

## *Short Communications*

### Piezoelectricity in Bone as a Function of Age

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The piezoelectric constant of mature and immature bone (defined herein) has been measured in an effort to determine whether it varies with age. It was found that the average value of the piezoelectric constant  $d_{14}$  of femur from three week old calves was 58% of the value of femur from three year old bulls. The results were interpreted to indicate qualitative differences in the corresponding collagen matrices. Mature human tibia from males ranging in age from 21 to 53 years of age showed a small but significant increase in  $d_{14}$  with age. Some data concerning diseased human bone, and well-preserved human bone excavated in Peru are also presented.

*Key words:* Bone — Piezoelectricity — Collagen — Aging.

#### **Introduction**

Previous work has suggested the importance of piezoelectricity as a control factor in certain kinds of bone growth, particularly modelling and remodelling [5]. Since bone growth processes are, in a general sense, a function of age, it seemed worthwhile to determine whether the piezoelectric property was also age dependent. See Sinex (1968) and the references cited there for a discussion of the age related chemical changes in bone and collagen.

Because of the difficulty in obtaining sufficient numbers of specimens of any one species covering its entire age span, we elected to proceed as follows. There is a natural division in age of the long bones depending on whether or not epiphyseal growth has ceased. In order to compare the piezoelectric effect between the two classes, which we shall call mature and immature respectively, we have employed bovine bone which was both suitable and available. There is a further question of whether age-dependent piezoelectric effects exist within either class. We have studied this possibility in the mature class only, using human tibiae from 21 to 53 years of age. These data and some related observations are presented below.

#### **Methods**

The human bone studied included specimens of tibia from seven males. The specimens were clinically normal and were obtained during surgical amputation occasioned by factors other than direct bone pathology. The age recorded for each donor was that nearer the date of surgery. The specimens were cleaned of adhering soft tissue and marrow, degreased in acetone for 24 h and stored at room temperature until used. Specimens of femur and tibia from a 23-year-old female, obtained during surgery for osteosarcoma were similarly treated. Also studied were two specimens of mature human tibia of unknown age and sex excavated at a burial site of a pre-Columbian civilization of Peru [1] which existed from approximately 400 to 1600 A. D.

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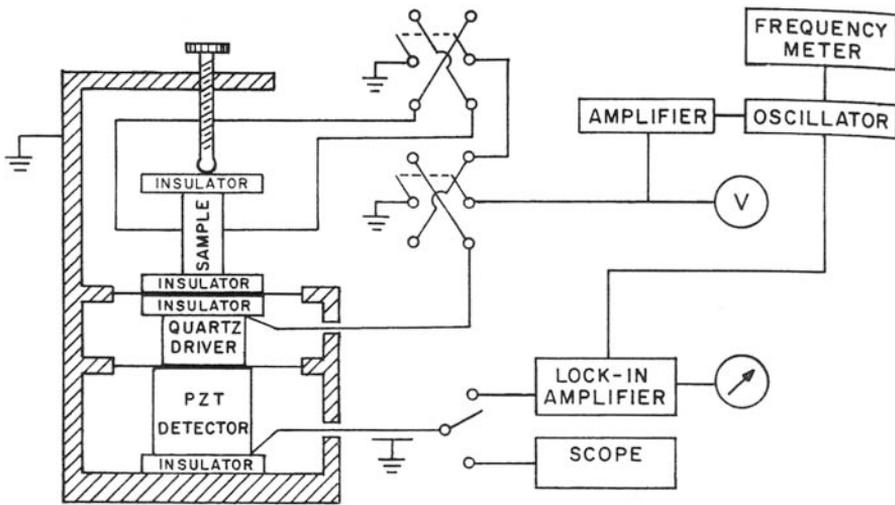


Fig. 1. Apparatus employed in the measurement of the piezoelectric effect.  $V_8$  applied to the sample causes a deformation by virtue of the converse piezoelectric effect. It is transmitted to the quartz detector which produces an output voltage  $V_{out}$  by means of the direct piezoelectric effect of quartz. With the switch reversed  $V_q$  is applied to the X-cut quartz driver so as to produce  $V_{out}$ . The apparent piezoelectric constant for the samples studied was then calculated as:

$$d_{14} = \frac{2d_q c V_q}{a V_s}$$

where  $d_q$  is  $d_{11}$  of quartz ( $d_{11} = 6.5 \times 10^{-8}$  cgs esu) and  $c$  and  $a$  are the sample thickness and height respectively. All values of  $V_8$  were averages obtained by reversing the field across the sample so as to produce the same  $V_{out}$

Bovine bone was obtained at a slaughterhouse shortly after death. The specimens consisted of seven femura, one from each of four 3-week-old bull calves and one from each of three bulls which were 3 to 4 years old. The specimens were cleaned and stored as described above.

From 4 to 10 samples were cut from each specimen with the aid of a specially designed table saw. The samples were nominally 10 x 5 x 2 mm and were composed solely of cortical bone. To permit measurement of the piezoelectric coefficients,  $d_{14}$ , the samples were cut so that their long axis was 45 degrees from the original long axis of the bone. Silver paint was used both as electrodes and to hold in place the thin wire leads from each sample. To assess the effect of absorbed water vapor, some measurements were made on samples equilibrated to room humidity. Subsequently, all samples were dried by heating to 100° for 24 h prior to measurement in order to provide a common base line for water content. The piezoelectric measurements were then made at room temperature in a time sufficiently short to preclude reabsorption. The piezoelectric constants were computed by Fukada's method [3], employing a slightly modified circuit which displayed the converse effect (See Fig. 1). Depending on the sample, the clamped system had two resonances between 2 kHz and 3kHz which were utilized as measuring frequencies.

The percent organic content of the specimens studied, which will be assumed here to be solely collagen, was determined by ashing dry bone in a muffle furnace at 625° for 24 h.

## Results

In Table 1 the piezoelectric constants and related data of mature and immature bovine femur are given. Also shown is the effect of drying the samples at 100°

prior to measurement. Piezoelectric constants and related data from mature human tibia of different ages are given in Table 2. Piezoelectric constants relating to a case of osteosarcoma with the lesion in the tibia, are presented in Table 3 along with values obtained from the Peruvian specimens.

### Discussion

The effect of absorbed water on the measured value of the piezoelectric constant of bone is shown by the differences observed in specimens 1 and 5 in Table 1. Since bone is hygroscopic [4], measurement of the converse effect in bone containing absorbed water is accompanied by a reduction of the applied electric field inside the sample. The magnitude of the change depends on the model assumed for water absorption [2] and the result is a lower apparent piezoelectric constant in the presence of water. From Table 1, the average value  $d_{14}$  of dry immature bovine femur is about 58% of the value for mature femur. Although piezoelectricity in bone originates in the collagen component [6], the difference in  $d_{14}$  is not due to a quantitative difference in the collagen. For samples of mature bovine femur, the average amount of collagen present can be seen from Table 1 to be  $2.00 \text{ g bone/cm}^3 \times 0.25 \text{ g collagen/g bone} = 0.50 \text{ g collagen/cm}^3$ . The average collagen density ( $\rho_c$ ) of immature bovine bone is also  $0.50 \text{ g collagen/cm}^3$ . Thus mature bone exhibits both a higher piezoelectric constant and a higher piezoelectric constant per unit of  $\rho_c$ . The latter observation, which may bear on the question of the ultimate origin of piezoelectricity in collagen, indicates that structural changes occur within the collagen matrix during maturation.

The piezoelectric constant of mature human tibia varied over a relatively narrow range (Table 2). Nevertheless, the constants of the youngest four specimens are significantly lower suggesting that a larger specimen population might show an increase in  $d_{14}$  with age. If the piezoelectric constant of bone is taken to be constant over the range studied, since  $\rho_c$  decrease with age, the results indicate that structural changes must occur within the collagen matrix accompanying aging. These changes tend to increase  $d_{14}$ , thereby preventing what would otherwise be a decrease. Some evidence for this can be seen in the last column of Table 2 which shows that the piezoelectric constant of bone per unit of  $\rho_c$  increases with age. Thus, there is either an increase in  $d_{14}$  of bone with age of a qualitative age related change in the bone matrix, or both. If  $d_{14}$  increases with age, its significance in accounting for age-related growth characteristics within the mature class must be assessed against its relatively narrow range for the age group studied.

The values of  $d_{14}$  in the Peruvian specimens (Table 3) are within the range shown in Table 1 and illustrate the stability and relative constancy of the piezoelectric effect in mature human bone. In accord also, are the data of Table 3 showing that even in bone containing a neoplastic lesion, the value of  $d_{14}$  of the non-involved bone falls within the same narrow range. At the site of the lesion the histological picture is one of disorganization and this is reflected in the low value of  $d_{14}$  found for samples prepared from this site.

In conclusion, immature bovine bone has been found to have a lower piezoelectric constant than mature bone, suggesting the possibility that differences between them in growth characteristics are related to piezoelectricity. In contrast,

Table 1. Piezoelectric constant  $d_{14}$  and related data of mature (3 year) and immature (3 weeks) bovine femur. The errors given here and in subsequent illustrations are standard deviations. The two groups differ significantly at  $P < 0.005$

Specimen No.	Age	No. of samples	$d_{14}$ ( $\times 10^{-9}$ cgs esu)		Bone density ( $\text{gm}/\text{cm}^3$ )	Collagen (%)	$\rho_c^a$	$d_{14}/\rho_c$
			room	dry				
1	3 years	7	$4.25 \pm 0.66$	$5.60 \pm 0.62$	2.00	25.6	0.51	11.0
2	3 years	7		$5.11 \pm 0.82$	2.00	24.6	0.49	10.4
3	3 years	8		$5.63 \pm 0.48$	2.00	24.8	0.50	11.3
4	3 weeks	6		$2.99 \pm 0.36$	1.74	29.1	0.51	5.9
5	3 weeks	6	$2.60 \pm 0.43$	$3.80 \pm 0.46$	1.83	29.9	0.55	6.9
6	3 weeks	9		$2.57 \pm 0.53$	1.68	28.2	0.47	5.5
7	3 weeks	4		$3.28 \pm 0.51$	1.71	28.9	0.49	6.7

<sup>a</sup> g collagen/cm<sup>3</sup>.

Table 2. Piezoelectric constants  $d_{14}$  and related data of dry mature human tibia of different ages. Specimens were clinically normal and obtained from males. The values of  $d_{14}$ ; of the youngest four specimens are significantly lower at  $P < 0.005$

Specimen No.	No. of samples	Age	$d_{14}$ $\times 10^{-9}$ cgs esu	Bone density ( $\text{gm}/\text{cm}^3$ )	Collagen (%)	$\rho_c^a$	$d_{14}/\rho_c$
1	5	21	$7.29 \pm 0.53$	1.81	33.3	0.87	8.38
2	6	26	$5.68 \pm 0.61$	1.94	31.7	0.62	9.16
3	7	27	$5.80 \pm 0.52$	1.86	31.4	0.58	10.00
4	5	30	$5.98 \pm 0.65$	1.70	34.2	0.58	10.31
5	5	35	$7.96 \pm 0.30$	1.86	29.1	0.54	14.74
6	5	36	$8.23 \pm 0.42$	1.86	29.2	0.54	15.24
7	4	53	$6.07 \pm 0.70$	1.82	29.2	0.53	11.45

<sup>a</sup> g collagen/cm<sup>3</sup>.

Table 3. Piezoelectric constants  $d_{14}$  of dry human tibia excavated in Peru and of human bone with osteosarcoma

Specimen No.	Condition	Age	Sex	Bone	No. of samples	$d_{14}$ $\times 10^{-9}$ cgs esu
1	Osteosarcoma	23	F	Femur	2	$5.8 \pm 0.5$
				Tibia	3	$5.9 \pm 0.5$
				Tibia at lesion	4	$0.5 \pm 0.3$
2	Peruvian	?	?	Tibia	4	$7.8 \pm 1.2$
3	Peruvian	?	?	Tibia	4	$8.2 \pm 0.2$

the piezoelectric constant of mature human bone, depends little on age within the interval studied. In the absence of degradation the piezoelectric constant of bone does not seem to depend on the lapse of time which follows either death or

removal of the bone from the donor individual. Also, judging from the results obtained from one set of specimens, the piezoelectric constant of mature bone is independent of sex, and is unaffected by the presence of neoplasia.

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