FINAL REPORT

ELECTRIC FIELDS AND CALCIUM MOBILITY IN BONE

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A - Area ($meters^2$)

C - Capacitance (farads)

E - Electric Intensity (volt/meter)

K - Dielectric Constant

Q - Charge (coulombs)

S - Length (meters)

ε_o - Permittivity of Free Space (farad/meter)

1 - Muscle Tissue

2 - Bone

3 - Muscle Tissue

INTRODUCTION TO THE PROBLEM

Prior to our first manned space flight, many people expressed legitimate concern about man's possible response to the space flight environment. This concern was based upon information obtained from aircraft experience and from conjecture about the effects of man's exposure to the particular environmental variables known to exist at the time. From these experiences and suppositions some of the predicted effects were anorexia, nausea, disorientation, sleeplessness, fatigue, restlessness, euphoria, hallucinations, decreased G-tolerance, gastrointestinal disturbance, urinary retention, diuresis, muscular incoordination, muscle atrophy, and demineralization of bones.

First probing of the space environment by this nation was made in the Mercury spacecraft which reached mission duration of 34 hours. The actual situation following the completion of the Mercury program may be summarized as follows:

No Problem: Launch and reentry acceleration, psychomotor performance, eating and drinking, orientation, and urination.

Remaining Problem: Extended weightlessness, defecation, sleep, and orthostatic hypotension.

Now that the Gemini program is almost at an end we know that sleep and defecation present no problems in space. The questions pertaining to orthostatic hypotension and extended weightlessness still have not been resolved completely.[1]

This first encounter with the weightless environment has provided encouragement about man's future in space. From the Gemini VII flight we know now that there are no physiological hazards in a weightless environment for at least 14 days. One long range danger of weightlessness may lie in a prolonged alteration of calcium metabolism.

Before manned space flights clinical experiments with fracture-patients, polio-patients, and experimental studies with normal subjects in casts have demonstrated that immobilization causes marked disturbances in calcium metabolism even under normal conditions of gravity.[2] This change in metabolic activity appears as an increase of urine calcium. (Figure 1) A few hours after an astronaut enters a weightless environment an increase in urine calcium can be detected.[3]

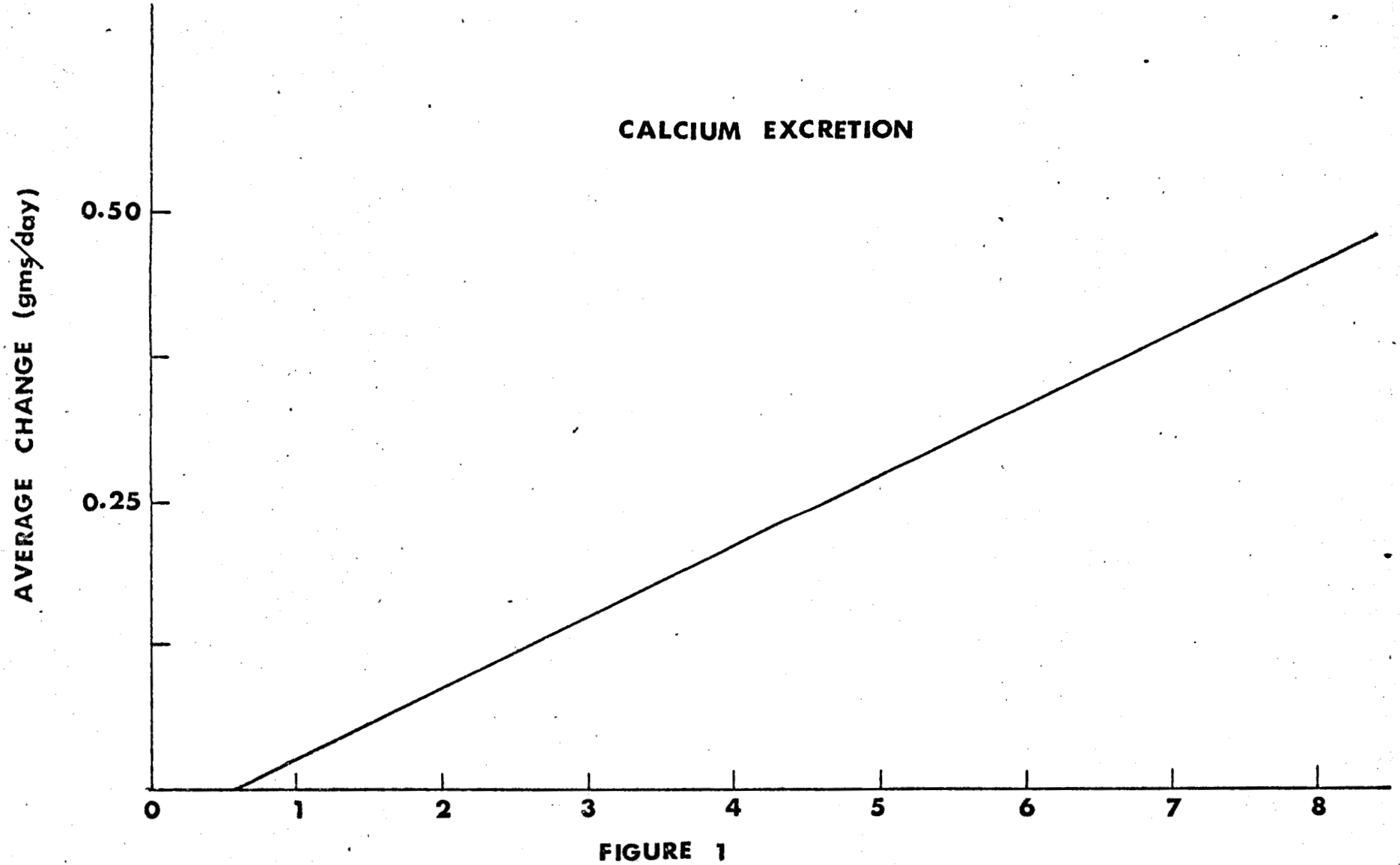

CALCIUM EXCRETION
AVERAGE CHANGE (gms/day)
0.50
0.25
0
1
2
3
4
5
6
7
8
IMMOBILIZATION TIME (weeks) (REFERENCE 3)

FIGURE 1

OSTEOATROPHOSIS

Bone is not static, but is in a constant state of change. Always new bone is being formed by cells called osteoblasts. Cells that maintain the bone as a living tissue are called osteocytes, while old bone is being reabsorbed by cells called osteoclasts. (Figure 2) In normal individuals this process is in equilibrium.[4]

Osteoporosis is caused by an interruption of this equilibrium process, and has been defined as a reduction in bone density with little change in chemical composition.[5] From immobilization studies carried out in the Biomechanics Laboratories of the Theoretical and Applied Mechanics Department it has been shown that rat bone can lose up to 15% of its original weight over a period of about one month. Of this 15%, only about 2% is due to a change in the density. The remaining 13% weight change is due to a resorption of bone material from the surfaces of the bone. For this reason the word "osteoatrophosis" was formed to describe this condition. Osteoatrophosis is defined as a reduction of bone volume with little

or no change in density or chemical composition of the bone. Osteoatrophosis may be over the entire body or it may be localized in only one limb.

When an astronaut is in a weightless environment the osteoclast activity becomes greater than the osteoblast activity.[2,1] Therefore, calcium homeostasis of the body is upset, and osteoatrophosis develops.

Plasma calcium, the controlling factor of calcium homeostasis, plays a vital role in many processes in the tissues including blood clotting, enzyme activation, neuromuscular excitability, membrance permeability, and muscle contraction. Severe hypercalcemia can cause intestinal and cardiac disturbances, and may result in serious kidney damage, while hypocalcemia may lead to tetany and death. Because of this the level of calcium in body fluids is one of the most critical physiological constants and is regulated with great precision.

The control of plasma calcium on a day to day basis is regulated primarily by hormones of the thyroid and parathyroid. A fall in plasma calcium will stimulate the parathyroid to increase its

production of parathormone(PTH) which will stimulate osteoclast activity and will elevate the plasma calcium level to normal. A rise in plasma calcium will stimulate the thyroid to increase its production of calcitonin which will stimulate osteoblast activity and will lower the plasma calcium level to normal.[6] These two hormones, parathormone and calcitonin, regulate normal daily changes in plasma calcium, but are not able to regulate abnormal physiological conditions such as prolonged bed-rest or weightlessness.

THEORY

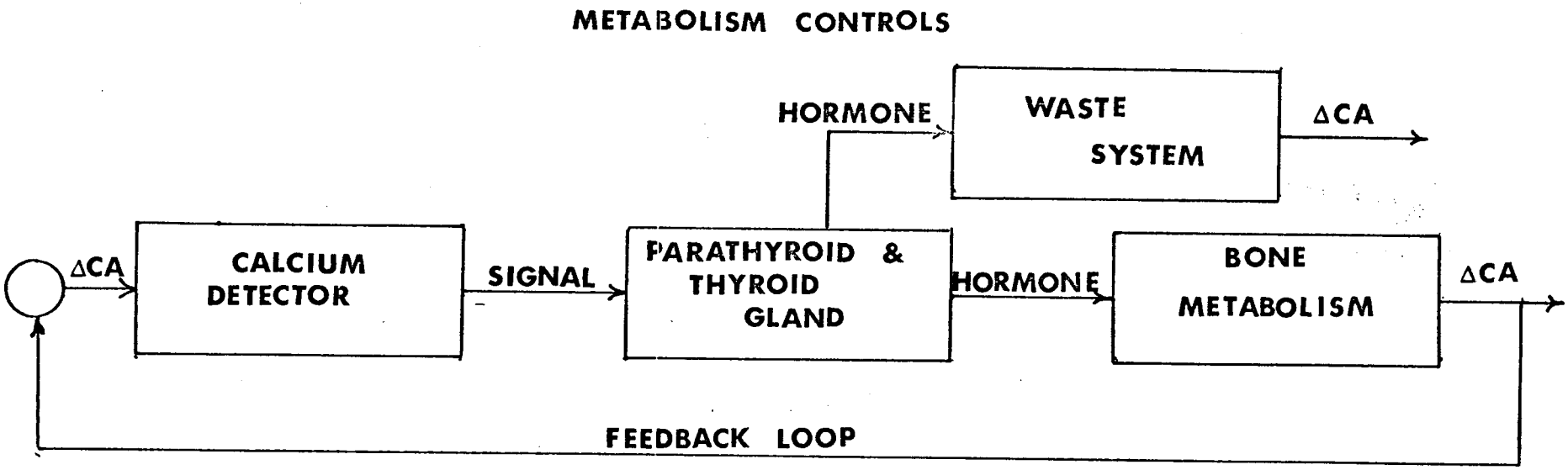
Presently a round trip journey to Venus would require about 12 months. A human space proble to Mars and back would require approximately 18 months. With our present knowledge in a flight of this duration, severe renal damage would occur from hypercalcemia.[7] Also demineralization of bone, if not checked, could possibly reach the point where the bones would not be able to withstand reentry gravitational forces. Therefore, if flights of this duration are to be endeavored, osteoatrophosis must be controlled. However, before control can be achieved, at least a general understanding of the mechanism involved must be obtained. The purpose of this thesis is to study a proposed mechanism and control of osteoatrophosis.

For many years it has been known that when bones are not stressed they will become weak, and bones that are stressed will stay strong.[8] The way the bone cells are able to measure the stress in the bone and adapt to it is not known to date.

In recent years a team of Japanese scientists found that bone was peizoelectric.[9] That is, if a bone is squeezed,an electric charge will appear on the surface of the bone. This charge is proportional to the stress,or load, on the bone.

Since cell metabolism is almost entirely ionic then it would not be unreasonable to think that an electric field caused by the charge may change the cell metabolsim.(Figure 3)

The method by which this proposed interaction was to be studied was to immobilize a rat's limb thus giving the rat osteoatrophosis and try to correct the condition by placing an electric charge on the surface of the bone. Parallel electric plates, with the rats femur between them, were used to produce the electric charge on the bone, and the immobilization was caused by a plaster of Paris cast placed on the right leg of the rat.(Figure 4)


METABOLISM CONTROLS
HORMONE
WASTE SYSTEM
ΔCA
ΔCA
CALCIUM DETECTOR
SIGNAL
PARATHYROID & THYROID GLAND
HORMONE
BONE METABOLISM
ΔCA
FEEDBACK LOOP

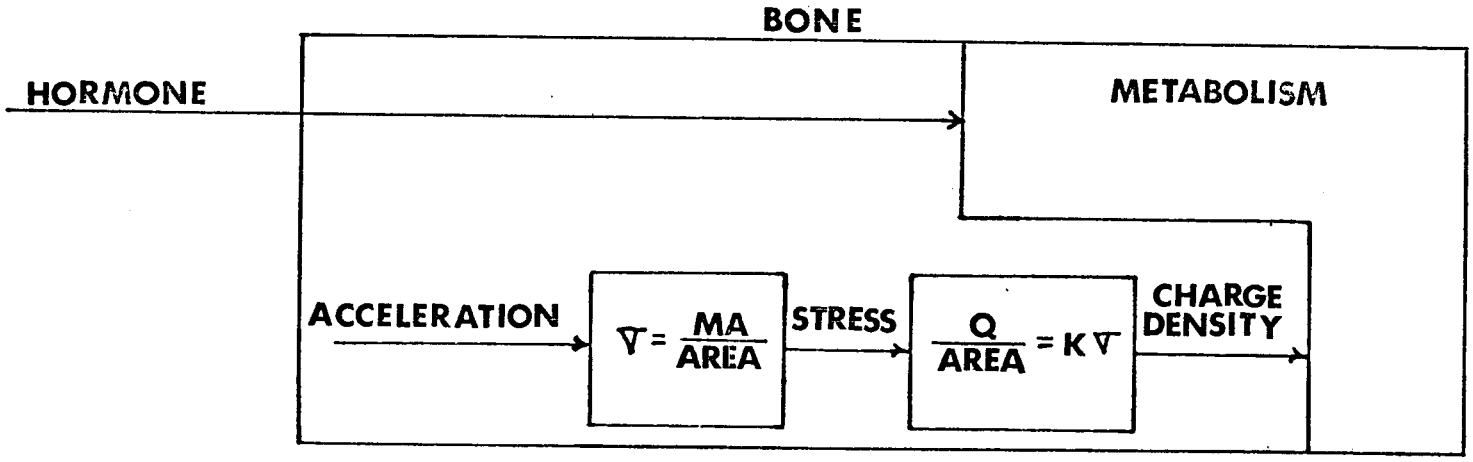
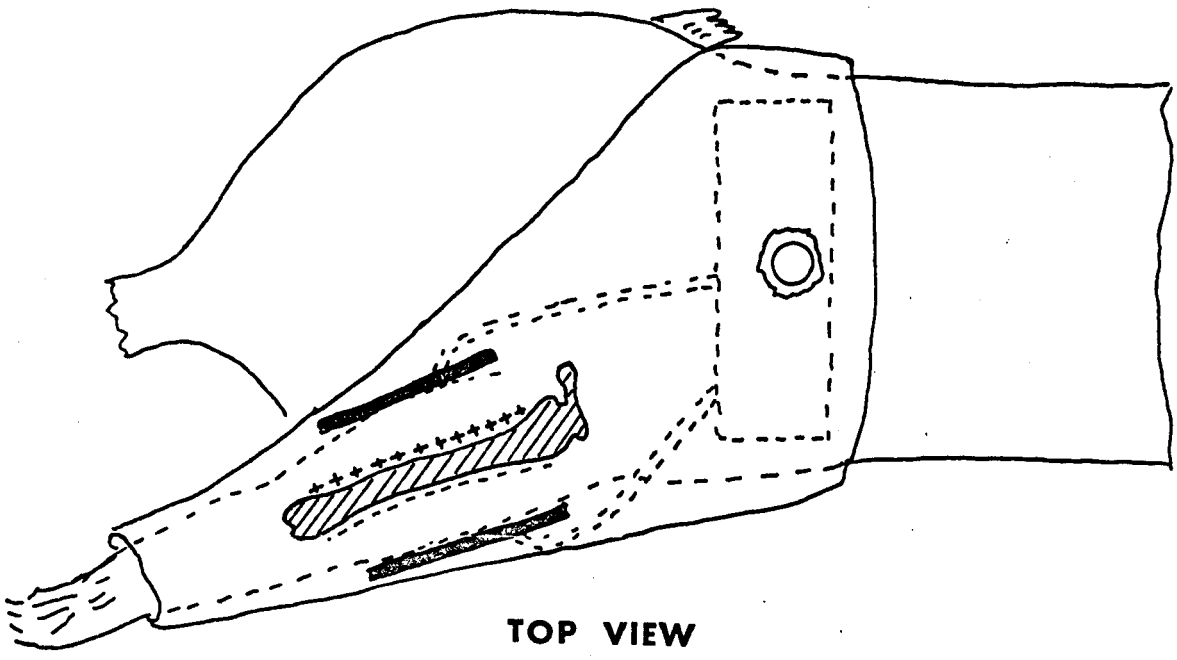
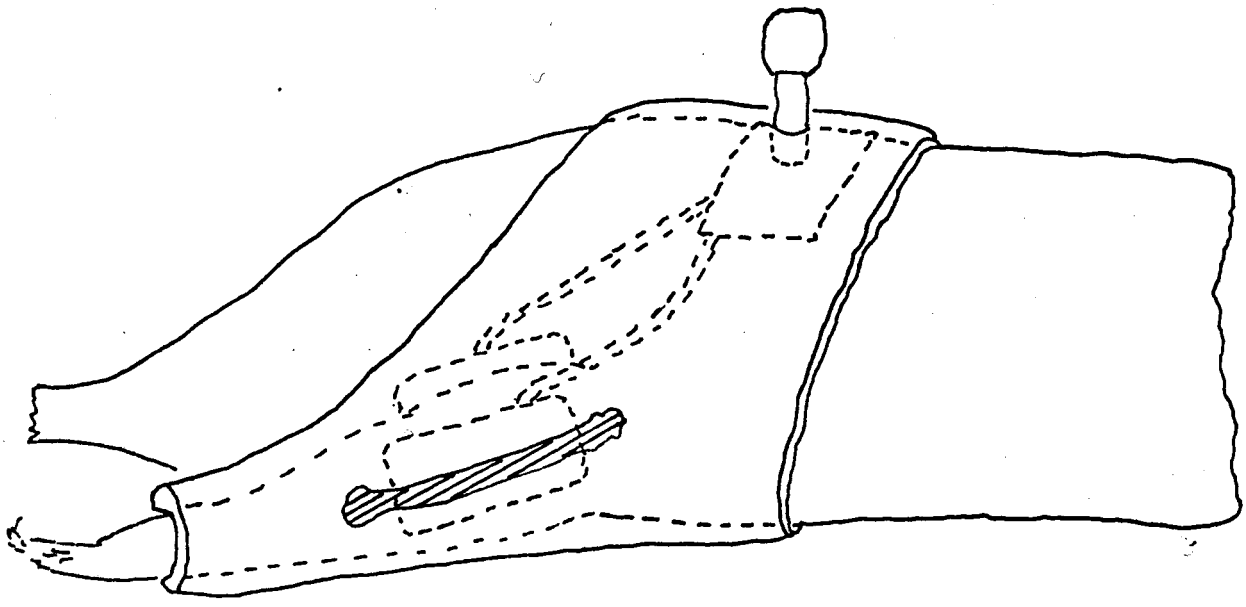

BONE
HORMONE
METABOLISM
ACCELERATION
∇ = MA / AREA
STRESS
Q / AREA = K∇
CHARGE DENSITY

FIGURE 3

CAST & PLATE LAYOUT



TOP VIEW



SIDE VIEW

FIGURE 4

FEASIBILITY STUDY

The first question to be answered was whether it would be possible to simulate a charge similar to the one found on the bone under normal physiological conditions. This was done by determining the voltage necessary to generate a charge equal to the charge generated by vigorous exercise.

Rat bone has an average ultimate stress of about 12,000 psi in compression. Since the safety factor of bone is about 4, a stress of 3,000 psi was believed to be representative of vigorous exercise. From a stress-charge density curve in John Dainora's thesis,[10] a charge density of $0.85 \times 10^{-9} \frac{\text{coul.}}{\text{in}^2}$ was determined to be the charge density generated by this exercise.(Figure 5)

A relationship between the voltage on the plates and the charges on the bone had to be developed. The derivation of this equation was obtained by using Gauss's Law and the definition of capacitance.

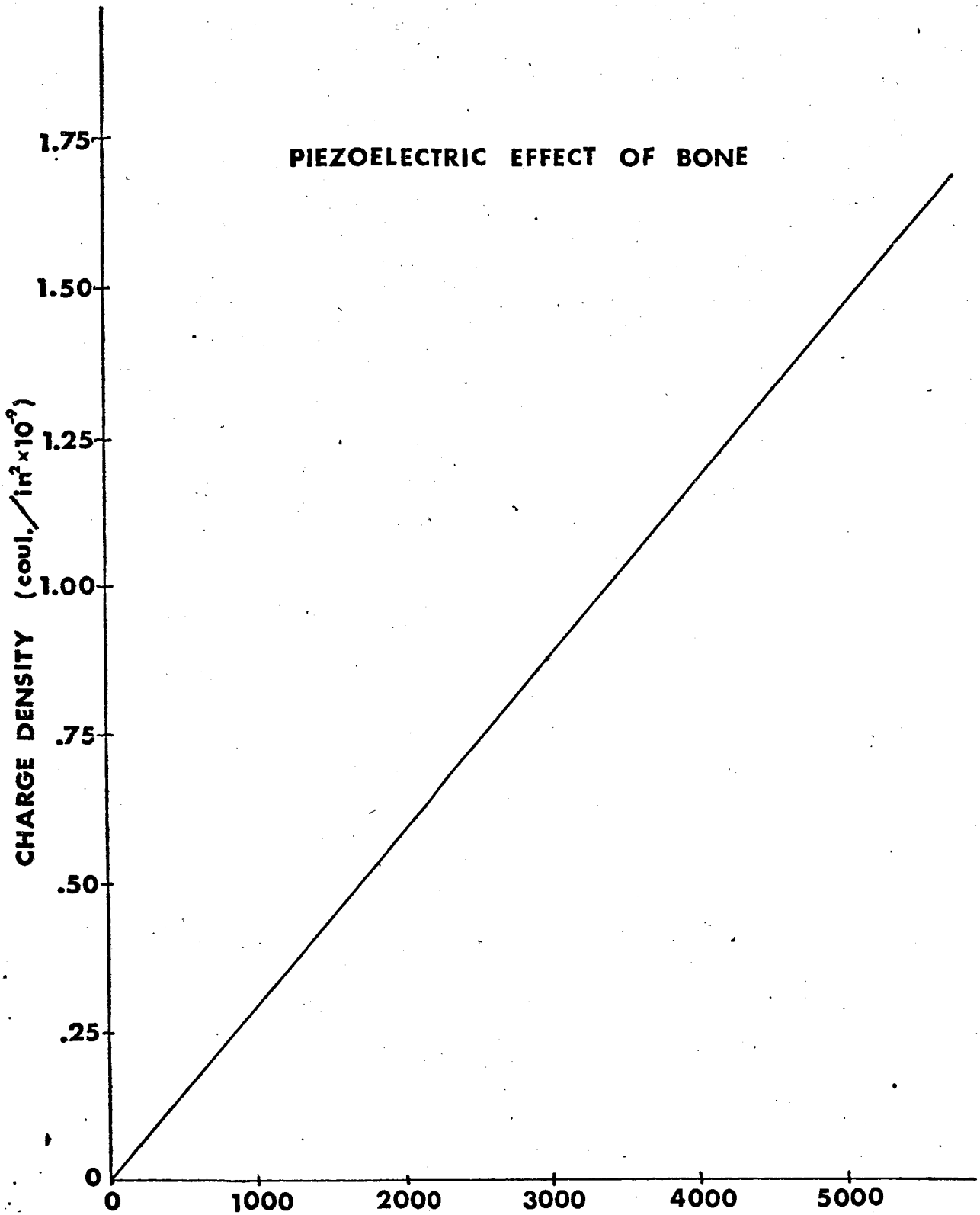
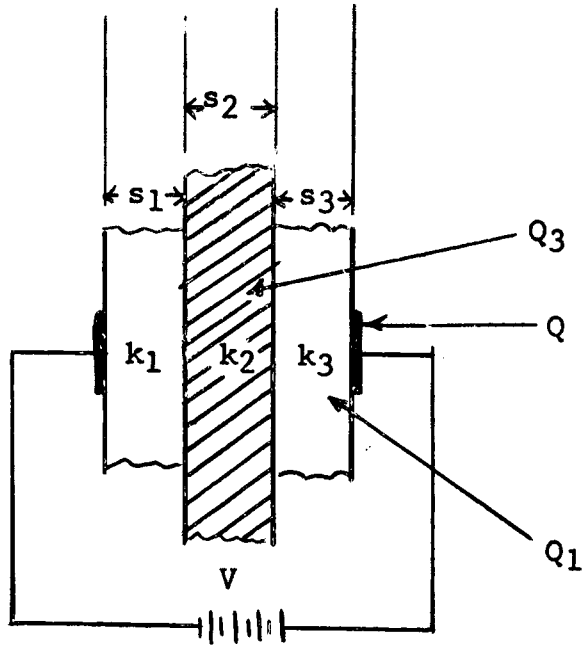

PIEZOELECTRIC EFFECT OF BONE
CHARGE DENSITY (coul./in^2×10^{-9})
1.75
1.50
1.25
1.00
.75
.50
.25
0
0
1000
2000
3000
4000
5000
STRESS (psi) (REFERENCE 12)

FIGURE 5



Field Generator Schematic

Figure 6

$$V = \frac{Q}{C} \tag{1}$$

But $$\frac{1}{C} = \frac{1}{C_1} + \frac{1}{C_2} + \frac{1}{C_3} \tag{2}$$

$$V = \frac{Q}{C_1} + \frac{Q}{C_2} + \frac{Q}{C_3} \tag{3}$$

We know that $C_1 = \frac{k_1 \epsilon_o A}{s_1}$ (4a)

And $C_2 = \frac{k_2 \epsilon_o A}{s_2}$ (4b)

Put into equation (3) and let $s_1 = s_3$, $k_1 = k_3$

$$V = \frac{1}{\epsilon_o}\left[\frac{2s_1}{k_1} + \frac{s_2}{k_2}\right]\frac{Q}{A} \tag{5}$$

Now from Gauss's Law

$$\epsilon_o \oint \vec{E} \cdot \vec{ds} = Q \tag{6}$$

For a dielectric between two parallel-plates Gauss's Law gives:

$$\epsilon_o \oint \vec{E} \cdot \vec{ds} = \epsilon_o EA = Q - Q_1 \tag{7}$$

So $$E = \frac{Q}{\epsilon_o A} - \frac{Q_1}{\epsilon_o A}$$

With no dielectric between two parallel-plates we get:

$$E_0 = \frac{Q}{\epsilon_o A} \tag{8}$$

But $$E_0 = k_1 E \tag{9}$$

So $$E = \frac{Q}{k_1 \epsilon_o A} \tag{10}$$

Now putting into equation(7)

$$Q_1 = Q\left[1 - \frac{1}{k_1}\right] \tag{11a}$$

And $$Q_2 = Q_1\left[1 - \frac{1}{k_2}\right] \tag{11b}$$

Or $$Q_2 = Q\left[1 - \frac{1}{k_1}\right]\left[1 - \frac{1}{k_2}\right] \tag{12}$$

Dividing by A

$$\frac{Q}{A} = \frac{Q_2}{A}\left[\frac{k_1 k_2}{(k_1-1)(k_2-1)}\right] \tag{13}$$

Putting this into equation (5)

$$V = \frac{1}{\epsilon_o}\left[\frac{2s_1}{k_1} + \frac{s_2}{k_2}\right]\left[\frac{k_1 k_2}{(k_1-1)(k_2-1)}\right]\frac{Q_2}{A} \qquad (14)$$

With the above equation and assumptions on s_1, s_2, k_1, k_2, we are able to calculate the necessary voltage to produce the desired charge density on the bone.

Let $s_1 = 7 \times 10^{-3}$m, $s_2 = 4 \times 10^{-3}$m,

$k_1 = 70$, $k_2 = 10^{11}$

Putting into equation (14)

$$V = 1.18 \times 10^{11} \frac{Q_2}{A}$$

Now for $\frac{Q_2}{A} = 0.85 \times 10^{-9} \frac{\text{coul.}}{\text{in}^2}$

$V = 100$ volts

Since it was not known whether the metabolic activity was effected by the charge on the bone or the rate of change of the charge on the bone four groups of 12 were picked giving three test groups and one control group. The first test group of rats was treated with a DC voltage of 100 volts. The second test group was treated with an AC voltage of 200 volts at 3 cps peak-to-peak, the approximate

frequency at which rats run, while the final test group was treated with 200 volts at 30 cps peak-to-peak, a frequency well above the physiological range.

A thousand volt DC power supply was designed and built to furnish the electrical power for the experiment. Two AC amplifiers were designed to operate with two sign-wave generators giving 3 cps and 30 cps frequencies at 200 volts peak-to-peak.

The electrodes were made of 3/4 x 1/2 x 1/16 brass shim stock. To make the electrical connections between the power supply and the plates, micro plugs and jacks were used. To prevent the rat from being shocked,the plates, wires and jack had to be insulated from the rat. Also, waterproofing of these parts was necessary to prevent any shorts caused by urination on casts.

TREATMENT OF THE RATS

For this experiment forty-eight male Spraque-Dawley rats each weighing approximately 100 grams were obtained. The rats were anesthetized with ether. Cotton cast padding was wrapped around the right leg and waist of each rat. The electrodes were taped to the rats thigh. Then quick drying plaster of Paris cast material was wrapped around the leg and waist of each rat.(Figure 7)

Each cage housing a rat was electrically insulated from all the other cages. Each cage had an individual electrical lead running to it with a 6 inch long brass protective shield around the end of the wire to prevent the rat from chewing into the wires. The first 12 cages were used as controls while the second 12 cages were connected to the direct voltage. The high frequency group and the low frequency group voltages were connected to the remaining 24 cages.

A vacuum tube voltmeter was used to set the correct voltage on the DC group, while a dual beam oscilloscope was used to obtain the correct peak-to-

peak voltage and to monitor the wave form on the alternating groups. Then the rats were treated for periods of 28 days one hour in the morning and one hour in the afternoon.(Figure 8)

At the end of 28 days the rats were sacrificed. After sacrificing the rats, the right and left femurs were removed for analysis, placed in saline, and stored in a refrigerator.

WEIGHT AND SPECIFIC GRAVITY

The preparations and material property testings were carried out in the Biomechanics Division of the Theoretical and Applied Mechanics Department. Each bone was cleaned thoroughly to remove all traces of tendons and muscle tissue. Because the electric field was applied only to the shaft, all tests were conducted on this portion of the bone. Then for comparing the weights between the left and right femur the sections were taken from the same location on the bone.

The same procedure was employed for each test rat. The anterior edge of the patellar surface was used as a reference point for cutting the right and left femur of each rat. Then these two bones were cut again near the lesser trochanter. Each bone section was measured with the micrometer and the longest one was sanded down from its proximal end until both bones were of equal length. The marrow was cleaned from both bones.

To obtain the specific gravity of each bone it was necessary to weigh the bones in air and sub-

merged in water. Each bone was dried in the air for one minute and weighed on a Christian Becker Analytical Scale. This weight was called the wet weight and was used for the weight comparisons.

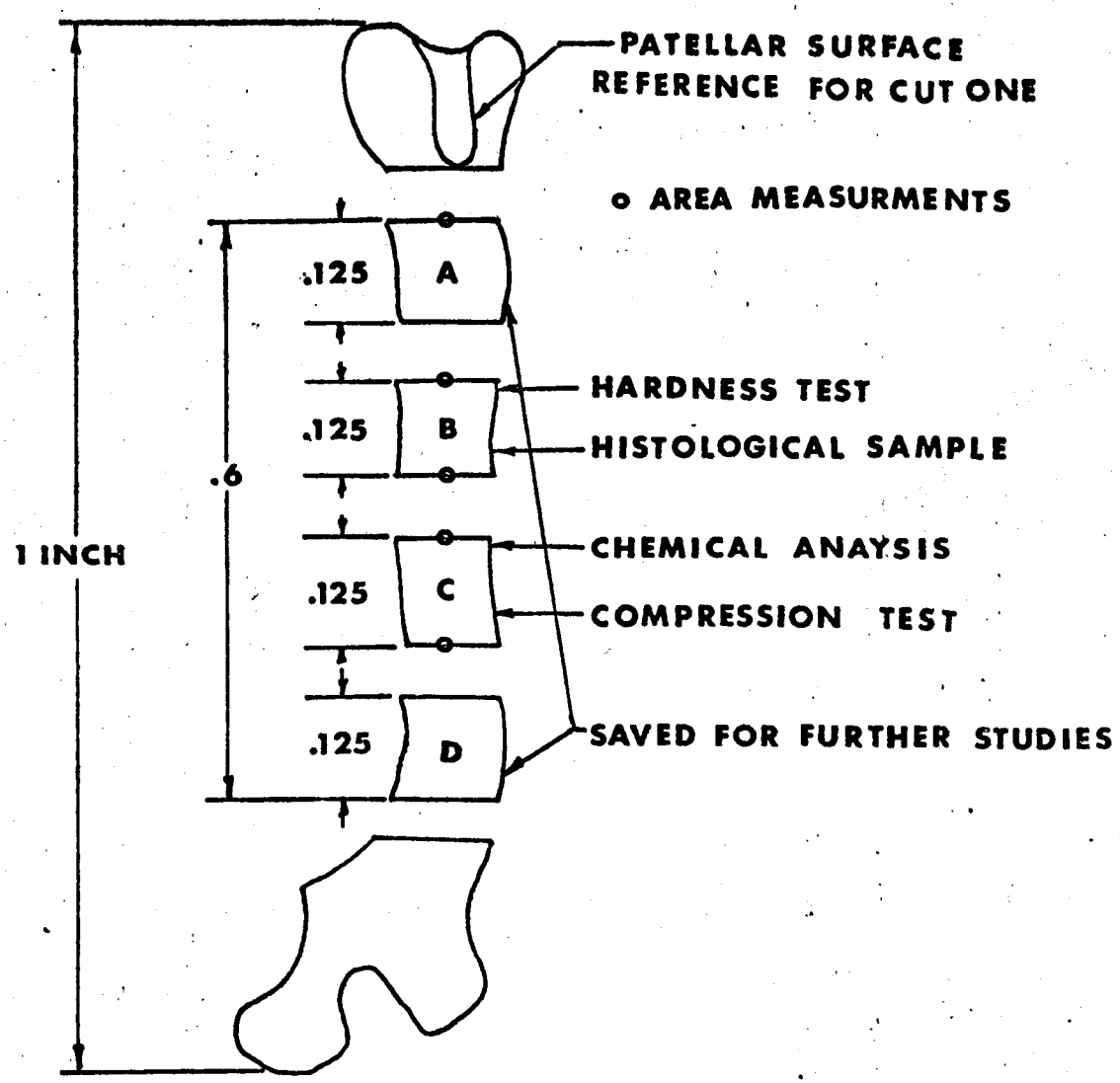
The air was removed from the water to be used for the submerged weighings by boiling. Before weighing the submerged bone, the scales were balanced with the tray submerged in the water. Each bone was weighed twice and averaged. This weight was called the submerged weight.

The difference between the wet weight and the submerged weight gave the boyancy of the bone. Dividing this into the wet weight gave the specific gravity of the bone.

CROSS-SECTIONAL AREA DETERMINATION

Before any other tests could be made the bones had to be cut into smaller pieces. A special cutting device was built to cut the bones into sections which were parallel and of approximately equal lengths. Each bone was mounted in a pin vise by its proximal end. Then the bone was cut into three pieces 1/8 of an inch long and allowing the piece in the vise to vary in length depending on the original length of the bone. Next, each section of bone was assigned to particular tests.(Figure 9)

Both ends of section C, and proximal end of section D, and the distal ends of section A and B were photographed. These photographs were enlarged. The enlargements were planimetered and the actual cross-sectional area calculated. The cross-sectional areas of sections A, B, C, and D, were averaged together to obtain an average cross-sectional area for the entire bone.



RIGHT RAT FEMUR
FIGURE 9

MICRO-HARDNESS

The micro-hardness tests were conducted on a Wilson Tukon Hardness Tester. The Vickers scale was used for the comparison of the relative hardness of each bone. The Vickers Number was calculated from the following equation:

$$\text{Vickers Number} = \frac{2L\sin\frac{a}{2}}{d^2}$$

Where:
- L = Load in kilograms
- a = 136^{o} apex angle of the diamond
- d = Length of average diagonal in milometers
- 1 Filar unit = 0.4719 microns

The hardness tests were run on section B of each bone. Four indentions about 90 degrees apart were placed in the end of each bone. A 20x microscope objective was used to measure the length of the diagonal of the diamond indentions. To obtain an average Vickers Number for each bone the four Vickers Numbers for each bone were averaged together.

OSTEONE COUNT

Osteones are microscopic cylinders inside the cortical bone. These cylinders contain the blood vessels and nerves which maintain the normal activity of bone cells.(Figure 2) If the activity of the bone cells were altered by the electric fields, the number of osteone per unit may have been changed. That is, if the cells activity were to increase, the blood supply to the cells would have to increase. This would mean that more osteones would be formed. The opposite would occur if the cell's activity was to decrease, and then the osteones would be reabsorped.

A 1/16 inch sample of bone from two specimens of each group were cut out. These samples were sanded down to about 5-10 microns thick. The following staining procedure was used.

1. Place into Villaneuva Osteochrome stain 48 hours for permanent stain
2. Differentiate with gentile agitation in 0.01% glacial acetic acid in 95% alcohol. Differentiation takes 20-25 minutes for the maximum stained specimens (48 hours)
3. Place specimen in 95% alcohol for 4 minutes

4. Place specimen in 95% alcohol for 4 minutes

5. Place specimen in absolute alcohol for 3 minutes

6. Place specimen in absolute alcohol for 3 minutes

7. Clear in equal parts of absolute alcohol and xylol for 2 minutes

8. Clear in one part absolute alcohol and 3 parts xylol for one minute

9. Clear in one part absolute alcohol and 9 parts xylol for one minute

10. Place in xylol for one minute

11. Place in xylol for one minute

12. Mount in any of the neutral synthetic resins

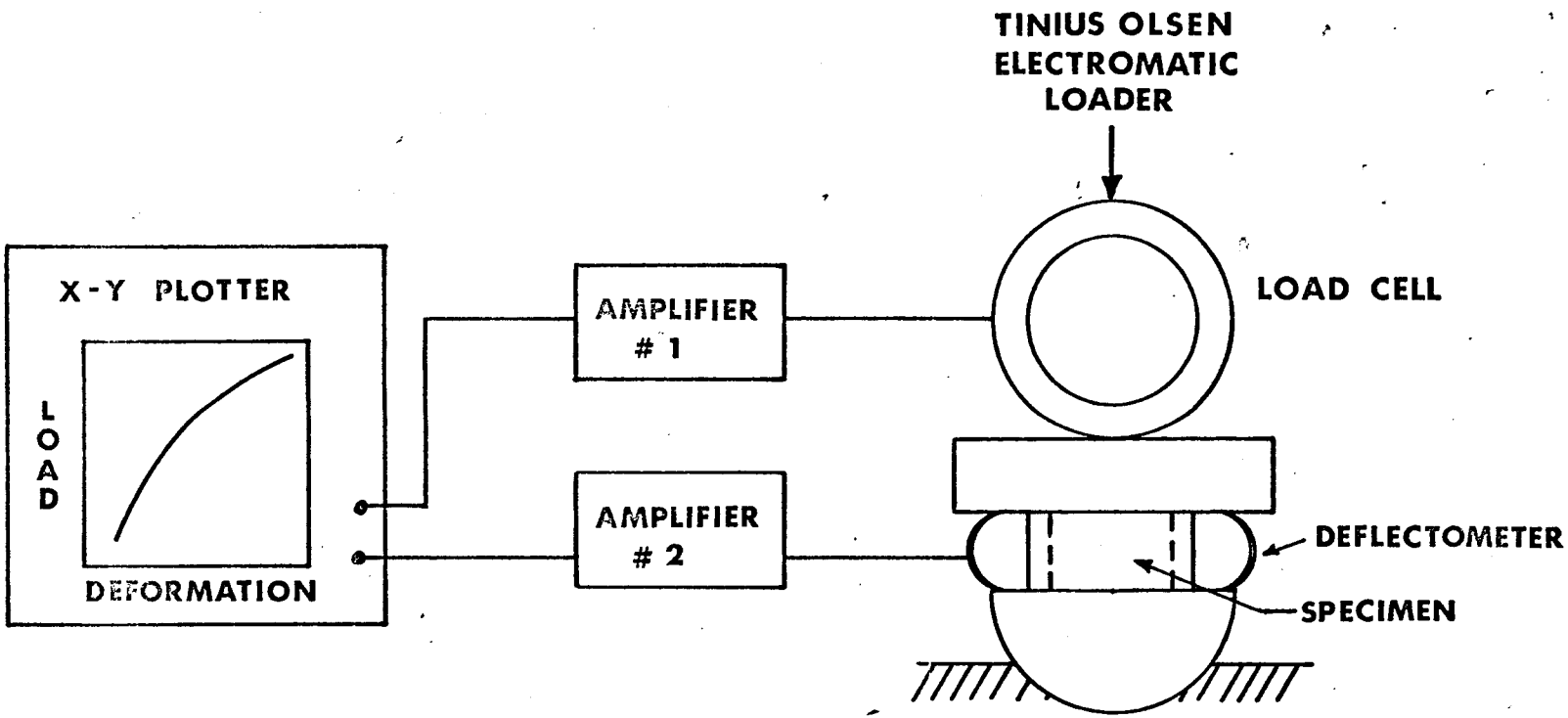
By using a microscope the osteones were counted. The area was obtained in a similar manner as in the cross-sectional area study. From this the osteones per unit area were calculated.

COMPRESSION TESTS

The compression tests were conducted on section C of each bone. The preparation of these sections was important in obtaining good test results. The ends of each section were cut so that they were perpendicular to the longitudinal axis of the bone, then hand sanded until they were parallel. When the bone sections were parallel and perpendicular their length was measured with a micrometer. Because past study showed that drying of only 3-5 minutes would change the bones strength properties. The tests were conducted on wet bones.

The test set-up consisted of a Tinius Olsen Testing Machine, a ring load cell, a strainmeter, (Figure 10) two Daytronic Model 800, and Moseley Model 20 X-Y Recorder.(Figure 11)

A dial indicator was placed on the loading machine in such a way as to measure the movement of the loading machine head with respect to the base. The load cell was mounted to the head and then moved into contact with the strainmeter.(Figure 12) The deflections were read on the dial



BLOCK DIAGRAM FOR RAT FEMUR

COMPRESSION TEST

FIGURE 11

indicator and the corresponding strain gage amplifier reading recorder to plot the calibration curve shown in Figure 13.

For a typical test the following procedure was followed. The load cell was brought into contact with the strainmeter. A pre-deflection of about 0.006 in. was applied to the strainmeter. The test specimen was placed between the load cell and the base of the strainmeter and shimmed up until the specimen was in contact with the load cell. A preload of 10 pounds was then placed on the test specimen. The loading machine, strain gage indicators, and X-Y Recorder were all zeroed. Next, the loading machine speed was set at 0.25 inches/minute, and the tests were run until the specimen failed. Then the load was released and the bone was put in saline for the chemical analysis. To prevent drying of the bone each test was run less than one minute long. 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.

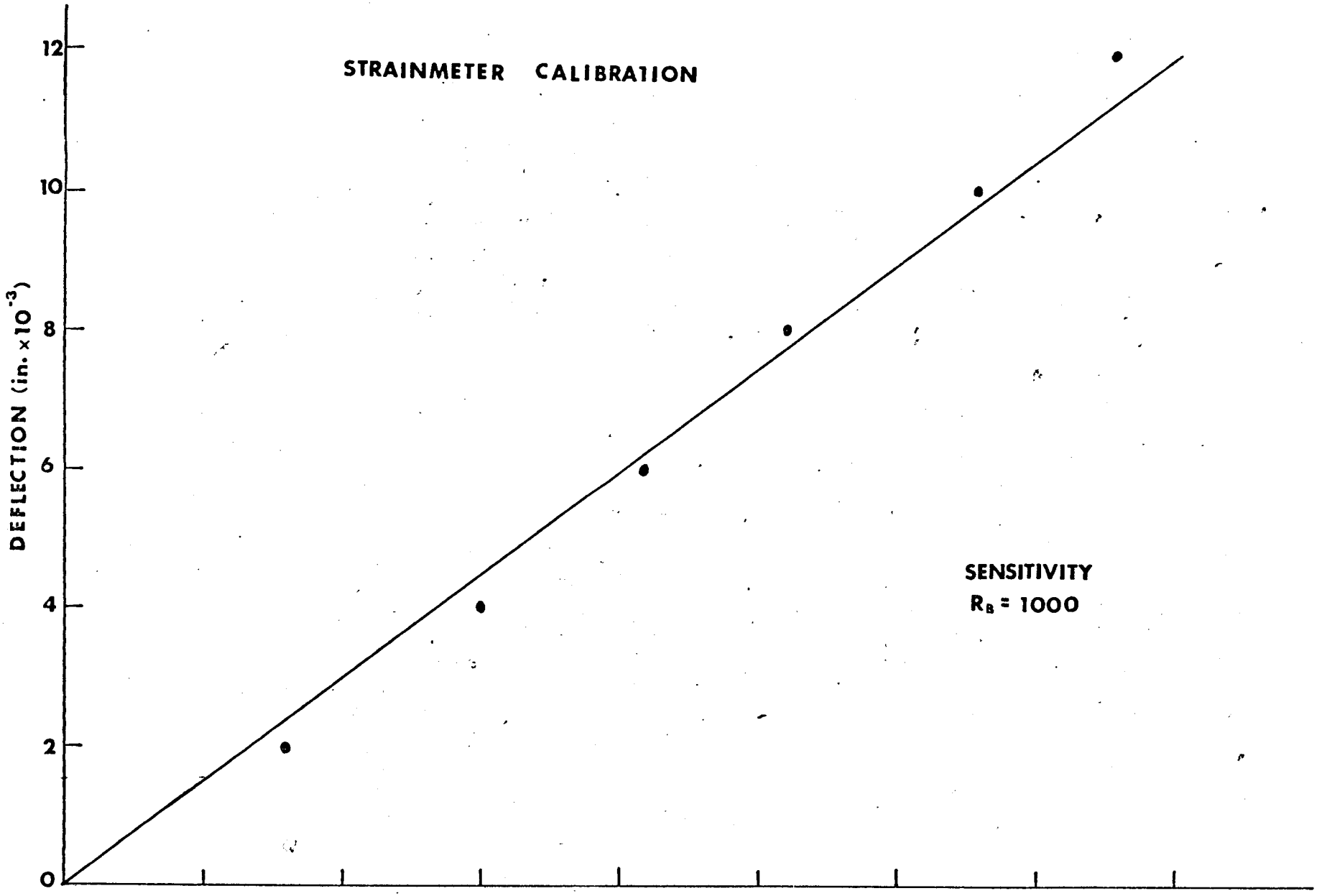

STRAINMETER CALIBRATION
DEFLECTION (in. x 10⁻³)
0
2
4
6
8
10
12
250
500
750
1000
1250
1500
1750
2000
STRAIN GAGE RECORDER READING (μ)
SENSITIVITY
R_B = 1000

FIGURE 13

CHEMICAL ANALYSIS

These tests were conducted in the laboratory of Dr. T. R. Bullard at the University of Virginia. This chemical analysis was designed to determine the per cent mineral content, organic content, calcium content, and phosphorus content in section C of each bone specimen.

All the bones were dried in an oven at 105°C. for three days. Sample weighings were made each day until no weight change could be determined. This weight was recorded and called the dry weight.

These bones were placed in porcelain crucibles and put into a muffle furnace at 600°C. for 12 hours. After the crucibles had cooled the bones were weighed again and the weight recorded. This weight was called the ash weight.[12]

The difference between the dry weight and the ash weight is the organic content of that section of bone. Because all of the bone sections do not weigh the same, the absolute weight of the organic content is not of much use. But if the weight of the organic material for that section is divided by the dry weight

for that section the per cent of organic content becomes relevant. Dividing the ash, which is the mineral in the section, by the dry weight will give the per cent mineral content in that section.

The calcium method that was used was adapted from reference 13. The method outline is listed below:

1. Dissolve the bone ash section in one milliliter of 5N HCL and diluted to 25 milliliter with deionized water
2. 0.1 milliliter sample diluted to 10 milliliter with deionized water
3. Add 1 drop 20% octanol
4. Add 7 drops 9N NAOH
5. Add 1 milliliter alcoholic ammonium purpurate
6. Place tub in spectrophotometer and titrate with (λ) wavelength at 510 m μ. Set Coleman Jr. Spectrophotometer at 40%T. Add 0.2 milliliter of 0.018% EDTA, mix with air bubbling device, and record % transmittance. Add another 0.2 milliliter EDTA, mix and record. Repeat until 2.0 milliliter EDTA have been added.

Plotting the milliliter of EDTA against the spectrophotometer readings gives the breaking point which is the point where all the calcium in the sample is taken out of solution by the EDTA. With this

value and a standard curve (Figure 14) the total weight of calcium was calculated in the section. Next, the ash weight was divided into the calcium weight giving the per cent calcium in the section.

The phosphorus method that was used was adapted from reference 14. The method outline is listed below:

1. Add 0.2 milliliter sample to tube and dilute to 10.7 milliliter with deionized water.
2. Add 1.3 milliliter of molybdate I solution and mix by shaking.
3. Add 0.5 milliliter of aminonaphthol-sulfonic acid and mix by shaking.
4. Let stand about 10 minutes, then read in Coleman Jr. Spectrophotometer at 660 m μ.

With the spectrophotometer reading and a standard curve (Figure 15) the total weight of phosphorus in the section was calculated. The ash weight was then divided into the phosphorus weight giving the per cent phosphorus in the section.

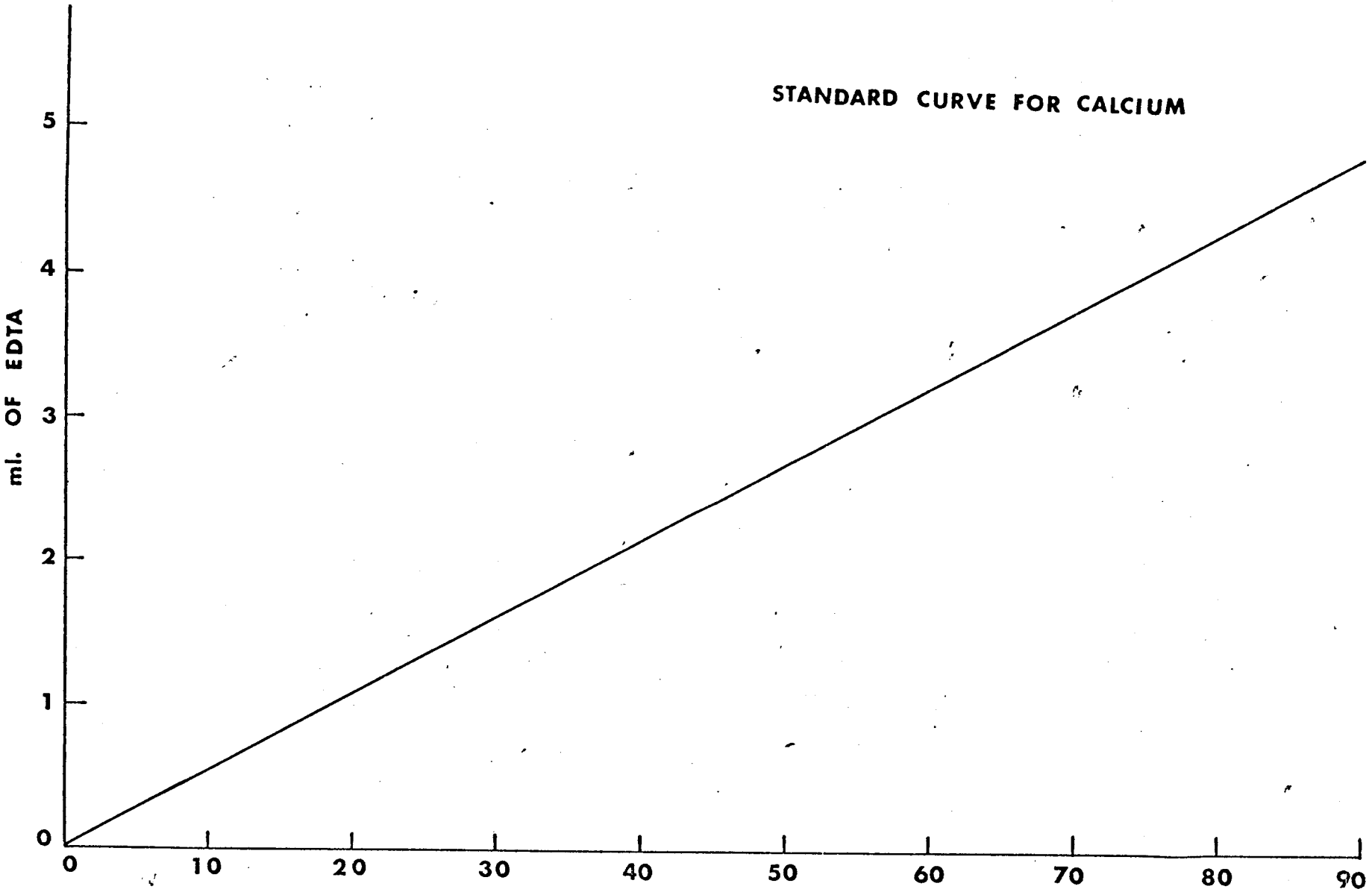

STANDARD CURVE FOR CALCIUM
ml. OF EDTA
0
1
2
3
4
5
0
10
20
30
40
50
60
70
80
90
μgm OF Ca IN 0.1 ml OF SOLUTION

FIGURE 14

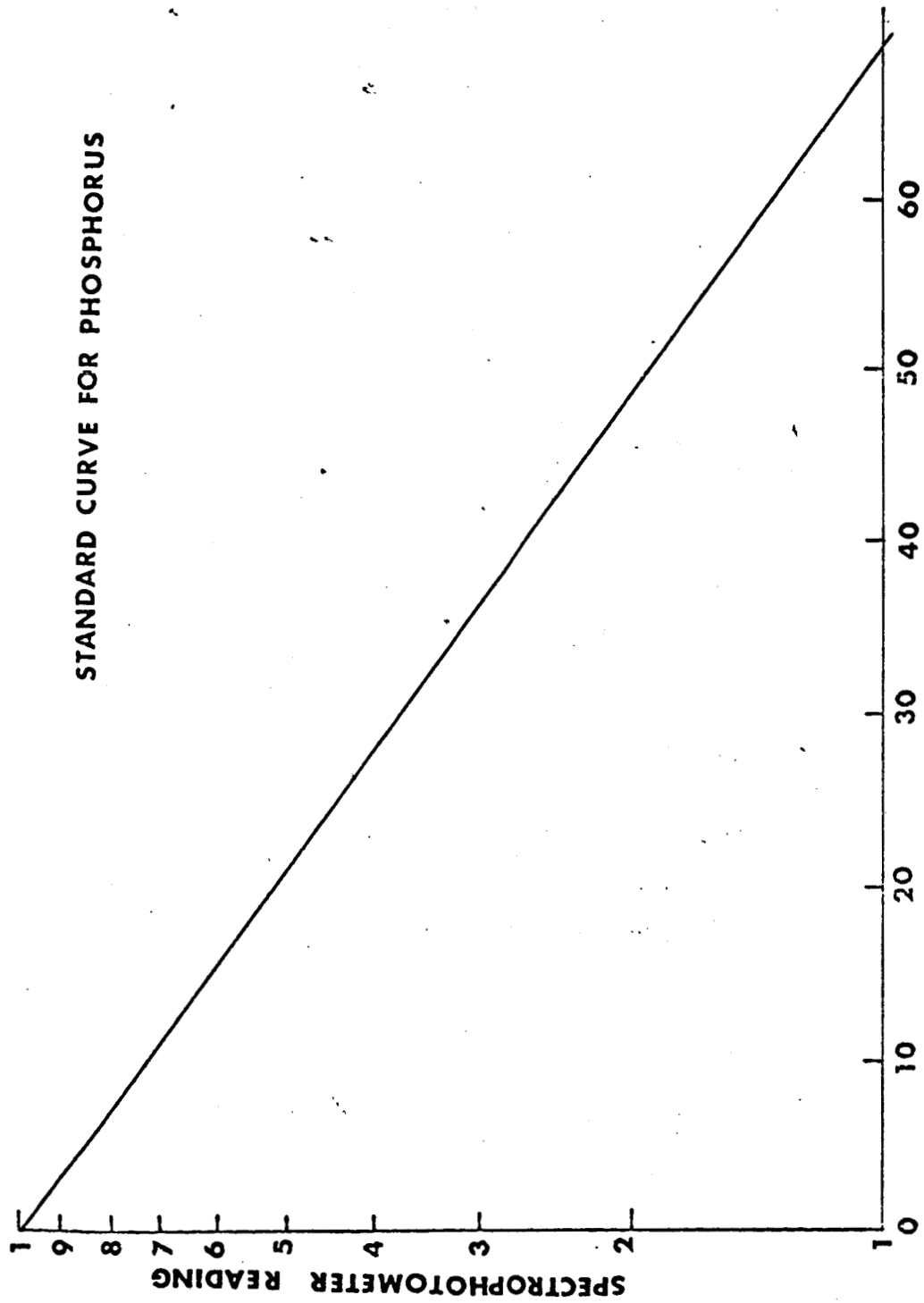

STANDARD CURVE FOR PHOSPHORUS
SPECTROPHOTOMETER READING
1
2
3
4
5
6
7
8
9
0
10
20
30
40
50
60
µgm OF P IN 0.2ml OF SOLUTION

FIGURE 15

RESULTS

Expression of Results

To assess the effect of inactivity the immobilized femur was compared by paired analysis to the normal femur. The effect of the electric field on the immobilized femur was determined by comparing the control rat data to that obtained from the animal subjected to the electric field. In order to determine what effect the electric field had on the osteoatrophosis the statistic relating to the immobilized femur was expressed as a percentage of the value of the normal limb. The nearer the percentage is to zero, the less is the difference between the two bones. The parameters evaluated were femur weight, specific gravity, cross-sectional area, hardness, compression properties, number of osteones per unit area, and chemical content. Histological slides were made and studied.

Treatment of the Rat

At the time of sacrifice only 32 of the original 48 rats were alive. The surviving rats were in the following four groups; eight control, nine direct

current, nine high frequency alternating current, and six low frequency alternating current. Of these 32 rats only 24 rats were suitable to be used in the study. (Table 1)

Mechanical and Chemical Properties

For each of the parameters evaluated, the actual value of the parameter and the per cent difference between the right (immobilized) femur and left (normal) femur for each rat was recorded on Tables 2 thru 16. The average of each of the parameters and the range over which it varied was also recorded on Tables 17 thru 21. The average per cent change of each group treated for each parameter was recorded on Tables 22 thru 24.

Physical Properties

On eight of the 18 rats treated with the electric field small tumors were detected. These tumors were distributed among the following groups; one in the DC group, five in the high frequency AC group, and two in the low frequency AC group. There were no tumors found on any of the controls.(Figures 16, 17, and 18)

TABLE 1
DAYS TEATED AND WEIGHT CHANGE

Bone No.	Weight Before Experiment	Weight After Experiment	Days of Field Treatment
C-1	105	150	--
C-2	110	146	--
C-3	104	74	Not
C-4	112	178	Treated
C-5	106	--	--
C-6	125	181	--
D-1	105	180	26.50
D-2	120	188	22.50
D-3	114	190	22.50
D-4	126	200	26.00
D-5	121	161	22.25
D-6	143	198	22.00
D-7	144	155	25.50
		Average	23.89
AH-1	159	234	24.00
AH-2	126	198	27.00
AH-3	136	187	27.00
AH-4	170	105	23.50
AH-5	157	224	22.50
AH-6	--	212	27.00
		Average	25.17
AL-1	165	167	21.00
AL-2	116	159	23.50
AL-3	131	121	26.00
AL-4	112	172	24.50
AL-5	145	172	22.50
		Average	23.50

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 2
BONE WEIGHT DATA

Bone No.	Weight Rt. (Gm.)	Weight Lt. (Gm.)	% Diff.	Bone No.	Weight Rt. (Gm.)	Weight Lt. (Gm.)	% Diff.
C-1	0.0783	0.0912	14.14	AH-1	0.1563	0.1664	6.07
C-2	0.0730	0.0884	17.42	AH-2	0.1173	0.1261	6.98
C-3	0.0487	0.0575	15.30	AH-3	0.1165	0.1412	17.49
C-4	0.0750	0.0838	10.50	AH-4	0.1043	0.1330	21.58
C-5	0.0890	0.0996	10.64	AH-5	0.1346	0.1515	11.16
C-6	0.1079	0.1182	8.71	AH-6	0.1470	0.1415	-3.88
D-1	0.0954	0.1060	10.00	AL-1	0.1220	0.1346	9.36
D-2	0.1150	0.1347	19.43	AL-2	0.0948	0.1193	21.21
D-3	0.1070	0.1273	15.95	AL-3	0.0878	0.0974	9.86
D-4	0.1280	0.1465	12.63	AL-4	0.1190	0.1371	13.20
D-5	0.1434	0.1353	-5.99	AL-5	0.1181	0.1273	7.23
D-6	0.1284	0.1384	7.23				
D-7	0.1003	0.1295	22.54				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 3
SPECIFIC GRAVITY DATA

Bone No.	Specific Gravity Rt.	Lt.	% Diff.	Bone No.	Specific Gravity Rt.	Lt.	% Diff.
C-1	1.938	1.936	-0.093	AH-1	1.841	1.679	-9.636
C-2	1.916	1.951	1.814	AH-2	1.886	1.911	1.293
C-3	1.917	1.943	1.303	AH-3	1.948	1.893	-2.927
C-4	1.948	1.958	0.501	AH-4	1.928	1.889	-2.048
C-5	1.828	1.937	5.673	AH-5	1.804	1.829	1.323
C-6	1.829	1.833	0.207	AH-6	1.880	1.949	3.551
D-1	1.842	1.840	-0.076	AL-1	1.863	1.959	4.931
D-2	1.904	1.905	0.063	AL-2	1.843	1.841	-0.114
D-3	1.904	1.917	0.694	AL-3	1.848	1.862	0.746
D-4	1.861	1.901	2.146	AL-4	1.886	1.848	-2.067
D-5	1.824	1.908	4.397	AL-5	1.834	1.826	-0.411
D-6	1.880	1.795	-4.724				
D-7	1.878	1.885	0.355				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 4
CORTICAL AREA DATA

Bone No.	Cortical Area Rt. ($In.^2$)	Cortical Area Lt. ($In.^2$)	% Diff.	Bone No.	Cortical Area Rt. ($In.^2$)	Cortical Area Lt. ($In.^2$)	% Diff.
C-1	0.00470	0.00532	11.80	AH-1	0.00646	0.00668	3.24
C-2	0.00431	0.00564	23.78	AH-2	0.00599	0.00635	5.59
C-3	0.00290	0.00405	28.64	AH-3	0.00540	0.00648	16.72
C-4	0.00530	0.00600	11.80	AH-4	0.00536	0.00638	16.05
C-5	0.00614	0.00737	16.31	AH-5	0.00630	0.00785	19.60
C-6	0.00599	0.00668	10.32	AH-6	0.00694	0.00682	-1.73
D-1	0.00596	0.00713	16.30	AL-1	0.00538	0.00678	20.64
D-2	0.00565	0.00654	13.55	AL-2	0.00452	0.00623	27.53
D-3	0.00725	0.00710	-2.22	AL-3	0.00487	0.00523	6.79
D-4	0.00641	0.00770	16.88	AL-4	0.00649	0.00703	7.84
D-5	0.00724	0.00761	4.92	AL-5	0.00580	0.00650	10.91
D-6	0.00665	0.00720	7.40				
D-7	0.00552	0.00720	23.29				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 5
ULTIMATE LOAD DATA

Bone No.	Load Rt. (Lb.)	Load Lt. (Lb.)	% Diff.	Bone No.	Load Rt. (Lb.)	Load Lt. (Lb.)	% Diff.
C-1	50	65	23.07	AH-1	69	85	18.82
C-2	*--	67	*--	AH-2	68	85	20.00
C-3	40	*--	*--	AH-3	75	90	16.60
C-4	70	58	-20.69	AH-4	61	88	30.68
C-5	41	69	40.58	AH-5	71	94	24.46
C-6	64	80	20.00	AH-6	93	99	6.06
D-1	60	74	18.92	AL-1	59	*--	*--
D-2	68	90	24.44	AL-2	49	56	12.50
D-3	71	99	28.28	AL-3	*--	65	*--
D-4	61	101	39.60	AL-4	64	59	-8.47
D-5	99	87	-13.79	AL-5	*--	66	*--
D-6	81	85	4.71				
D-7	56	72	22.22				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 6
ULTIMATE STRESS DATA

Bone No.	Ultimate Stress Rt. (PSI)	Lt. (PSI)	% Diff.	Bone No.	Ultimate Stress Rt. (PSI)	Lt. (PSI)	% Diff.
C-1	10,395	10,987	5.40	AH-1	10,670	12,600	15.31
C-2	*--	11,498	*--	AH-2	11,951	13,323	10.30
C-3	16,136	*--	*--	AH-3	13,393	13,787	2.90
C-4	11,913	9,163	-30.00	AH-4	10,557	13,543	21.90
C-5	7,163	9,798	26.90	AH-5	11,255	12,217	7.87
C-6	10,303	11,330	9.10	AH-6	13,928	14,591	4.54
D-1	9,804	10,071	2.60	AL-1	11,510	*--	*--
D-2	12,495	13,913	10.20	AL-2	10,624	9,743	-9.04
D-3	10,069	12,500	19.40	AL-3	*--	12,143	*--
D-4	11,036	13,160	16.10	AL-4	10,908	8.613	-26.82
D-5	12,711	12,319	-3.20	AL-5	*--	11,232	*--
D-6	12,616	12,139	-3.90				
D-7	9,811	10,125	3.10				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 7
ULTIMATE DEFLECTION DATA

Bone No.	Ultimate Deflection Rt. (In.)	Lt. (In.)	% Diff.	Bone No.	Ultimate Deflection Rt. (In.)	Lt. (In.)	% Diff.
C-1	0.00221	0.00232	-4.74	AH-1	0.00453	0.00268	-67.91
C-2	* --	0.00304	* --	AH-2	0.00350	0.00264	-32.58
C-3	0.00371	* --	* --	AH-3	0.00335	0.00324	-3.40
C-4	0.00350	0.00294	-19.05	AH-4	0.00438	0.00278	-57.55
C-5	0.00386	0.00515	24.85	AH-5	0.00318	0.00345	8.97
C-6	0.00517	0.00762	32.15	AH-6	0.00305	0.00345	11.65
D-1	0.00263	0.00304	13.49	AL-1	0.00309	* --	* --
D-2	0.00317	0.00283	-12.01	AL-2	0.00227	0.00365	37.79
D-3	0.00314	0.00335	6.27	AL-3	* --	0.00252	* --
D-4	0.00310	0.00608	49.01	AL-4	0.00376	0.00200	-88.00
D-5	0.00350	0.00319	-9.72	AL-5	*--	0.00360	*--
D-6	0.00536	0.00546	1.83				
D-7	0.00376	0.00407	7.62				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 8
ULTIMATE STRAIN DATA

Bone No.	Ultimate Strain Rt. (In./In.)	Lt. (In./In.)	% Diff.	Bone No.	Ultimate Strain Rt. (In./In.)	Lt. (In./In.)	% Diff.
C-1	0.0175	0.0189	7.40	AH-1	0.0381	0.0214	-78.0
C-2	*--	0.0220	*--	AH-2	0.0282	0.0210	-34.3
C-3	0.0294	*--	*--	AH-3	0.0272	0.0261	-4.2
C-4	0.0269	0.0230	-16.90	AH-4	0.0342	0.0226	-51.3
C-5	0.0297	0.0399	25.60	AH-5	0.0238	0.0278	14.4
C-6	0.0438	0.0573	23.50	AH-6	0.0242	0.0276	12.3
D-1	0.0204	0.0230	11.30	AL-1	0.0245	*--	*--
D-2	0.0244	0.0225	-8.40	AL-2	0.0189	0.0292	35.3
D-3	0.0253	0.0266	4.90	AL-3	*--	0.0205	*--
D-4	0.0248	0.0456	44.90	AL-4	0.0294	0.0163	-80.3
D-5	0.0278	0.0259	-7.30	AL-5	*--	0.0286	*--
D-6	0.0422	0.0430	1.90				
D-7	0.0294	0.0308	4.50				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 9
MODULUS OF ELASTICITY DATA

Bone No.	Modulus of Elasticity Rt. (PSI)	Lt. (PSI)	% Diff.	Bone No.	Modulus of Elasticity Rt. (PSI)	Lt. (PSI)	% Diff.
C-1	0.72	0.85	15.3	AH-1	0.44	0.78	43.6
C-2	* --	0.43	* --	AH-2	0.68	0.90	24.4
C-3	0.79	*--	*--	AH-3	0.75	0.66	-13.6
C-4	0.63	0.61	-3.3	AH-4	0.39	0.86	54.7
C-5	0.30	0.30	0.0	AH-5	0.67	0.66	-1.5
C-6	0.23	0.28	17.9	AH-6	0.80	0.73	-9.6
D-1	0.57	0.64	10.9	AL-1	0.79	* --	* --
D-2	0.75	0.89	15.7	AL-2	0.79	0.40	-97.5
D-3	0.52	0.64	18.8	AL-3	* --	0.77	* --
D-4	0.59	0.34	-73.5	AL-4	0.51	0.34	-50.0
D-5	0.53	0.60	11.7	AL-5	* --	0.52	* --
D-6	0.34	0.35	2.9				
D-7	0.43	0.42	-2.4				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 10
ENERGY OF DISTORTION DATA

Bone No.	Energy of Distortion Rt. (PSI)	Lt. (PSI)	% Diff.	Bone No.	Energy of Distortion Rt. (PSI)	Lt. (PSI)	% Diff.
C-1	106.8	92.3	-15.7	AH-1	261.5	165.6	-57.9
C-2	*--	148.1	*--	AH-2	216.1	183.9	-17.5
C-3	312.1	*--	*--	AH-3	240.5	213.1	-12.9
C-4	200.8	140.8	-42.6	AH-4	212.8	195.2	-9.0
C-5	123.2	228.1	46.0	AH-5	169.0	221.4	23.7
C-6	235.1	393.2	40.2	AH-6	211.5	240.9	12.2
D-1	113.7	145.6	21.9	AL-1	181.2	*--	*--
D-2	196.1	202.9	3.4	AL-2	121.2	160.2	24.4
D-3	157.9	209.8	24.7	AL-3	*--	151.4	*--
D-4	173.3	336.4	48.5	AL-4	200.5	160.0	-25.0
D-5	198.3	193.8	-2.3	AL-5	* --	196.5	* --
D-6	298.5	297.2	-0.4				
D-7	157.7	194.7	19.0				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 11
MICRO-HARDNESS DATA

Bone No.	Micro-Hardness Rt. (Vicker) No.	Lt. (Vicker) No.	% Diff.	Bone No.	Micro-Hardness Rt. (Vicker) No.	Lt. (Vicker) No.	% Diff.
C-1	48.2	40.1	-20.20	AH-1	29.7	33.0	10.00
C-2	33.9	40.1	15.46	AH-2	34.8	31.3	-11.18
C-3	46.7	37.8	-23.54	AH-3	29.7	32.1	7.48
C-4	40.1	41.3	2.91	AH-4	26.9	33.0	18.48
C-5	30.0	39.0	23.07	AH-5	24.0	31.3	23.32
C-6	35.8	36.8	2.72	AH-6	29.7	40.1	25.94
D-1	39.0	28.3	-37.81	AL-1	40.1	53.3	24.77
D-2	30.5	36.8	17.12	AL-2	27.6	32.1	14.02
D-3	33.9	34.8	2.59	AL-3	26.3	25.7	-2.33
D-4	39.0	40.1	2.74	AL-4	33.0	28.3	-16.61
D-5	26.3	33.0	20.30	AL-5	25.1	28.3	11.31
D-6	25.1	32.1	21.81				
D-7	30.5	42.6	28.40				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 12
OSTEONE COUNT

Bone No.	Osteone Count Rt. Osteone/In.^{2}	Lt. Osteone/In.^{2}	% Diff.
C-3	56,300	48,800	-16.7
C-4	45,200	55,701	18.9
D-4	41,122	45,085	8.8
D-5	40,473	38,456	-5.3
AH-4	53,396	35,031	-52.4
AH-6	45,275	53,813	15.2
AL-1	60,408	60,919	0.8
AL-3	53,500	62,800	14.8

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 13
% MINERAL CONTENT DATA

Bone No.	% Mineral Content Rt.	Lt.	% Diff.	Bone No.	% Mineral Content Rt.	Lt.	% Diff.
C-1	68.0	68.2	0.29	AH-1	71.4	71.8	0.56
C-2	69.2	67.7	-2.22	AH-2	76.8	70.0	-9.71
C-3	66.7	67.7	1.48	AH-3	Test was not run		
C-4	67.7	70.1	3.42	AH-4	68.9	71.0	2.96
C-5	67.6	70.0	3.43	AH-5	68.4	69.3	1.30
C-6	69.2	70.1	1.28	AH-6	70.1	70.8	0.99
D-1	69.3	70.0	1.00	AL-1	68.2	72.7	6.19
D-2	69.2	69.0	-0.29	AL-2	70.0	70.6	0.85
D-3	68.8	71.4	3.64	AL-3	Test was not run		
D-4	71.1	71.5	0.56	AL-4	68.8	73.0	5.75
D-5	72.3	70.6	-2.41	AL-5	70.9	70.5	-0.57
D-6	74.7	71.5	-4.48				
D-7	70.3	69.2	-1.59				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 14
% ORGANIC CONTENT DATA

Bone No.	% Organic Content Rt.	Lt.	% Diff.	Bone No.	% Organic Content Rt.	Lt.	% Diff.
C-1	32.0	31.8	-0.63	AH-1	28.6	28.2	-1.42
C-2	30.8	32.3	4.64	AH-2	24.2	30.2	19.87
C-3	33.3	33.2	-0.30	AH-3	Test was not run		
C-4	33.2	29.9	-11.04	AH-4	31.1	29.0	-7.24
C-5	32.4	30.0	-8.00	AH-5	31.6	30.7	-2.93
C-6	30.8	29.9	-3.01	AH-6	29.9	29.2	-2.40
D-1	30.7	30.0	-2.33	AL-1	31.8	27.3	-16.48
D-2	30.8	31.0	0.65	AL-2	30.0	29.4	-2.04
D-3	31.2	28.6	-9.09	AL-3	Test was not run		
D-4	28.9	28.5	-1.40	AL-4	31.2	27.0	-15.56
D-5	27.7	29.4	5.78	AL-5	29.1	29.5	1.36
D-6	25.3	28.5	11.23				
D-7	29.7	30.8	3.57				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

TABLE 15
% CALCIUM CONTENT DATA

Bone No.	% Calcium Content Rt.	Lt.	% Diff.	Bone No.	% Calcium Content Rt.	Lt.	% Diff.
C-1	41.6	43.0	3.25	AH-1	38.4	39.3	2.29
C-2	45.5	39.6	-15.40	AH-2	36.1	36.6	1.36
C-3	43.8	46.4	5.17	AH-3	Test was not run		
C-4	45.8	40.3	-11.20	AH-4	40.2	* --	* --
C-5	43.3	39.6	-9.34	AH-5	41.6	36.8	-13.04
C-6	40.5	34.9	-16.10	AH-6	38.8	36.7	-5.72
D-1	33.6	35.6	5.61	AL-1	38.2	34.2	-10.90
D-2	*--	40.6	*--	AL-2	38.9	41.6	6.49
D-3	43.8	37.5	-16.80	AL-3	Test was not run		
D-4	39.7	37.8	-5.03	AL-4	39.4	38.5	-2.33
D-5	38.5	41.9	8.12	AL-5	37.9	37.3	-1.61
D-6	38.1	39.9	4.51				
D-7	40.1	42.2	4.98				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 16
% PHOSPHORUS CONTENT DATA

Bone No.	% Phosphorus Content Rt.	Lt.	% Diff.	Bone No.	% Phosphorus Content Rt.	Lt.	% Diff.
C-1	19.0	18.1	-4.97	AH-1	19.2	19.4	1.03
C-2	19.4	21.0	7.61	AH-2	18.4	19.9	7.54
C-3	20.0	19.9	-0.50	AH-3	Test was not run		
C-4	*--	19.7	*--	AH-4	18.4	19.0	3.16
C-5	19.5	19.4	-0.51	AH-5	20.8	19.4	-7.22
C-6	20.3	18.8	-7.97	AH-6	18.3	18.4	0.54
D-1	20.6	18.3	-12.56	AL-1	20.1	22.2	9.46
D-2	19.5	19.6	0.51	AL-2	19.2	20.4	5.88
D-3	18.6	*--	*--	AL-3	Test was not run		
D-4	19.1	18.9	-1.05	AL-4	20.4	18.8	-8.51
D-5	19.7	18.7	-5.34	AL-5	18.8	17.3	-8.67
D-6	19.8	18.4	-7.60				
D-7	20.9	21.5	4.18				

C - Control
D - Direct
AH - Alternating High Frequency
AL - Alternating Low Frequency

* Bad test

TABLE 17
SUMMARY OF ABSOLUTE VALUES AND RANGES

	Average Weight Rt. (Gm.)	Lt. (Gm.)	Average Specific Gravity Rt.	Lt.	Average Cortical Area Rt. ($In.^2$)	Lt. ($In.^2$)
Control	0.0787	0.0898	1.896	1.926	0.00490	0.00584
	Range 0.1182-0.0575		Range 1.958-1.828		Range 0.00737-0.00290	
Direct	0.1168	0.1311	1.870	1.879	0.00638	0.00721
	Range 0.1465-0.0954		Range 1.795-1795		Range 0.00770-0.00552	
Alternating Low Frequency	0.1083	0.1231	1.855	1.867	0.00540	0.00654
	Range 0.1371-0.0878		Range 1.959-1.826		Range 0.00703-0.00452	
Alternating High Frequency	0.1293	0.1433	1.881	1.858	0.00605	0.00606
	Range 0.1664-0.1043		Range 1.949-1.679		Range 0.00785-0.00536	

TABLE 18
SUMMARY OF ABSOLUTE VALUES AND RANGES

	Average Ultimate Load Rt. (Lb.)	Lt. (Lb.)	Average Ultimate Stress Rt. (PSI)	Lt. (PSI)	Average Ultimate Deflection Rt. (In.)	Lt. (In.)
Control	53	68	11,182	10,555	0.00369	0.00421
	Range 80-40		Range 16,136-7,163		Range 0.00762-0.00221	
Direct	71	87	11,220	12,032	0.00352	0.00400
	Range 101-56		Range 13,913-9,811		Range 0.00546-0.00263	
Alternating Low Frequency	57	62	11,014	10,430	0.00304	0.00294
	Range 66-49		Range 12,143-9,743		Range 0.00376-0.00200	
Alternating High Frequency	73	90	11,959	13,344	0.00367	0.00304
	Range 99-61		Range 14,591-10,557		Range 0.00453-0.00264	

TABLE 19
SUMMARY OF ABSOLUTE VALUES AND RANGES

	Average Ultimate Strain Rt. (In./In.)	Lt. (In./In.)	Average Modulus of Elasticity Rt. (PSI)	Lt. (PSI)	Average Energy of Distortion Rt. (PSI)	Lt. (PSI)
Control	0.0295	0.0322	0.53	0.49	195.6	200.5
	Range 0.0573-0.0220		Range 0.85-0.23		Range 393.2-92.3	
Direct	0.0278	0.0311	0.53	0.55	175.1	225.7
	Range 0.0456-0.0204		Range 0.89-0.34		Range 336.4-113.7	
Alternating Low Frequency	0.0243	0.0237	0.70	0.51	167.6	167.0
	Range 0.0294-0.0163		Range 0.79-0.34		Range 200.5-121.2	
Alternating High Frequency	0.0293	0.0244	0.62	0.77	218.6	203.4
	Range 0.0381-0.0210		Range 0.90-0.44		Range 261.5-165.6	

TABLE 20
SUMMARY OF ABSOLUTE VALUES AND RANGES

	Average Micro-Hardness Rt. Vicker No.	Lt. Vicker No.	Average Osteone Count Rt. Osteones/In.^{2}	Lt. Osteones/In.^{2}	Average % Mineral Content Rt.	Lt.
Control	39.1	39.2	50,750	52,250	68.1	69.0
	Range 46.7-30.0		Range 56,300-45,200		Range 70.1-66.7	
Direct	32.0	35.4	40,798	41,771	70.8	70.6
	Range 42.6-25.1		Range 45,085-38,456		Range 74.7-68.8	
Alternating Low Frequency	30.4	33.5	56,954	61,859	69.5	71.7
	Range 53.3-25.1		Range 62,800-53,500		Range 73.0-68.2	
Alternating High Frequency	29.1	33.5	49,335	44,422	71.1	70.6
	Range 40.1-24.0		Range 53,813-35,031		Range 76.8-68.4	

TABLE 21
SUMMARY OF ABSOLUTE VALUES AND RANGES

	Average % Organic Content Rt.	Lt.	Average % Calcium Content Rt.	Lt.	Average % Phosphorus Content Rt.	Lt.
Control	32.1	31.2	43.4	40.5	19.6	19.4
	Range 33.3-29.9		Range 46.4-34.9		Range 21.0-18.1	
Direct	29.2	29.5	38.8	39.3	19.7	19.4
	Range 31.2-25.3		Range 42.2-33.6		Range 21.5-18.3	
Alternating Low Frequency	30.5	28.3	38.0	37.8	19.7	19.6
	Range 31.8-27.0		Range 41.6-34.2		Range 22.2-17.3	
Alternating High Frequency	29.1	29.5	39.0	37.3	19.2	19.0
	Range 31.6-24.2		Range 41.6-36.1		Range 20.8-18.3	

TABLE 22
AVERAGE % CHANGE BETWEEN
LEFT AND RIGHT FEMUR

	Weight	Specific Gravity	Cortical Area	Ultimate Load	Ultimate Stress
Control	12.79	1.57	17.12	*22.06	*-5.94
Direct	11.00	0.41	11.45	17.7	6.33
Alternating Low Frequency	12.17	0.62	14.74	*8.06	*-5.60
Alternating High Frequency	9.90	-1.41	9.91	19.44	-10.47
Test Accuracy	0.25%	1%	1%	0.5%	2%

* - Per cent change between average left and average right femur

TABLE 23
AVERAGE % CHANGE BETWEEN
LEFT AND RIGHT FEMUR

	Ultimate Deflection	Ultimate Strain	Modulus of Elasticity	Energy of Distortion	Micro-Hardness
Control	*12.35	*8.39	*-8.16	*2.44	-0.90
Direct	8.07	7.40	-2.30	16.40	7.88
Alternating Low Frequency	*-3.40	*-2.53	*-37.30	*-0.36	6.23
Alternating High Frequency	-23.47	-23.50	16.30	-10.20	12.34
Test Accuracy	3%	3%	6%	6%	5%

* - Per cent change between average left and average right femur

TABLE 24
AVERAGE % CHANGE BETWEEN
LEFT AND RIGHT FEMUR

	Osteone Count	% Mineral Content	% Organic Content	% Calcium Content	% Phosphorus Content
Control	1.1	1.28	-3.06	-8.65	-1.03
Direct	1.8	-0.51	1.20	*1.27	*-1.55
Alternating Low Frequency	7.8	3.06	-8.18	-2.08	-0.46
Alternating High Frequency	-18.3	-1.18	1.18	-4.61	1.01
Test Accuracy	5%	2%	2%	3%	3%

* - Per Cent change between average left and average right femur

DISCUSSION

Treating of the Rats

The use of casts in this experiment was one of the biggest sources of trouble. The rats chewed the casts off or pulled themselves out of them. If the plates, wires, and plug were not completely insulated from the cast wet from urination the rats could be shocked.

Out of the 48 rats that began the experiment 16 of them died from three causes. Three rats could not adjust to having the cast on, and would chew it off as soon as it was put on them. After three or four days these rats were found dead of exhaustion in their cages. All of the casts had to be replaced at least once because the cast would wear thin from being moved around in the cages. Shorts did develop in some of the casts and these had to be changed. In changing the casts the rats were anesthetized with ether. Eight of these rats died from overdoses of ether. The last five rats died from unknown causes. None of the deaths were believed to be directly related to the electric field treatment.

Each time a cast was changed a day of treatment on that rat was lost. It was decided that any rat not being treated for at least 21 days would not be included in the experiment. Of the 32 rats sacrificed eight were disregarded because they had been treated less than 21 days , this gave the 24 rats that were tested in this experiment.

Mechanical and Chemical Properties

The weight, specific gravity, and cortical area all showed an improvement in osteoatrophosis with treatment of the electric field. In these three tests the alternating high frequency electric field prevented osteoatrophosis more than any of the other groups.

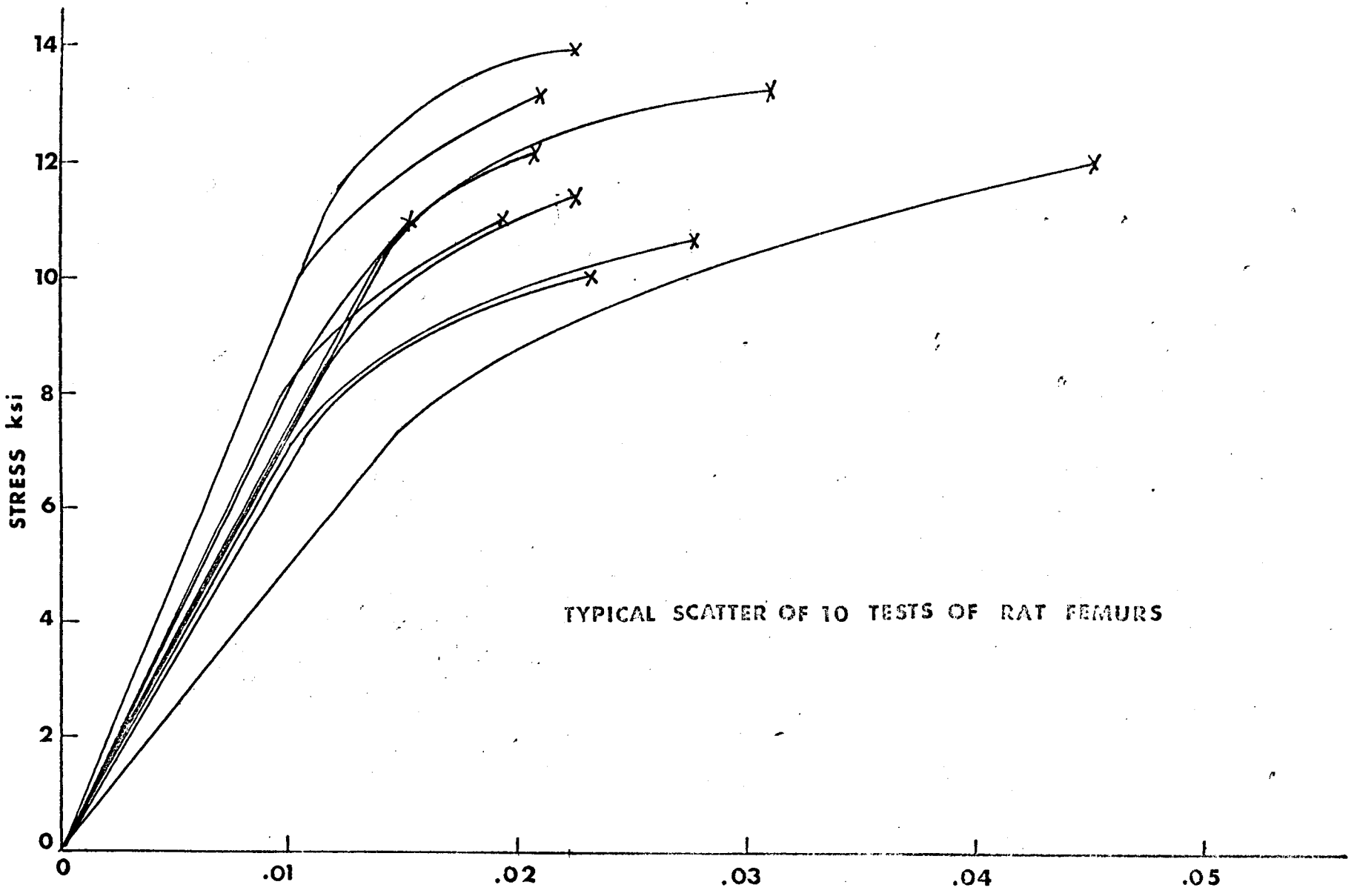
Great care was taken in the conduction of the compression tests. But because of the low number of test specimens and small size, these tests were not considered to be as relevant as the weight, specific gravity, and cortical area measurements. Some of the right femurs, even though their cross-sectional areas were less than the cross-sectional areas of the left femurs, were found to be much stronger. Because of this scattering the confidence level is low for these

tests.(Figure 16) Of the six parameters measured in the compression tests three showed the alternating high frequency electric field prevented osteoatrophosis more than any of the other groups.

The micro-hardness tests were surprising because the electric field treatment seemed to decrease the hardness of the treated femur. Because this is opposite to the weight, specific gravity, and cortical area results and because it does not seem reasonable for these results to be in opposition to each other, this test was looked upon with some reservations.

Poor lighting of the bone and difficulties in getting the diamond tip of the hardness tester to strike the bone surface perpendicular contribute to scattering of the data. Even with this scattering of the data the alternating high frequency electric field shows the most change over the control.

Because no studies of osteone counts in osteoprosis or atrophy of disuse could be found in the literature the osteone count was carried out with no idea of the results. The results on a small sample of bones seemed to indicate that there were no


14
12
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STRESS ksi
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STRAIN in/in
TYPICAL SCATTER OF 10 TESTS OF RAT FEMURS
STRAIN RATE = 0.2/sec.

FIGURE 16

changes in the osteone count between the right and left femurs of the control, direct, or alternating low frequency groups, but an increase was found in the osteone count of the treated femur over the normal femur in the alternating high frequency group.

The chemical analysis shows so much variation between each bone sample that it was impossible to arrive at a valid conclusion. Even though some of the changes were large enough to be significant the number of bones were not. Therefore from this and Dr. Bullard's previous experience no change in any of the chemical properties were considered to have taken place due to the immobilization or electric field treatment.

Of the 15 measured parameters four of them showed no change between the right and left femur in any of the test groups. Of the remaining 11 measured parameters eight of them were effected the most by the alternating high frequency group. Even though there were not enough rats tested to make any diffident conclusions, trends seemed to indicate that the electric field did effect the bones metabolism.(Tables 25 and 26)

TABLE 25
QUICK SUMMARY OF RESULTS

Group	Weight	Specific Gravity	Cortical Area	Ultimate Load	Ultimate Stress
Direct	2	2	2	2	2
Alternating Low Frequency	3	3	3	1	3
Alternating High Frequency	1	1	1	3	1

Group	Ultimate Deflection	Ultimate Strain	Modulus of Elasticity	Energy of Distortion	Micro-Hardness
Direct	3	3	3	1	2
Alternating Low Frequency	2	2	1	3	3
Alternating High Frequency	1	1	2	2	1

1 - Most change from control
2 - Second most change from control
3 - Least change from control

TABLE 26
QUICK SUMMARY OF RESULTS

Group	Osteone Count	% Mineral Content	% Organic Content	% Calcium Content	% Phosphorus Content
Direct	*	*	*	*	*
Alternating Low Frequency	*	*	*	*	*
Alternating High Frequency	1	*	*	*	*

1 - Most change from control
2 - Second most change from control
3 - Least change from control
* - No significant change from control

Bone Tumors

The most significant thing to come out of this research was the finding of tumors on eight of the 18 femurs treated. These tumors were found in two locations on the bone. Six of them were found in the region just below the greater trochanter and on the lateral side of the bone.(Figure 17) The other two tumors were found on the lateral side of the center portion of the shaft. There were no ulcers on the skin of any of these rats. No tumors were found on the control group.

After histological slides were made and the bones studied under the microscope it was decided there were two types of tumors.[15] One type stained green while the other type stained red by the Villanueva stain. The section of the bone that stained green (Figure 18) is believed to be young bone but very unorganized. The second type stained red (Figure 19) had osteones and well organized bone cells in the tumor tissue. Aside from being able to determine that there are bone cells in these tumors no further identification of these tumors could be made by any one at West Virginia University or

University of Virginia Medical Schools. Slides and photographs of these sections are being prepared to be sent to several well-known bone pathologist in the country.

It is again interesting to note that of the eight tumors five of them were found on the alternating high frequency groups.

CONCLUSION

1. The tumors found on the treated bones may have been caused by the cast or the electric plates in the cast, but the pathologists and orthopedists at the West Virginia University Medical Center indicated they did not believe the tumors were caused by either the cast or the electric plates. Since there were no tumors on the control group it is believed that the electric field caused the tumors.

2. Since 73% of the measured parameters indicated that the alternating high frequency electric field caused the greatest change from the control in the parameters measured and 63% of the tumors were found on the right femur treated with the alternating high frequency electric field, it is concluded that the alternating high frequency electric field treatment did cause a change in the bone metabolism.

3. The direct electric field and alternating low frequency electric field also caused changes from the control in the parameters measured the degree of change was much less. Effects of these two electric fields on metabolism was less than that of the alternating high frequency electric field.

RECOMMENDATIONS

The recommendations are that this experiment be repeated in an attempt to verify its results. About 100 kittens should be used in the repeat experiment, and instead of using a cast a strap on device to hold the electric plate should be designed to fit on the right leg of the kittens. The voltage on the plates should be increased to give a charge density equivalent to a stress of about one-half or two-thirds of the ultimate stress, and the high frequency alternating field should be used. The daily treatment of two hours a day should remain unchanged, but the duration of the experiment should be increased from 28 days to at least 42 days. All of the mechanical and chemical tests conducted in this experiment should then be made. It is hoped that this larger experiment will serve to amplify the very promising results obtained in this preliminary study.

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ABSTRACT

The object of this research was to investigate the effect of electric fields on calcium mobility of bone. Forty-eight 100 gram male rats were purchased for this study. Each rat had electric plates and a plaster of Paris cast placed on his right leg. The 48 rats were divided into four groups of 12 each. The first group of rats were used for a control. The second, third and forth groups of rats were treated with 100 peak voltages at frequencies of zero, three, and 30 cycles per second respectively. The three groups were treated two hours a day for 28 days. Their right and left femurs were dissected for analysis.

A comparison was made between the right and left femurs of the treated rats to determine the per cent difference in the measured parameters. The parameters measured were weight, specific gravity, cross-sectional area, compression properties, hardness, osteone count, and chemical properties.

A comparison was then made between the control and the treated rats to determine the effects of the electric field.

Seventy-three per cent of the measured parameters indicated that the 30 cps group caused the greatest change from the control. Tumors were found on 63% of the right femur treated with the 30 cps electric field.

The results indicate that the treatment with the alternating high frequency electric field cause a change in the bone metabolism.