biologic activity may also, in part, have been measured. Finally, the presence of the metallic Kirschner wire in the tail could have had unknown effects, although these effects also should have been identical between Groups I and II.

Becker<sup>4</sup> pointed out that there are three types of growth normally exhibited by bone: epiphyseal plate growth in length; appositional growth in response to mechanical stress; and growth following fracture. It appears probable that all three are influenced, and perhaps controlled, by various electrical signals. The data reported here are valid only for the experimental conditions studied, which probably involve the second type of growth observed in normal bone remodeling in response to stress or lack of stress.

Information is accumulating on the effects of various types of electrical stimuli. Histologic and mechanical data have been available, and the results presented here provide biochemical evidence that electromagnetic fields of the types used in these experiments can diminish abnormal rates of bone resorption and increase rates of bone formation. Bioelectric effects therefore appear to provide a link between mechanical stimuli and cellular behavior. How the electrical effects bring about the demonstrated alterations in cellular behavior remains unclear, with increased production of osteoblasts,<sup>2</sup> alterations in cAMP,<sup>8</sup> membrane permeability,<sup>12</sup> O<sub>2</sub> tension,<sup>6</sup> or other unknown mechanisms having been proposed. The data reported here, however, provide one more indication of the exciting potential of electrical means of influencing cellular behavior.

#### SUMMARY

The effects of electromagnetic fields on bone formation and bone resorption in ex-

perimental disuse osteoporosis were investigated in rats treated with pulsed electromagnetic fields. Compositional studies, including hydroxyproline and proteoglycan content, as well as percent ash, showed no difference among the three groups. Bone formation as estimated by synthesis rates of collagen and proteoglycan appeared to be normal in immobilization osteoporosis, but bone resorption as measured by collagenolytic activity and release of prelabelled calcium was increased. Treatment with pulsed electromagnetic fields increased the rate of synthesis of proteoglycan and collagen and diminished the rate of bone resorption utilizing the same biochemical measurements. Thus, the data on this experimental system in rats indicate that pulsed electromagnetic fields diminish abnormal levels of resorption in disuse osteoporosis and increase rates of bone formation.

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