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ELECTRIC FIELDS AND BONE LOSS OF DISUSE*

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Abstract – The object of this research was to investigate the effect of electric fields on bone loss due to disuse. Plaster casts embedded with electric field generating plates were applied to the right legs of forty-eight male rats. Fields of various types were applied over a period of 28 days. A paired analysis of the properties of the right and left femurs was compared to a sham group which received no field treatments. Parameters measured included bone weight, specific gravity, cortical area, ultimate strength, modulus of elasticity, hardness, osteon count, and chemical analysis. It was found that the bone weight loss and cortical area reduction caused by immobilization were reduced by electric field treatments. Eight bone tumors were observed on eighteen femurs treated with the electric fields. No tumors were observed on the sham group.

INTRODUCTION

THE INFLUENCE of functional loads on bone remodeling has long been recognized. Hermann von Meyer (1867) suggested a stress trajectorial theory of bone form while Wolff's (1870) law of bone architecture is well known. More recently Frost (1964) has proposed a guidance system based on negative feedback of the physical loads on bone. Concurrently, Bassett and Becker (1962) have demonstrated that electric currents influence bone growth, and have suggested that stress-generated electrical phenomena are capable of directing the activity of bone cells and are in some way responsible for the orientation and/or aggregation pattern of macromolecules in the extracellular space. Still, the immediate controlling factors have not been identified from a possible list that includes stress-sensitive P/N junctions, classical piezoelectricity, availability of electron donors and electrical pumping effect.

Fukada and Yasuda (1957) and more recently Shamos and Lavine (1964) reported the measurement in bone of an electrical effect similar to the classical piezoelectricity found in certain crystals, while McElhaney has measured charge distributions on the human femur and identified regions of positive and negative bone (in press). The results of these experiments support the view that the highly oriented collagen matrix gives rise to an electrical phenomenon that is well described by classical piezoelectricity. In a piezoelectric material a given strain field will produce a corresponding electric field and conversely a given electric field will produce a corresponding strain field.

This paper describes a series of experiments exploring the hypothesis that strains resulting from the application of electric fields will affect the ordered growth of bone. Specifically, it is hypothesized that due to the converse piezoelectric effect, the application of electric fields will cause the bone to deform in a manner consistent with the strength and direction of the field: and thus the bone will respond to the electric field in the same way as it would to an appropriate external load giving rise to electrical exercising.

MATERIALS AND METHODS

Forty-eight male Sprague–Dawley rats each weighing approximately 100 g were used. Cotton cast padding was first wrapped around

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the waist and right leg of each rat, the opposing copper plates were taped on each side of the rat's femur, and plaster of paris cast material wrapped around the waist and leg of each rat (Fig. 1). When a voltage was applied to the plates with wires brought out through the cast, an electric field was generated that engulfed the femur.

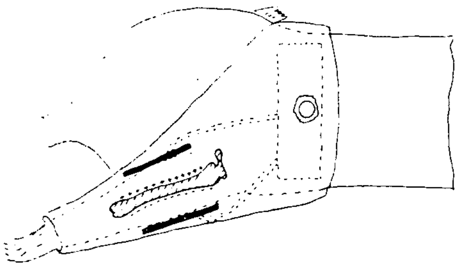

Fig. 1. Cast and plate positions.

The test animals were divided into four groups of twelve rats each. Group 1 was used as a sham and was given no electric field treatment. A d.c. potential of 100 v was applied to the plates of rats in group 2. An a.c. potential of 200 V peak to peak and 3 H was applied to the third group's electrodes, and an a.c. potential of 200 V and 30 H was applied to the fourth group's plates. These voltages correspond to the electric fields generated by stresses of approximately 3000 psi as calculated from Gauss' law and the stress–charge density curve for bone (Dainora, 1965).

The electrodes were made of brass $\frac{3}{4}\times\frac{1}{2}\times\frac{1}{16}$ in., and carefully insulated and waterproofed before installation.

The rats were given an electric field treatment for 1 hr every 12 hr for 28 days, after which the animals were sacrificed and the right and left femurs removed for testing and comparison.

A specially designed high-speed cutting machine was used to prepare segments of the femur shaft for mechanical property tests. This machine, incorporating micrometer-type feed and positioning devices, allowed precise control of the lengths and produced smooth parallel cuts. Since the plates were situated around the femur shaft, all tests were conducted on this section. The anterior edge of the patellar surface was used as a reference point for the first cut. A second cut was made 0·6 in. from the first, near the lesser trochanter. The shaft was then carefully cleaned and the marrow removed. The specific gravity of this section was determined by weighing in air and submerged in deairified water. The bone material was stored in saline and refrigerated to prevent deterioration. The shaft was then cut into four pieces and tested in accordance with the code of Fig. 2.

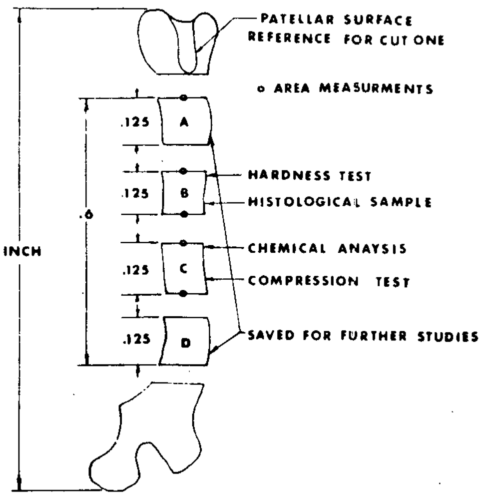

Fig. 2. Test sections.

AREA MEASUREMENT

The total, the cortical, and the medullary canal areas were measured at five sections along the shaft by enlarging photographs of the cross section to 8·5 × 11 in. and planimetering the photographs. In this way an accurate, repeatable area measure was obtained. Such measurements aided in the location of regions of active bone resorption or deposition and, in conjunction with the density measurements, allowed comparisons of the changes on the periosteal and endosteal surfaces with the interior of the bone.

HARDNESS TEST

Micro-hardness measurements were made with a Wilson Tukon Hardness Tester. The

Vickers scale was used for comparisons of the relative hardness of each bone. Four indentions approximately 0·005 in. on a side were made on the end of section *B* with a pyramid-shaped diamond indenter. A bifilar measuring microscope was used to measure the length of the diagonal of the indentation. The Vickers Harndess Number was computed from the diagonal length and the indenting load of 100 g. With this method the hardness of individual osteons may be measured.

HISTOLOGICAL STUDY

A 10–20 μ section of the shaft cross section was made by wet sanding. This was then stained using the undecalcified Villaneuva, Hoffer and Frost (1964) method. Osteons were counted and osteoid seams and overall cellular activity noted. The areas of these sections were also measured using the previously mentioned method so that the osteons per unit area could be calculated.

COMPRESSION TESTS

The compression tests were conducted on section *C* of each bone. The specimens were carefully prepared so that the ends were smooth, flat, and parallel. Since drying affects the material properties, all bones were kept wet during this regime. A Tinius Olsen electromatic testing machine was used to apply the load. A specially designed load cell and strainmeter were used to supply load-deformation data to an x–y plotter which automatically plotted the load–deflection curve. (see Fig. 3) The loading machine speed was 0·25 in./min and the tests were run until the specimen failed. From the curve plotted on the x–y recorder, the ultimate load, ultimate stress, ultimate deflection, ultimate strain, modulus of elasticity, and energy absorption were obtained.

CHEMICAL ANALYSIS

A chemical analysis was made to determine the per cent mineral, organic, calcium, and phosphorus content in section *C* of each bone specimen after the compression test had been made. The bones were dried in an oven at 105° for 72 hr and then ashed in a muffle furnace at 600° for 12 hr to determine the organic content. Calcium was determined according to Copp (1963) while phosphorus was measured according to Fiske (1925).

RESULTS

To assess the effect of inactivity, the immobilized right femur was compared by paired analysis to the untreated left femur. The effect of the electric field on the immobilized femur was determined by comparing the sham rat data to data obtained from the animals subjected to the electric field.

Table 1 shows the values and ranges of the parameters measured. Table 2 shows the average per cent change between the left and

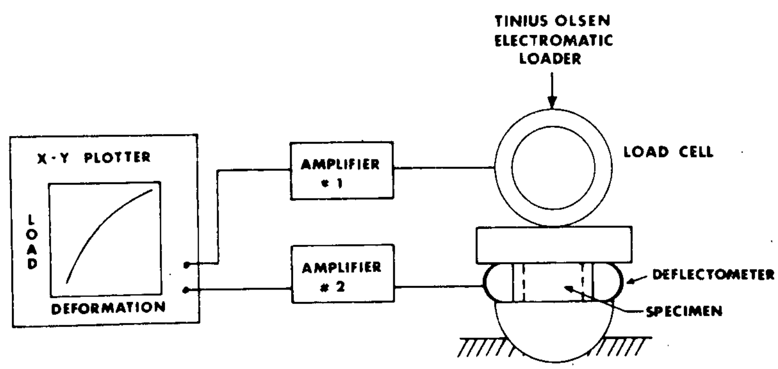

Fig. 3. Block diagram for rat femur compression test.

Table 1. Summary of absolute values and ranges for rat femur shaft sections

		Sham	c.c. field	3-Hz field	30-Hz field
Average wt. g	right	0·079	0·117	0·108	0·129
	left	0·090	0·131	0·123	0·143
	range	0·11–0·058	0·147–0·095	0·137–0·088	0·167–0·104
Average sp. gr.	right	1·90	1·87	1·86	1·88
	left	1·93	1·88	1·87	1·86
	range	1·96–1·83	1·93–1·80	1·96–1·83	1·95–1·68
Average cortical area in.^{2}	right	0·0049	0·0064	0·0054	0·0061
	left	0·0058	0·0072	0·0065	0·0061
	range	0·0074–0·0029	0·0077–0·0055	0·0070–0·0045	0·0079–0·0054
Average ultimate load, lb	right	53	71	57	73
	left	68	87	62	90
	range	80–40	101–56	66–49	99–61
Average ultimate stress, psi	right	11,200	11,200	11,000	12,000
	left	10,600	12,000	10,400	13,300
	range	16,100–9200	13,900–9800	12,100–9700	14,600–10,600
Average modulus of elasticity, lb/in^2 × 10^6	right	0·53	0·53	0·70	0·62
	left	0·49	0·55	0·51	0·57
	range	0·85–0·23	0·89–0·34	0·79–0·34	0·90–0·44
Average energy of distortion lb/in^2	right	195	175	167	218
	left	200	225	167	203
	range	393–92	336–113	200–121	261–165
Average μ-hardness No.	right	39·1	32·0	30·4	29·1
	left	39·2	35·4	33·5	33·5
	range	46·7–30·0	42·6–25·1	53·3–25·1	40·1–24·0
Average osteon count, in.^{2}	right	50,750	40,800	56,954	49,335
	left	52,250	41,800	61,860	44,422
	range	56,300–45,200	45,100–38,500	62,800–53,500	53,813–35,031
Average % organic content	right	32·1	29·2	30·5	29·1
	left	31·2	29·5	28·3	29·5
	range	33·3–29·9	31·2–25·3	31·8–27·0	31·6–24·2
Average % Ca content	right	41·4	38·8	38·0	39·0
	left	40·5	39·3	37·3	37·3
	range	46·4–34·9	42·2–33·6	41·6–34·2	41·6–36·1
Average % P content	right	19·6	19·7	19·7	19·2
	left	19·4	19·4	19·6	19·0
	range	21·0–18·1	21·5–18·3	22·2–17·3	20·8–18·3

right measurements based on the left or untreated active leg. Of the original forty-eight rats, thirty-two survived the treatment and only twenty-four were judged normal based on an average body wt. gain of 50 per cent. The results given in the tables are average values for six shams, seven treated with d.c. field, six with the 3-Hz field, and five with the 30-Hz field.

BONE TUMORS

Of significance in this research was the

(*facing p. 50*)

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	30-Hz field
	0·129
	0·143
3	0·167–0·104
	1·88
	1·86
	1·95–1·68
	0·0061
	0·0061
5	0·0079–0·0054
	73
	90
	99–61
	12,000
	13,300
	14,600–10,600
	0·62
	0·57
	0·90–0·44
	218
	203
	261–165
	29·1
	33·5
	40·1–24·0
	49,335
	44,422
	53,813–35,031
	29·1
	29·5
	31·6–24·2
	39·0
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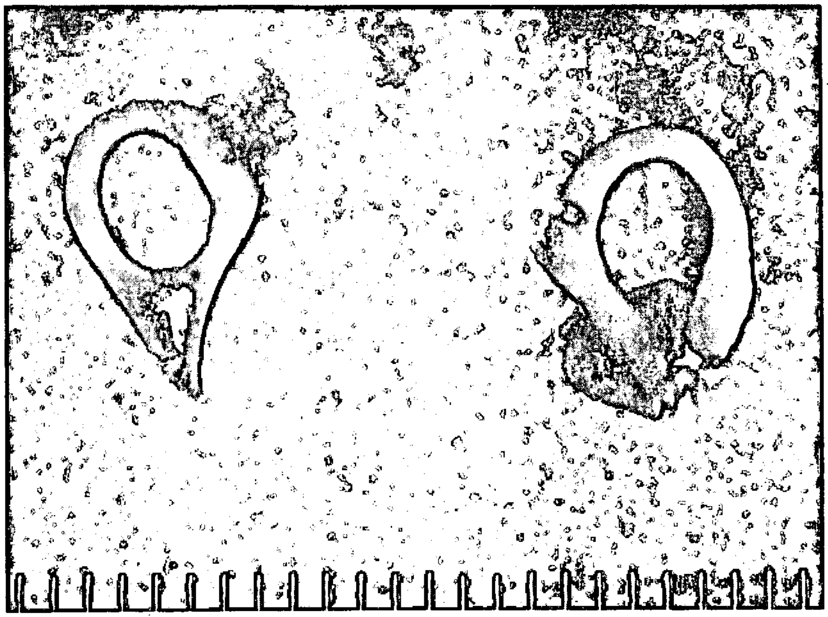

Fig. 4. Normal shaft and tumor.

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Table 2. Average per cent change between left and right femur

	Sham	d.c. field	3-Hz field	30-Hz field	Estimated accuracy (%)
Wt.	12·8	11·0	12·2	9·9	0·3
Sp. gr.	1·6	0·4	0·6	−1·4	1
Cortical area	17·1	11·5	14·7	9·9	1
Ultimate load	22·0	17·7	8·1	19·4	0·5
Ultimate stress	−6·0	6·3	5·6	−10·5	2
Modulus of elasticity	8·2	−2·3	−37·3	16·3	6
Energy of distortion	2·4	16·4	−0·4	−10·2	6
μ-Hardness	−0·9	7·9	6·2	12·3	5
Osteon count	1·1	1·8	7·8	−18·3	5
% Mineral content	1·3	−0·5	3·1	−1·2	2
% Organic content	−3·1	1·2	−8·2	1·2	2
% Ca content	−8·7	1·3	−2·1	−4·6	3
% P content	−1·0	1·6	−0·5	1·0	3

finding of bone tumors on eight of the eighteen femurs treated with electric fields. (See Fig. 4) These tumors were found in two locations on the bone. Six were in the region below the greater trochanter on the lateral side of the bone and two were on the lateral side of the central portion of the shaft. No tumors were found in the sham group. The tumors showed well developed osteons outside the normal periosteal surface. No further identification has been made. It is interesting to note that of the eight tumors found, five were in the 30-Hz group, with one in the d.c. group.

DISCUSSION

Because of the high degree of variation inherent in biological measurements and the small sample studied in this experiment, the results must be considered as preliminary. However, it is believed that comparing the properties of the right immobilized femur with the untreated left femur from the same animal in a paired analysis minimizes this natural variability.

Table 3 .ndicates that the 30-Hz field caused the greatest change from the sham group in the parameter measured while the d.c. field caused the least. Only small differences were noted in those parameters that typify bone as a material, i.e. chemical composition, specific gravity, hardness, ultimate strength, and modulus of elasticity, while significant differences occurred in structural or shape-related properties such as weight, cortical area, and ultimate load. This indicates that the major effect of immobilization with casts is a

Table 3. Summary of results

	d.c. field	3-Hz field	30-Hz field
Height	2	3	1
Sp. gr.	2	3	1
Cortical area	2	3	1
Ultimate load	2	1	3
Ultimate stress	2	3	1
Ultimate strain	3	2	1
Modulus of elasticity	3	1	2
Energy of distortion	1	3	2
μ-Hardness	2	3	1

Key: 1 – Most change from control; 2 – Second most change from control; 3 – Least change from control.

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loss of bone from the surfaces or a remodeling of the external features with lesser effects on the interior bony material. Of significance, therefore, is the lessening of these effects of immobilization by the electric field treatments.

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