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Graduate School

THE PIEZOELECTRIC EFFECT OF BONE IN VITRO

by

Lloyd E. Gauntt

A Thesis in Partial Fulfillment
of the Requirements for the Degree
Master of Science in the Field of Orthodontics

96410

June 1965

Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Orthodontic tooth movement is predicated upon the resorption and deposition of bone under induced stress. The mechanism of this bone deposition and resorption is incompletely understood. Several theories are currently advanced. Sicher proposes that this mechanism involves the intensity of stress on the bone and a concomitant interference in blood circulation. An increase of stress beyond the limits of tolerance leads to destruction of bone, while an increase of stress within the limits of tolerance leads to formation of new bone. When pressure interferes with the blood supply or blood drainage of bone, resorption results.[20] Frost postulates that the deformation in surface curvature of bone, as a result of stress, controls the location of bone deposition and resorption. When a bone surface becomes more concave bone deposition takes place, and when a bone surface becomes less concave bone resorption takes place. He suggested that the stimulus for this mechanism may be electrical in nature.[6]

Both concepts imply a transformation of stress into biomechanical, biochemical and bioelectrical phenomena. A variety of research methods are currently being employed to study these relationships. The direct piezoelectric properties of bone have been suggested as playing an active role in the internal morphology of bone tissue.

This study has been designed to develop a technique to measure, quantitatively, the direct piezoelectric effect of the crystallites of bone. Such a technique would be of projected value in further studies related to the biomechanical and bioelectrical phenomena of bone formation and resorption under stress loading.

Objectives of the Study

(1) Design and fabricate an apparatus to measure piezoelectricity.

(2) Demonstrate the direct piezoelectric effect of bone in vitro.

(3) Investigate the hypothesis that the piezoelectric effect of bone is due to the organic fraction of the bone.

CHAPTER II

REVIEW OF THE LITERATURE

I. LITERATURE ON PIEZOELECTRICITY

Piezoelectricity is electricity or electric polarity generated in response to pressure. The term is derived from the Greek word "piezein" meaning "to press." The piezoelectric effect refers to the phenomenon by which mechanical and electrical properties are interconnected in a crystal.[19]

The piezoelectric phenomenon was demonstrated for the first time in 1880 by the Curie brothers. In response to a weight applied to the surface of a crystal, a voltage was generated which was directly proportional to the weight. This is termed the direct piezoelectric effect. The following year Lippman hypothesized that if a voltage was applied to the crystal surface the converse effect would occur, namely, physical displacement of the crystal. The Curie brothers later confirmed the hypothesis of Lippman. This is termed the converse piezoelectric effect.[10]

Piezoelectric properties are exhibited by a number of crystal substances of which the most important are quartz, Rochelle salt, and tourmaline. These crystals find a broad application due to their ability to convert mechanical energy into electrical energy, and conversely, electrical energy into mechanical energy. Microphones, hearing aids, oscillators, loud speakers, audiometers and sonar devices are examples of some of their uses.[8]

II. LITERATURE ON THE PROPERTIES OF CRYSTALS

Crystals are distinguished and classified into six fundamental systems. The classification is based on the geometry of the plane surfaces which determine the crystal shape and the angles of adjoining crystal faces.

The hexagonal system, to which quartz,[19] collagen[14] and hydroxyapatite[4, 12, 13, 16] belong, is of special interest to this study. In this system the prism consists of six sides which meet the top and bottom surfaces at right angles. There are, correspondingly, four axes, three of which are of equal length and the fourth longer or shorter. The corresponding pyramid has six sides.

A crystal must be asymmetrical to produce the piezoelectric effect. Crystals which are symmetrical do not generate a current in response to pressure because the stress application does not separate the positive and negative charges. Within the limitations of the system, the more asymmetrical a crystal the greater the piezoelectric effect produced.[10]

In practical application piezoelectric crystals are cut into plates or discs, and electrodes are placed on their major surfaces. Orientation of the major surface and the end surface of the disc, with respect to the axis of the crystal, depend on the type of crystal used and the vibratory action desired.[8]

III. LITERATURE ON THE STRUCTURE AND CHEMICAL COMPOSITION OF BONE

Bone may be considered to be divided into two major parts: the organic fraction and the inorganic fraction.

The organic fraction accounts for 35 per cent or more of the dry, fat-free weight of bone and has three components: (1) the cellular, (2) the ground substance, and (3) the collagen.[12] The cellular component consists of osteoblasts, osteocytes and osteoclasts.[20] The ground substance component is composed of hexoses in a mucopolysaccharide form.[12]

The most prominent component is the collagen which makes up 90-96 per cent of the dry, fat-free weight of the organic fraction.[12,20] Collagen is a complex macromolecule and is thought to be composed of three polypeptide chains arranged in a coiled-coil configuration.[15] It is so structurally regular that it produces a characteristic x-ray diffraction pattern and is considered to be crystalline in nature.[5,12,18]

Ramachandran and Kartha have proposed the hexagonal system for the unit cell of collagen.[14] The unit cell is a conceptual configuration, having no independent existence. It is the smallest expression of the ions found in the same ratios and in the same spatial relationships throughout the entire crystal. Imaginary lines connecting the ions outline the unit cell.

Chemically, collagen is characterized by a low content of aromatic amino acids and a high content of pyrrolidine amino acids and glycine. It is specifically hydrolyzed by the enzyme collagenase.[12]

The inorganic fraction of bone has two components: (1) the crystalline and (2) the non-crystalline. The non-crystalline inorganic component is composed of sodium, magnesium, calcium, phosphate and trace elements of other inorganic substances.[9]

The crystalline component is composed of hydroxyapatite crystals with a basic formula of $Ca_{10}P_2O_{26}H_2$. The crystals of bone are described in terms of the unit cell and are considered to be hexagonal in form.[4, 12, 13, 16] Robinson, on the basis of his work with the electron microscope, describes the apatite crystals as tabular in form with a regular hexagonal outline.[16] Engstrom reports, on the basis of x-ray crystallographic studies, that there is a close relationship between the apatite crystals and the collagenous fibers. These crystals are aligned with their long axis parallel to the collagen fibers.[21]

This close relationship of apatite crystals and collagen is further demonstrated by the sequence in bone formation. First collagen is laid down, and as the matrix matures and calcifies, the apatite crystals form between the collagen fibers with a resulting increase in strength and hardness.[11]

IV. LITERATURE ON THE PIEZOELECTRIC EFFECT OF BONE

Yasuda, Noguchi and Sata, in 1955, investigated "callus without fracture." In their experiment two rods of plastic were passed through the femur and joined by a spring to exert a compressive force on the bone. Callus was formed in response to this stimulus, and measurements showed negative potentials on the compressed parts and positive potentials on the parts under tension. From the results of this investigation the authors concluded that the dynamic energy exerted upon bones is transformed into electrical energy, and this electrical energy plays an important part in callus formation.[22]

Fukada and Yasuda, in 1956, demonstrated the piezoelectric effect in bone. Specimens of bone from the femur of man and ox were used. The specimens were dried completely by heating and the piezoelectric constants were measured by three different experiments: the static direct effect, the dynamic direct effect, and the dynamic converse effect. The piezoelectric effect appeared only when the shearing force applied caused the collagen fibers to slip past each other. The magnitude of the piezoelectric constant was dependent on the angle between the applied pressure and the axis of the bone. The maximum piezoelectric constant was about one-tenth that of the quartz crystal.

The specimens that were boiled in water and then completely dried showed little change in the piezoelectric effect. This observation indicated that the effect is not biological in origin. Fukada and Yasuda postulated that the observed piezoelectric effect of bone may be due to the collagen molecules.[7]

Bassett, in 1962, applied a bending force to both mammalian and amphibian bone. The bone developed a negative potential in the area of compression and a positive potential in the area of tension. The electrical potential generated was dependent upon the rate and magnitude of bony deformation. The results of experimentation did not differ significantly in fresh or dried bone. It is suggested that this was a piezoelectric effect generated by the crystals of hydroxyapatite. It is further suggested by Bassett that this stress potential, generated by bone, may direct the aggregation pattern of the macromolecules of the extracellular matrix.[2] Removal of the inorganic part destroys the ability of the bone to generate stress potentials.

Shamos, in 1963, observed a stress-induced electrical effect in a number of whole bones from different anatomical sites and species. Longer bones, such as ribs and femurs, were stressed by bending, while shorter bones such as the toe phalanx were stressed by compression. Sudden application of a static force resulted in a potential difference between the electrodes that was proportional to the stress applied. The decay time was about 0.5 seconds which was attributed to the relaxation phenomenon of the bone. On release of the stress the same voltage was generated but of the opposite polarity. The voltage generated was of the order of 0.3 mv/Kg of applied force.[17]

CHAPTER III

MATERIALS AND METHODS

I. MATERIALS

Bone Samples

The bone samples were taken from fresh preparations of bovine femur from which the marrow and soft tissues surrounding the bone shaft had been removed.

Small square plates of bone, 3 millimeters thick and 10 millimeters square, were cut from the outer surface of the femur. An angle of 45 degrees in the clockwise direction from the long axis of the bone was selected as shown in Figure 1. This angulation was reported by Fukada and Yasuda to give the maximum piezoelectric effect of bone. They postulated that the piezoelectric effect was due to the applied force causing the collagen fibers to slip past each other. It was shown that since the collagen fibers spiral along the axis of the bone, and are symmetrical with it, a 45 degree cut would produce the greatest piezoelectric effect due to the maximum slippage of collagen fibers.[7]

Care was taken not to coagulate the protein on cutting by first freezing the bone in distilled water and making frozen sections. Six samples, at an angle of 45 degrees from the long axis of the bone, were prepared. The samples were then dried to a constant weight and matched silver electrodes were attached to the major surfaces, which were then coated with silver conductive paint (general cement #21-1). Figure 2 shows a bone sample.

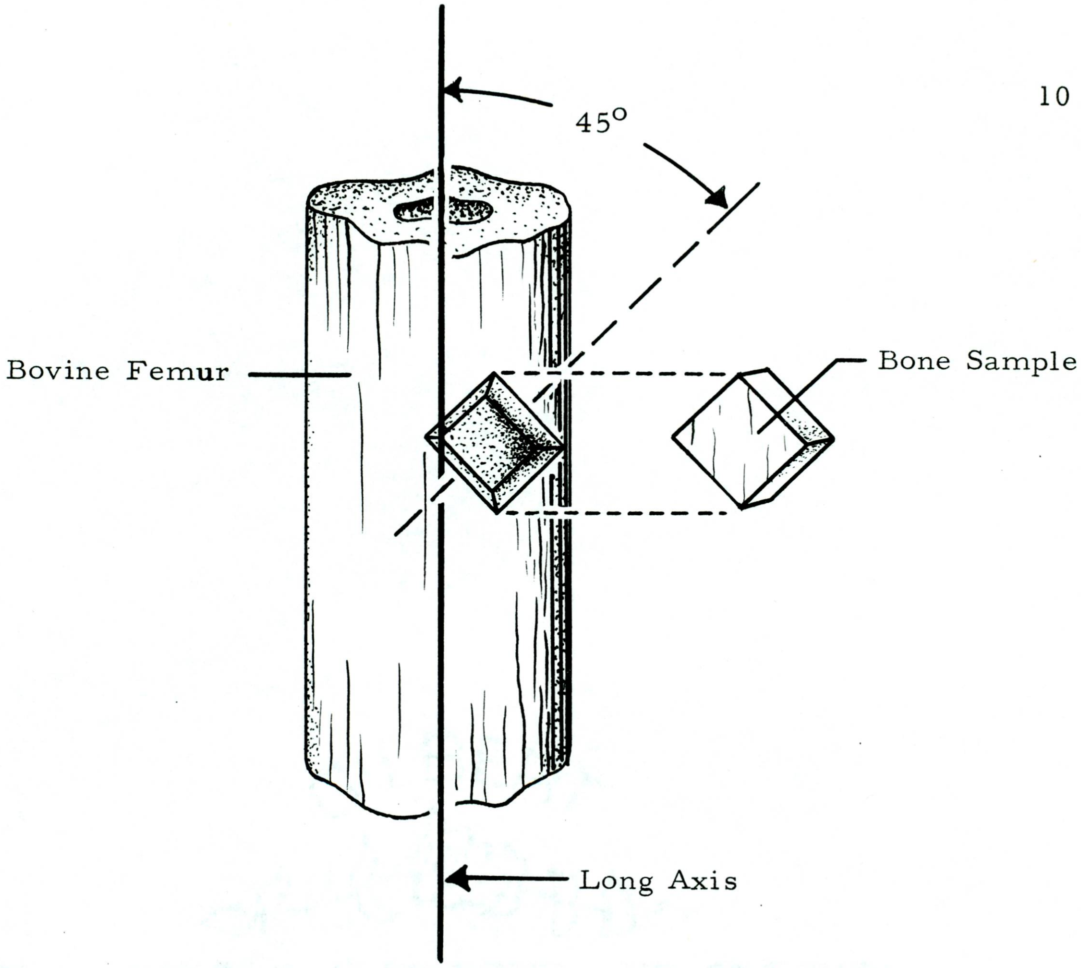


FIGURE 1

SELECTION OF BONE SAMPLE

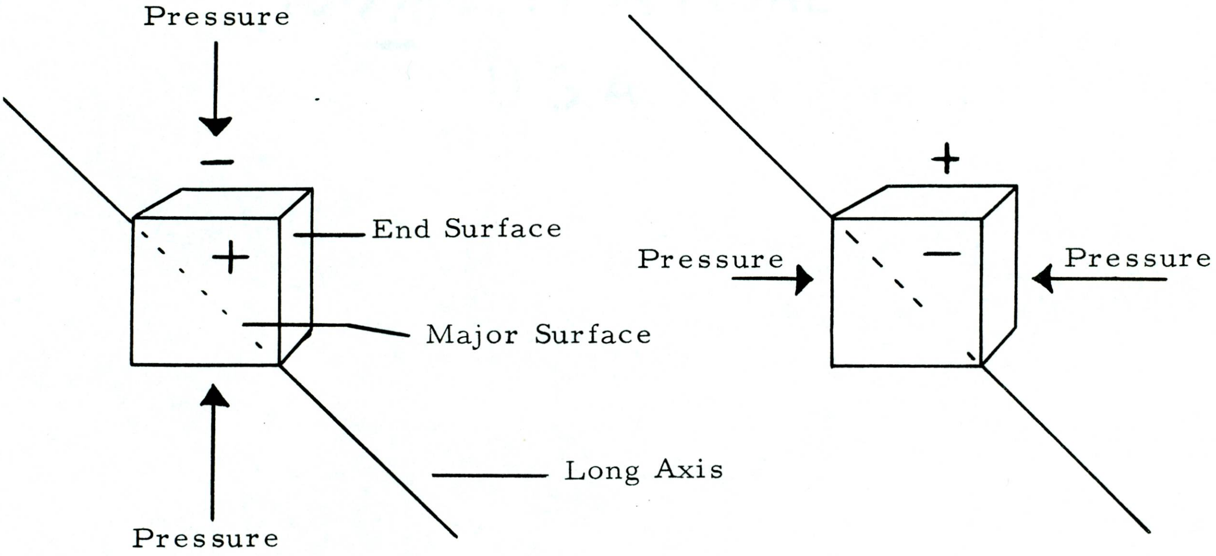


FIGURE 2

BONE SAMPLE AND POLARITY

Measuring Apparatus

Potentials were measured with a vibron electrometer (Electronics Instruments Limited, Model 33B) which were then led via a 1000:1 attenuator to a Grass polygraph (Model 5 PIAB low level D. C. preamplifier) for graphical display. This is a self-contained unit, equipped with a four-element magnetic ink-writing recorder with a motor driven paper feed as shown in Figures 3 and 4.

Calibration of the electrometer and low level D. C. preamplifier were carried out in the following manner. Precise voltages of various amplitudes, from a voltage calibrator (Exact Electric Inc. Type 105), were applied to the input of the electrometer. The deflection of the polygraph pen writer was then adjusted to conform to a suitable scale depending on the sensitivity settings of the electrometer and D. C. preamplifier. The system was found to be linear throughout the range of voltages used. The calibration table is shown in Table I.

A lever mechanism with a mechanical advantage of five, as shown in Figures 4 and 5, was used to increase the amount of force delivered to the bone sample. An adjustable table provided for vertical adjustment of the bone sample, so that the lever arm exerted the force at right angles to the bone. The force applied was measured indirectly by measuring the deformation of the bar within its elastic limit. A pair of strain gauges were attached to the surface of the bar at the point directly opposite the bone. The change in the resistance of the strain gauges was measured in a bridge type circuit. Calibration of the strain gauges was carried out in the following manner. The

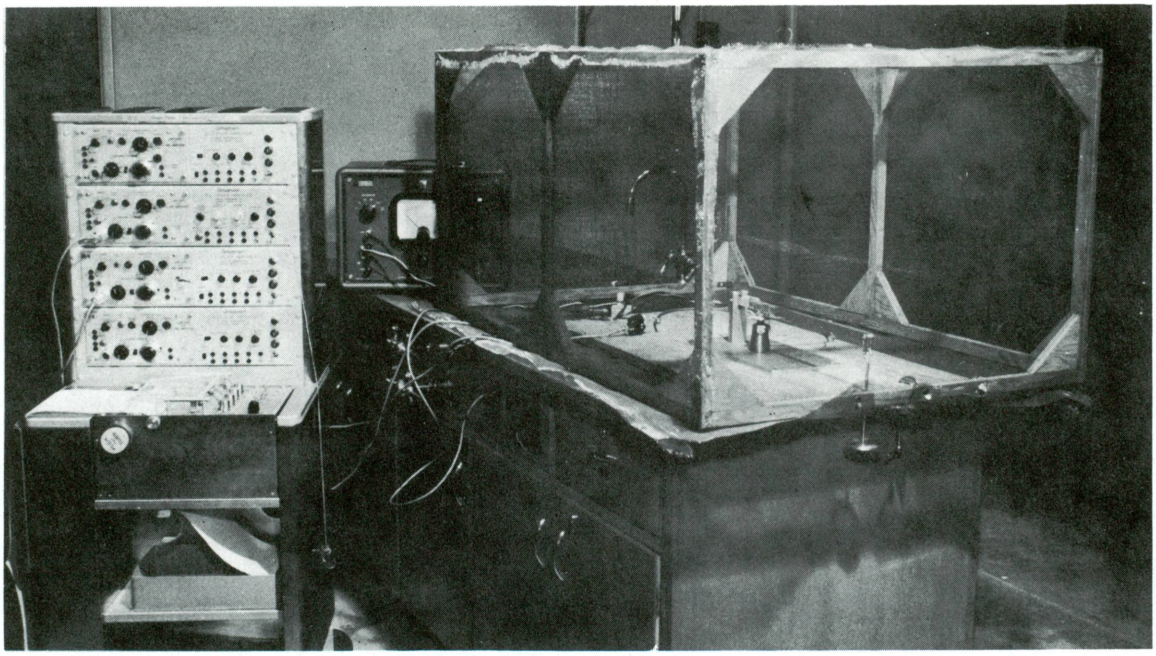


FIGURE 3

COMPLETE APPARATUS USED IN STUDY

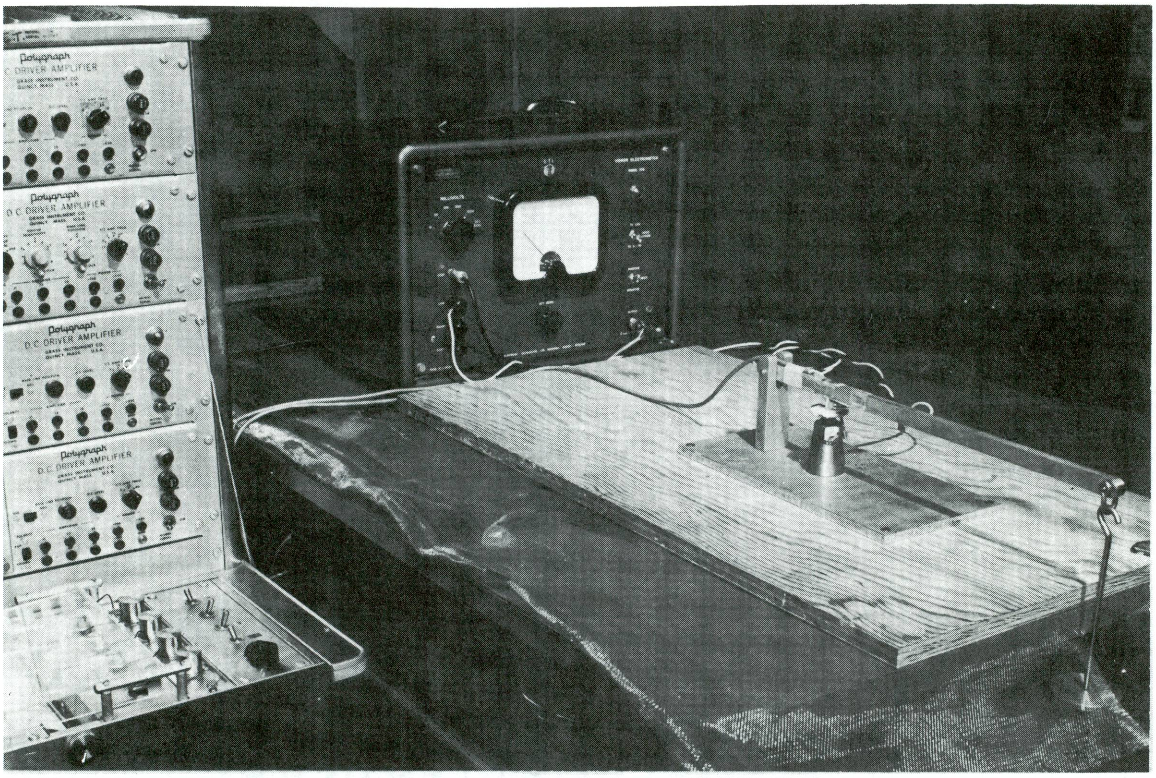


FIGURE 4

LEVER MECHANISM, ELECTROMETER AND GRASS POLYGRAPH

TABLE I

CALIBRATION OF VIBRON ELECTROMETER AND LOW LEVEL D.C. PREAMPLIFIER

Vibron Electrometer Sensitivity	Voltage Calibrator Input Voltage	Polygraph Sensitivity 1 unit/cm
100 mv	50 mv	2 cm
	100 mv	4 cm

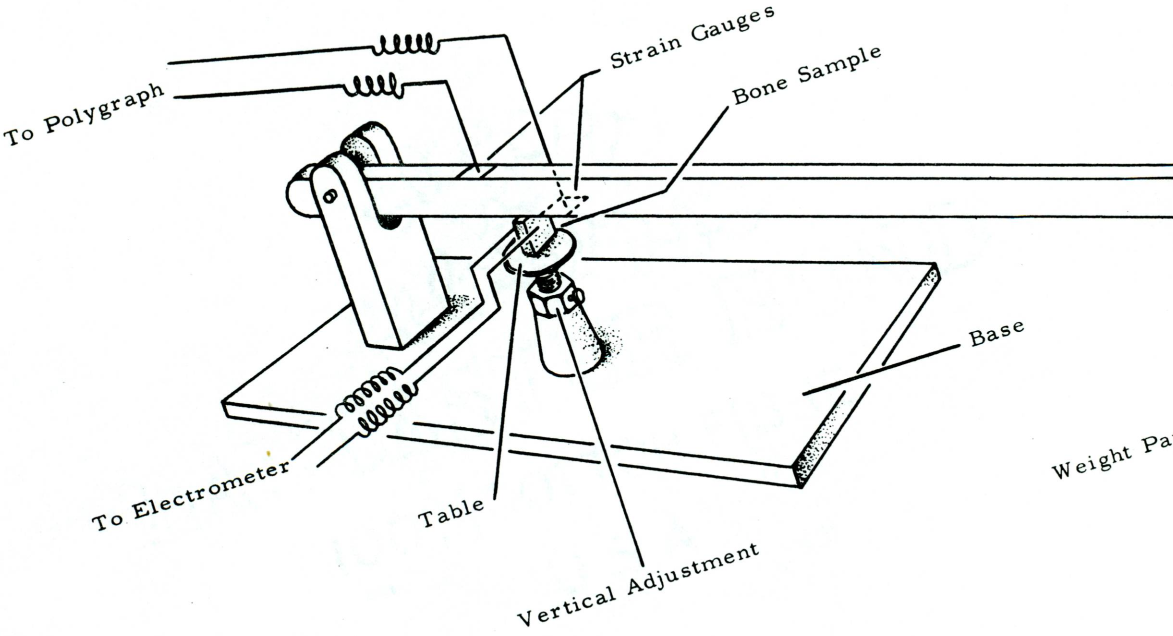


FIGURE 5
DIAGRAMMATIC ILLUSTRATION OF
LEVER MECHANISM

actual force applied to the bone was calculated in accordance with the laws of the mechanics of simple levers. A weight that would be equivalent to a force of 20 kilograms of force at the bone was placed on the weight pan and the bridge amplifier adjusted to give a suitable deflection on the polygraph pen writer, which was arbitrarily chosen to be 1 centimeter deflection equal to 20 kilograms of force. The force on the bone due to the lever arm alone was taken into account since it would be a constant. The calculated values were then accepted as being valid. The pan weight calculation table is shown in Table II. The lever mechanism was enclosed within a Faraday cage.

II. METHODS

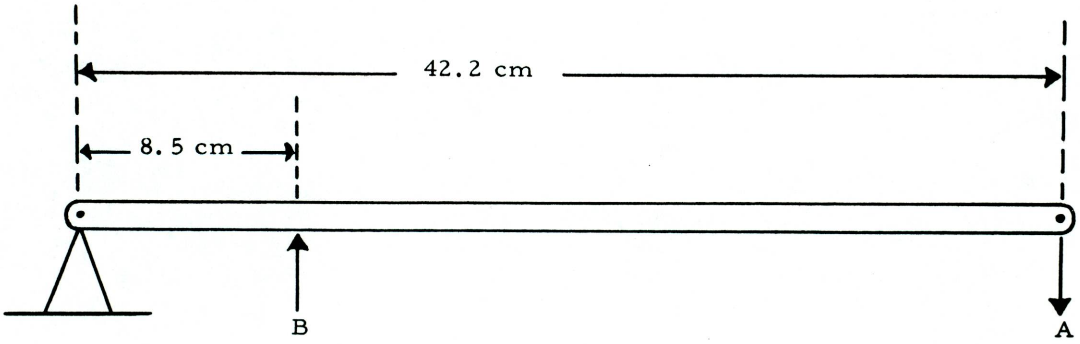
Pressure was applied to the bone sample on the end surfaces by a lever mechanism with a mechanical advantage of five, as shown in Figures 2, 4 and 5 (see pages 10, 12 and 14). This method of force application was utilized to increase the amount of force delivered to the bone sample. In response to the force applied to the bone sample, a voltage appeared on the major surfaces. The potential was measured with a vibron electrometer which was led via a 1000:1 attenuator to a Grass polygraph for graphical representation and potential determination.

The lever mechanism and bone sample to be stress-loaded were placed in a grounded Faraday cage to minimize the influence of static electricity on the electrometer as shown in Figure 3 (see page 12).

A series of five weights were used, to effect a pressure of 20,

TABLE II

PAN WEIGHT CALCULATION



Force at B = 1.0 Kg

Clockwise moment of force = counterclockwise moment of force

42.2 A cm = 8.5 cm x B = 8.5 cm x 1.0 Kg

42.2 A cm = 8500 gm - cm

Surface area of bone $.3\ cm^2$

Then for $.3\ cm^2$ surface area

201.4 gm x $.3\ cm^2$ = 60.42

For 20 Kg/cm^2 = 1208.4

A	B
1208.4 grams	20 kilograms
2416.8 grams	40 kilograms
3625.2 grams	60 kilograms
4833.6 grams	80 kilograms
6042.0 grams	100 kilograms

40, 60, 80 and 100 kilograms of force respectively, per square centimeter of bone surface. Two strain gauges were attached to the surface of the bar, at a point directly opposite the bone, and the input was connected to a second channel of the Grass polygraph.

The ink writer, as used in this study, was designed to provide an immediate and permanent record. It permitted simultaneous recording from two channels. With the calibration which was discussed earlier, this provided a measurable graphical representation of the force applied to the bone and the potential generated by the bone, as well as the relationships of force and potential.

The piezoelectric effect of the six bone samples was measured at 20, 40, 60, 80 and 100 kilograms of force respectively. The base lines A and B, as shown in Figure 6 (see page 20), were observed to oscillate initially on the sudden application of force and then stabilize and maintain a relatively constant level. This behavior of base lines was not observed on the removal of the force. Because of this resonance related problem, only the transient electrical output resulting from the removal of the force was used for potential determinations. An average was made of ten measurements and the figure recorded in Table III A (see page 19).

The bone samples were then treated with a 1 normal sodium hydroxide solution for 72 hours and at 37 degrees centigrade, to remove the organic fraction of the bone. The samples were then dried to a constant weight and remeasured using the same procedure as above. The bone samples each acted as its own control and the untreated values were compared with the treated values.

CHAPTER IV

RESULTS OF STUDY

By utilizing materials and methods outlined in the previous sections, polygraph tracings were obtained for the six bone samples at five different forces. An example of a tracing, from which potential values were determined, is shown in Figure 6. The relationship of pressure and potential is plotted in Figure 7 for the six bone samples stressed. Values of the direct piezoelectric effect for the six bone samples are shown in Table III A and B.

The bone samples exhibited a pressure-induced electrical phenomenon which varied considerably with the pressure application. The amplitude of the potential was observed to be dependent upon the magnitude of the bony deformation. Table III B shows the mean value of the potentials generated by each of the six bone samples. A mean value for the six samples was found to be 0.22 mv/Kg/cm^2.

Figure 7 shows that a linear relationship exists between the pressure applied and the potential generated by the bone sample.

It is observed that polarity is a function of the direction of force application. The polarity is found to reverse with the change in the direction of force application as shown in Figure 2 (see page 10). Also by rotating the bone sample one quarter of a turn the direction of force application is changed by 90 degrees and a reverse in polarity is observed. The initial potential generated by bone sample #2 on force application is shown in Figure 6 to be negative. This could be

TABLE III

VALUES FOR THE DIRECT PIEZOELECTRIC EFFECT OF BONE

A. Recorded Values

Pressure Kg/cm^2	Potential Millivolts Bone Samples					
	#1	#2	#3	#4	#5	#6
20	5.0	5.0	6.2	3.8	1.2	7.5
40	11.2	8.8	10.0	6.2	2.5	12.5
60	16.2	15.0	15.0	10.0	5.0	17.5
80	21.2	22.5	25.0	12.5	6.2	22.5
100	25.0	25.0	27.5	17.5	7.5	30.0

B. Computed Values mv/kg/cm^2

Pressure Kg/cm^2	Potential mv/Kg/cm^2					
	#1	#2	#3	#4	#5	#6
20	0.25	0.25	0.31	0.19	0.06	0.38
40	0.28	0.22	0.25	0.16	0.06	0.31
60	0.27	0.25	0.25	0.17	0.08	0.29
80	0.26	0.28	0.31	0.16	0.08	0.28
100	0.25	0.25	0.28	0.18	0.08	0.30
mean	0.26	0.25	0.28	0.17	0.07	0.31
mean - 0.22 mv/Kg/cm^2						

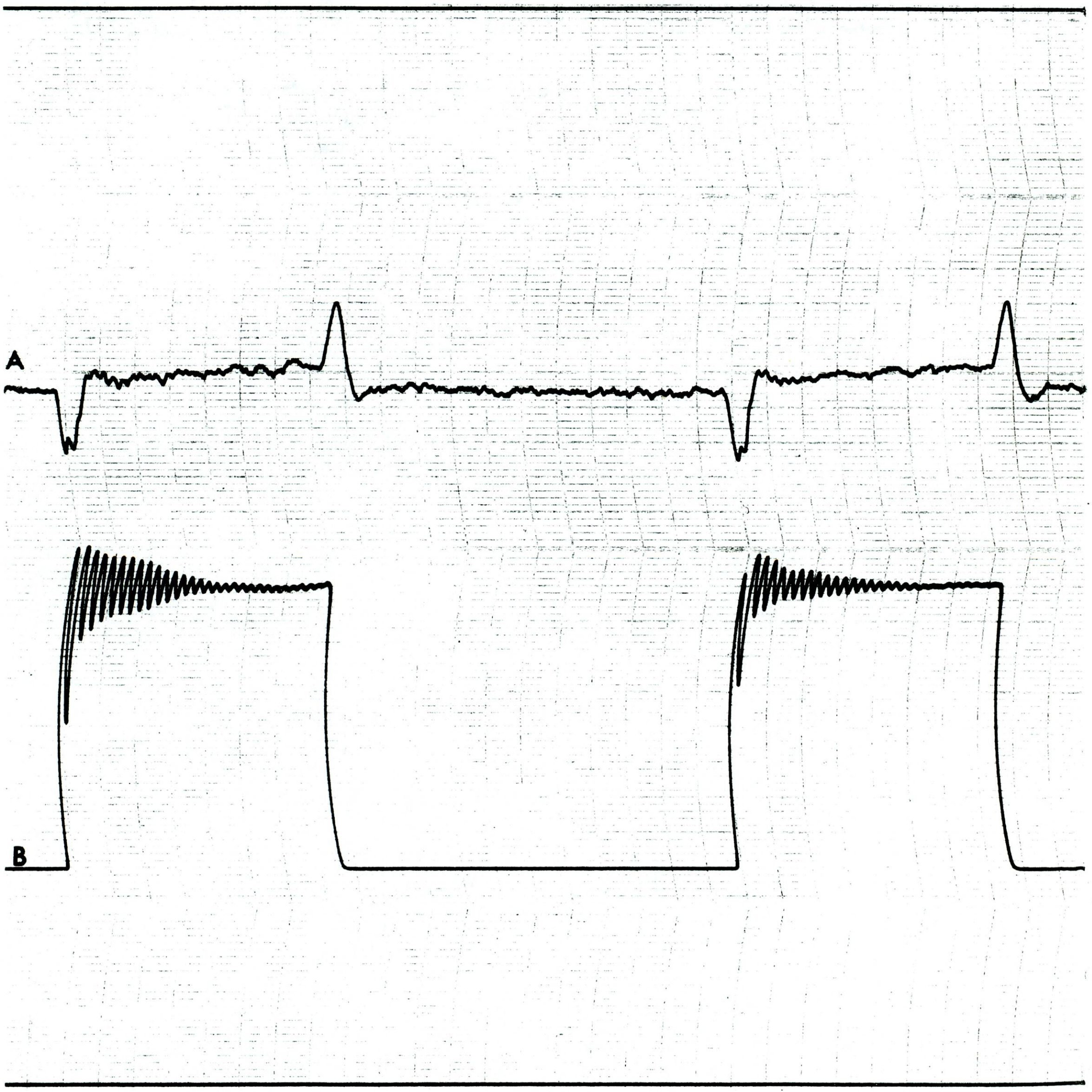


FIGURE 6

SAMPLE OF POLYGRAPH TRACINGS SHOWING
THE DIRECT PIEZOELECTRIC EFFECT
(Bone Sample #2 80 Kg/cm^2)

(A) Output from bone in millivolts (2.5 mv/mm of deflection)

(B) Output from strain gauge transducer measuring force applied to bone (20 Kg/cm of deflection)

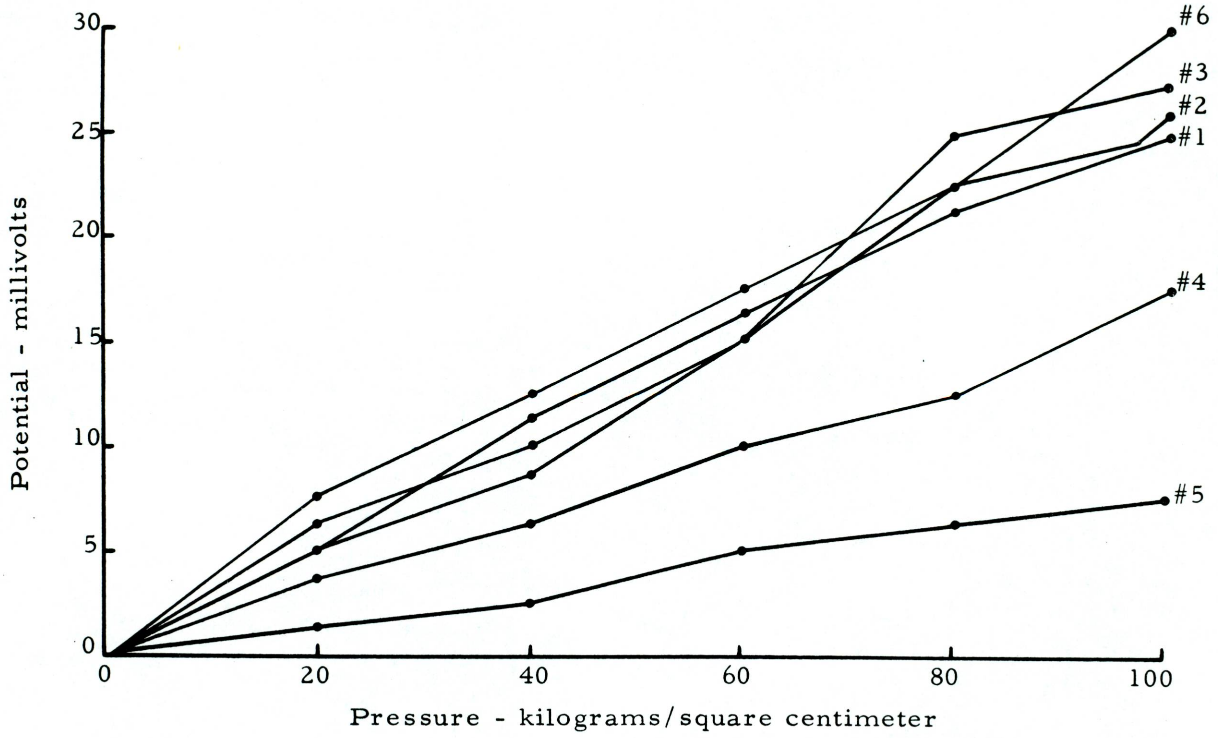


FIGURE 7

THE DIRECT PIEZOELECTRIC EFFECT OF THE SIX BONE SAMPLES made positive by reversing the electrodes or by rotating the bone sample one quarter turn in the lever mechanism. If the electrodes were placed on the sides and the pressure was applied to the major surface, the piezoelectric effect was not observed. Bassett found that, in deformation of bone by bending, polarity was determined by the direction of bending. Areas under compression developed negative potentials and those under tension developed positive potentials.[2]

When the organic fraction of the bone was removed with a 1 normal sodium hydroxide solution, the resultant "anorganic" bone was quite fragile. The bone samples were so fragile that fracture occurred when restressing of the bone samples was attempted.

Although each of the bone samples were cut at an angle of 45 degrees from the long axis of the femur, and from the same bone, the potentials obtained on force application differed slightly. This is shown in Figure 7 (see page 21).

CHAPTER V

DISCUSSION

In analyzing the transients of Figure 6 (see page 20) a number of phenomena are observed. The base line A shows a somewhat oscillating pattern. This is due to the inherent characteristics of the vibron electrometer and to the extreme sensitivity of the instrument. The double peaks noted on the initial deflection of base line A are due to the damped oscillatory force, as noted in base line B, which resulted from the spring-like effect of the lever arm on sudden application of force. This resonance effect is not maintained because the percentage of change in force is reduced after the first peak and also the resonance of the bone is inherently slower than that of the lever arm with the weight. Because of this problem, only the transient electrical output resulting from the removal of the force was used for potential determinations.

The steady state potential (noted after the initial deflection and throughout the duration of the applied stress) was observed to exhibit a slight rise as shown in Figure 6 A (see page 20). This may be a spring-like resistant force within the bone that is seeking to re-establish the original contour of the bone sample. This would also account for the overshoot in base line that is noted on application and removal of force. It could be postulated that if the force application was maintained this increase would reach a point of stabilization.

The potentials, generated by the bone on the application of stress, are similar to those of a quartz piezoelectric effect, but decay at a somewhat slower rate.[2]

Bone samples of the same size and cut at the same angulation to the long axis of the bone, exhibited differences in the potential generated. This was not considered to be unusual. Although the inorganic apatite crystals and collagen fibers are highly oriented, there are still differences in structure and ultrastructure between bone samples. Bone is a labile tissue, and it would not be expected to exhibit the piezoelectric effect in the same manner as a quartz crystal. A visual criterion was used to determine the angulation at which the samples were cut. It may very well be that the physical appearance of the bone does not correlate exactly with the crystalline structure of the bone.

Potentials generated in the bone samples apparently were not dependent upon cell viability. The bone sample potential could be determined after preparation and again after being dried to a constant weight with little change noted. The bone sample potential could be redetermined after being allowed to air dry for a month with little or no change detectable.

The potentials generated, as a result of force application to the bone samples, were found to be linear. This linear relationship, between pressure and potential generated, suggests that the phenomenon observed is piezoelectric in nature. As further evidence that this is a piezoelectric effect, the polarity of the electrical output from the bone sample should be cited. When the direction of force application is changed a change in polarity is observed and when the connections to the bone sample electrodes are reversed the polarity is reversed. On the release of the force application a reverse in polarity occurs. If this were electrostatic or electrostrictive in nature a polarity change would not be involved.[7] It seems reasonable and valid to conclude from the observation cited that this was a piezoelectric effect in bone.

It was not determined which of the two fractions, the organic or inorganic, generated the piezoelectric effect. When the organic fraction of the bone was removed the fragile inorganic bone could not withstand the pressure necessary for stressing. Although the piezoelectric effect is thought to be due to either the organic or inorganic portion, the problem is not quite that simple. When one fraction of the bone is disturbed, the interaction between the two fractions being so intimate, the resultant bone is entirely different and not just in the degree to which it was originally changed. Engstrom reported that a close relationship exists between the crystalline portions of the organic and inorganic fractions of bone.[21] It could very well be that the piezoelectric effect of bone is the result of an interaction between the organic and inorganic crystalline components.

These studies, in themselves, do not establish the relationship between cellular activity in bone and potential generated by pressure. Becker, in 1961, demonstrated that cells are responsive to alterations in d-c fields.[3] It seems reasonable that similar behavior might be expected of the cells in bone.

Little is known about the histological and functional features of bone during the time osteoblasts and osteoclasts are held in a state of rest due to selective inhibition. The nuclei of these osteoclasts and osteoblasts presumably are present and alive but are somehow prevented from fulfilling their usual function. One may postulate that the potentials generated play a direct role in activating or in selectively inhibiting the cells of the bone cell system. This postulate may be questioned, however, when the work of Bassett, Becker and Shamos is reviewed. They reported weak potentials on the surface of bones loaded in bending. The mean value of 0.22 mv/Kg/cm^2 obtained in this study is relatively weak. If the potentials do not play a direct role in activating or in selectively inhibiting the cells then they may have an indirect role. The potentials might be associated with unidirectional fluxes of anions and cations from the bone towards the bone surfaces. It is likely that these ion fluxes will effect cellular behavior more than weak electrical potentials.

CHAPTER VI

SUMMARY AND CONCLUSIONS

An apparatus was designed and fabricated, and a technique developed, to measure piezoelectricity in bone. The direct piezoelectric effect of bone in vitro was demonstrated and measured.

The organic fraction of the bone was removed in an attempt to ascertain if the piezoelectric effect observed was due to the organic fraction of the bone, as had been hypothesized. This method did not yield measurable results.

The following conclusions were drawn from this investigation:

1. Bone in vitro exhibits a pressure-induced electrical phenomenon.
2. Potentials generated by the bone samples vary considerably with the pressure applied.
3. The amplitude of the potential is dependent upon the magnitude of the bony deformation.
4. A linear relationship exists between the pressure applied and the potential generated.
5. Polarity is a function of the direction of force application.
6. Slight differences in potentials are observed for bone samples of the same size when equal pressures are applied.
7. Bone in vitro exhibits the piezoelectric effect.

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Graduate School

THE PIEZOELECTRIC EFFECT OF BONE IN VITRO

by

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The mechanism of bone deposition and resorption, under stress, is incompletely understood. The direct piezoelectric properties of bone have been suggested as playing an active role in the internal morphology of bone tissue.

This study was designed to fabricate an apparatus and develop a technique to measure, quantitatively, the direct piezoelectric effect of the crystallites of bone.

In vitro studies were done with bone samples taken from bovine femur. The samples were subjected to stress loading and were observed to exhibit a pressure-induced electrical phenomenon. The amplitude of the potentials generated was dependent upon the magnitude of the bony deformation. A linear relationship was observed to exist between the pressure applied and the potential generated. Polarity was found to be a function of the direction of force application. This may indicate that there is some interaction between internal surfaces of the bone that generate a potential on sliding past each other. From these observations it was reasoned that bone in vitro exhibits a piezoelectric effect.

An attempt was made to distinguish as to which of the two fractions, organic or inorganic, was responsible for the potentials generated.

One may postulate that the potentials, generated by the piezoelectric effect of bone, play a role in activating or in selectively inhibiting the cells of the bone cell system. Because the potentials measured were relatively weak, an alternate hypothesis may be that

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these potentials are associated with unidirectional fluxes of anions and cations from the bone towards the bone surfaces.