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PIEZOELECTRIC EFFECTS IN BIOLOGICAL TISSUES

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The aim of this paper is to analyze different contributions and differents points of view concerning the meaning of the piezoelectric effect in the bone. It is now obvious that this effect is overhelming in dry bone. In wet bone more important are streaming potentials.

Key words: bone, piezoelectricity

1. Introduction

The importance of electrical phenomena in physiology was distinctly realized in studies performed by von Haller (1708-1777), Swiss poet and doctor of medicine. In further research by Galvani (1737-1798) and Volta (1745-1827) the dependence of physiology of muscles and nervous cells from the action of electricity was demonstrated. Already in 1812, electricity was used to heal nonunions. We recall that a nonunion is a bone fracture that has failed to heal over the average period of time required for healing, cf. the papers by Peltier (1981) and House (2000) on this subject. The efforts of biologists and physicists of the next two centuries, among them of Helmholtz, consolidated this knowledge. The physiology is concerned with electrical processes at different stages, on the level of metabolism and level of growth, cf. Wojnar and Telega (1997).

The discovery of the piezoelectric effect in dry bones by Ivao Yasuda in 1953 was thought to solve positively long trials of looking for the explanation of bone remodelling. The history of analysis of the experimental data is quite drammatic.

Regling (2000) proposed a bioelectrical conception of connective tissue regulation in a bone, cartilage and tendon as well other connective tissues stressed by mechanical forces. The conception is based on the biological conjecture of a biosensor and nerve-like signal conducting function of the native collagen fibril in the extracellular matrix. The idea finds its support in the classical biophysics and physicochemistry. It allows pne to discuss the living state of the extracellular matrix and the biochemical aspects of acid and neutral protease activity, as well as nanoelectronic properties of connective tissue regulation.

In this review paper we are going to discuss only one aspect of the bone tissue, namely that the dry bone exhibits the piezoelectric effect. The phenomenon, discovered by Japanese scientists Fukada and Yasuda in the 1950's was initially regarded as a possible stimulus of the bone remodelling.

At the beginning, we give some preliminaries on the bone structure possessing biomechanical meaning, next describe the piezoelectric effect observed in a dry bone and finally provide comments on scientific discussion which led to the conclusion that in a wet bone mainly streaming potential are responsible for electric phenomena. However, we observe that the piezoelectric effect reveals its significance in other biological phenomena.

2. Bone

2.1. Bone activity

In the world of living tissues the bone belongs to the most remarkable. It houses the factory – the bone marrow that produces most of the cells of blood, it stores minerals and doles them out as needed to other parts of body, it repairs itself after injury, and it grows, as any other living tissue, until the body reaches adulthood. While it is growing and constantly building itself, it also serves as the rigid structural support for the body.

The key to the bone's growth and its hardness is its mineral building material, known as the "bone salt". The mineral is composed chiefly of calcium, phosphate and carbonate. The bone takes up, stores and also releases calcium into the blood in necessary amount to keep its optimum level. A bone grows in a continual process of building up and destruction. It grows in thickness, for example, as follows: bone destroying cells called osteoclasts erode inside of the bone, enlarging the marrow cavity, while bone forming cells called osteoblasts at the same time build up the bone on the outside.

The growth system and the structure of bone is designed to give it the greatest degree of support with minimum weight. According to the engineering principle that at a given weight a rigid material is stronger as a tube than as a solid rod, the long bone is the strongest at its boundary. The interior of bone is filled with the blood-forming bone marrow, see Fig. 1.

Fig. 1. Gross anatomy shown by longitudinal section of the upper end of a leg bone of a rat. The gray band running from left to right above center of the picture is the cartilage disk. The black material is the bone which is seen tunneling into the cartilage. The gray material running down the center of the bone is marrow, after Mc Lean (1955)

The most striking mechanical feature that differentiates the bone from other tissues is its hardness. The hardness of a bone results from the deposition within a soft organic matrix (collagen fibrils) of a complex mineral, composed mainly of calcium, carbonate and phosphate, and as a result the intercellular or interstitial part of bone is a calcified collagenous substance, cf. McLean (1955).

The bone mineral (the bone salt) is in the form of minute and hardly soluble crystals, about 500Å=50 nm in length. The volume of a single crystal (50 nm long, 25 nm wide and 10 nm thick) is of order of $12500\,\mathrm{nm}^3 = 125 \cdot 10^{-16}\,\mathrm{mm}^3$, so in one cubic milimeter we have $(1/125) \cdot 10^{16}$ crystals. The surface of one crystal is of the order of $50 \cdot (25 + 10) \cdot 2\,\mathrm{nm}^2 = 3500\,\mathrm{nm}^2$ and the surface of all crystals in $1\,\mathrm{mm}^3$ is of the order of 0.3 square meter. In cubic decimeter there are so many crystals that their surface adds up to 30ha. Such a great surface enables the rapid exchange of calcium in the blood. Under certain conditions as much as 100 per cent of calcium in the plasma may be replaced every minute.

The crystals of the bone mineral, as analyzed by X-ray diffraction, have chemical composition like that of fluorapatite, except of hydroxyl (OH) groups in place of the fluorine.

We recall that apatites are calcium phosphates described by the formula

$$3\,Ca_{10}(PO_4)_6X_2$$

When X stands for the hydroxyl group (OH) we deal with hydroxyapatite, known also as dahllite. Sixty-five per cent of the bone weight is due to the hydroxyapatite crystals, cf. Bourne (1971).

Thus, the crystal structure and chemical composition of the bone salt are approximated by the formula of hydroxyapatite

$$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$$

or

$$Ca_{10}(PO_4)_6(OH)_2$$

This mineral crystalizes in the form of thin, hexagonal tablets that are arranged in bands around the collagen fibres, cf. Fig. 2. The hydroxyapatite is centro-symmetrical and therefore is not piezoelectric, cf. Anderson and Eriksson (1968).

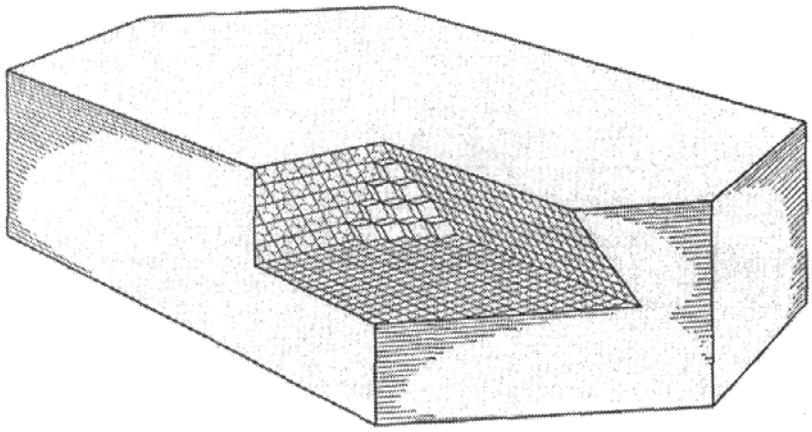

Fig. 2. Crystal of the bone is hydroxyapatite. Magnification of one milion times. Cutout illustrates the pattern of internal structure of the crystal, after Mc Lean (1955)

2.2. Collagen structure

The bone mineral is discontinuous and inhomogeneous in structure. The space between crystals of hydroxyapatite is in large part occupied by carbonate and citrate ions which are too large to be admitted to the crystals of

hydroxyapatite. These inorganic components are deposed on the fibers of the organic matrix, made up of a protein known as collagen. The remaining space between the crystals of hydroxyapatite is filled with semiliquid substance which transports materials to the bone mineral from the circulating blood and *vice versa.*

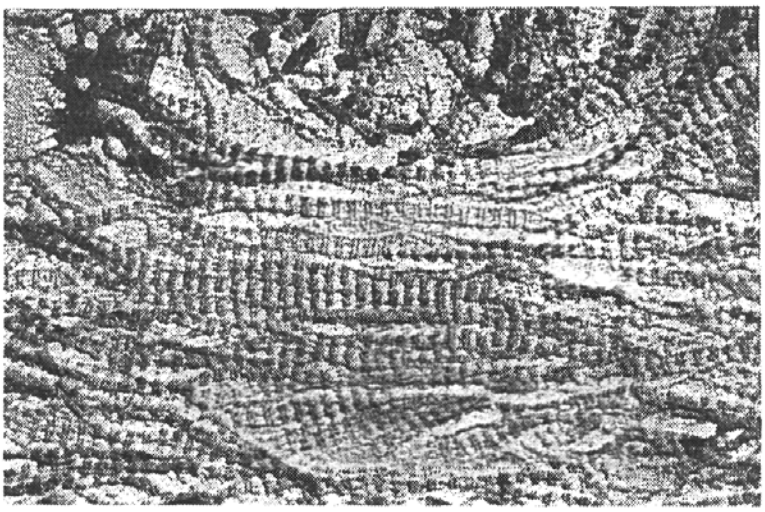

Fig. 3. Collagen fibres in a human leg bone shown by electron micrograph. The fibres have been made visible by removing calcium from the bone, after Mc Lean (1955)

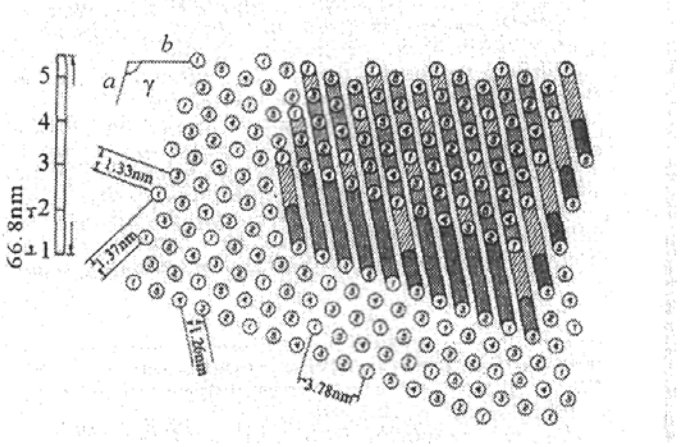

Fig. 4. Possible structure in one region of a collagen fibril, shown in projection down the fibril axis. Numbers refer to molecular segments (see upper left) in one 66.8 nm thick transverse section. The axes of molecules lie in the 1.26 nm separated Bragg planes, after Holmes (1979)

The analysis of X-ray data leads to a model for the crystalline regions of the fibril based on quasi-hexagonal molecular packing having the character of a molecular crystal, see Fig. 4.

X-ray diffractometry was also used to measure strain in the collagen molecule along the molecular axis as a response to the applied force. The experiment was performed on bovine Achilles tendon. The value of Young's modulus was found to be (2.9 ± 0.1) GPa. The measured sample was kept in 0.15 M NaCl solution, cf. Sasaki and Odajima (1996).

2.3. Bone structure

The bone tissue consists of a solid bone matrix and a fluid phase which contains blood and the extracellular fluid. As we have seen, the solid phase is composed of a crystalline mineral phase (calcium phosphate), an amorphous mineral phase, collagen fibers (which are made of sequences of amino acids) and a ground substance (mostly mucopolysaccharides).

Thus, from the mechanical point of view, the bone is a composite consisting primarily of three phases: collagen (which is piezoelectric), extracellular minerals and pores. In a compact bone collagen occupies about 40-50% of the volume, the mineral 40% and the pores – the rest, cf. Cowin (2001), Katz (1971), Piekarski (1973).

Two different types of normal compact bone tissue are called lamellar and osteonal. The greatest volume of the compact bone is osteonal. Osteons are typically about 200 μm in diameter and about 1-2 cm long. The main bone cells, osteocytes, occupy small cavities in the bone matrix which are called lacunae. The canaliculi which radiate from each lacuna, serve as a site for cytoplasmic activities, and they provide canal systems in the bone tissue which carry nutrients to the bone cells. The Volkmann canals, haversian canals and the canaliculi give a permeable structure to the bone matrix. The Volkmann canals are oriented radially and connect the marrow cavity to the haversian canals, cf. Fig. 5, Fig. 6 and Fig. 7.

The osteons and the interstitial lamellae provide the main structure of the bone matrix. The geometry of an osteon, which has a lamellar structure, is approximately a hollow cylinder, and is usually oriented parallel to the long axis of the bone shaft. Each lamella of the osteon consists of collagen fibers, calcium phosphate and osteocytes, and the central hollow part of the osteon (haversian canal) contains blood vessels and capillaries.

The bone tissue is laid down by the osteoblast cells. They secrete an amorphous material which in short time becomes fibrous; this is called osteoid. Osteoids provide space for calcium phosphate to crystallize. An osteoblast is surrounded by its own secretion, and as its surrounding hardens, it becomes an osteocyte. The other type of cell, an osteoclast, is responsible for the resorption of the bone tissue in remodelling.

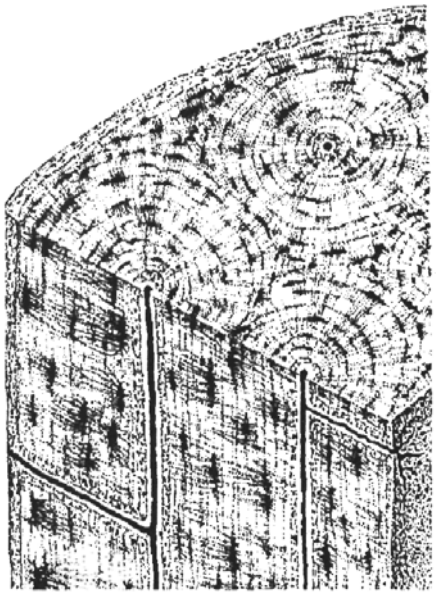

Fig. 5. Diagram illustrating the structure of the Haversian structure of a compact bone. Magnified 180 times, after Encyclopaedia Britanica (1959)

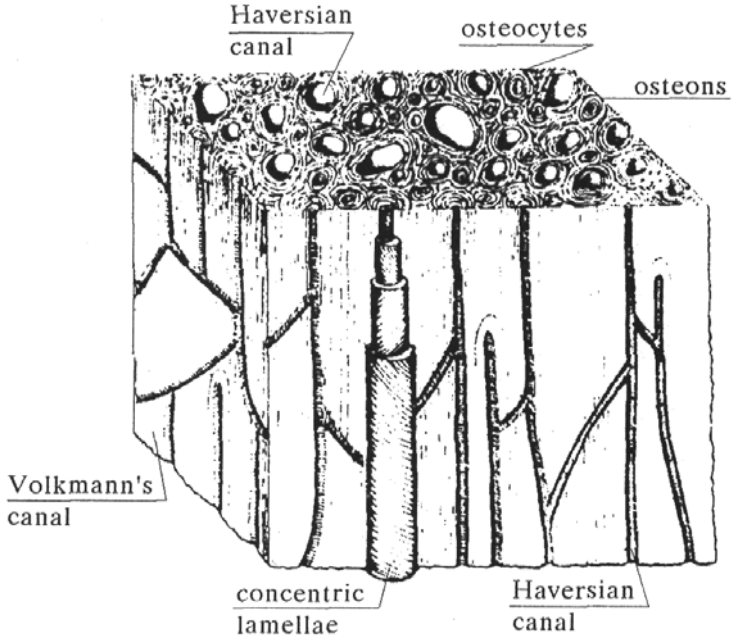

Fig. 6. Basic structure of an adult human's compact bone. The vertical axis corresponds to the long axis of the bone, after Güzelsu and Saha (1984)

Fig. 7. Ultrastructure of the compact bone. The single arrow shows an osteon and the double arrow shows a *lacuna* containing osteocyte, after Güzelsu and Saha (1984)

Bones adjust their densities and orientations according to their functions. Wolff's law states: Bone trabeculi are oriented so as to best resist the extrinsic forces which they must support, Wolff (1892). Wolff's law is summarized as a feedback mechanism, cf. Bassett (1971). However, the phenomenon of the bone remodelling is much more complicated than it was primarily believed.

At the macroscopic level there are two major forms of bone tissues called compact (or cortical) bone and spongy (or cancellous or trabecular) bone, see Fig. 8. It is the spongy bone that is filled with the marrow.

At the microstructural level both spongy and compact bones have lamellar organization. In compact bones the lamellae may be arranged in parallel fashion or in quasi-cylindrical structures of osteons (haversian systems).

The growth of a bone involves not only continuous accretion on the surface and at the ends, but also a continuous process of remodelling of the internal structure.

The remodelling, by deposition and resorption of the bone tissue is concerned with the bone tissue reactions to external effects. The hypothesis of conversion of information from functional forces to biological signals in the bone due to its electromechanical properties has been studied exhaustively after the discovery of piezoelectric properties of the bone, see the papers by Fukada and Yasuda (1957), Fukada (1968); cf. also Bassett (1971), Frost (1973), Brighton et al. (1979), Güzelsu and Saha (1984).

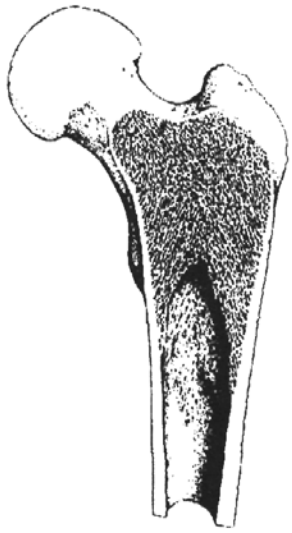

Fig. 8. Drawing of a longitudinal section through the neck and shaft of a human femur showing the structure of a spongy and compact bone, after Encyclopaedia Britanica (1959)

Measurements of the electrical conductance of living cells or tissues have been widely used to determine the parameters of the cells. Cole et al. (1969) made analogue measurements and found that the measured volume concentration agree with the corresponding Rayleigh and Maxwell formulae for the electrical resistance of suspensions of cylinders and spheres, within experimental accuracy of about 1% and within the concentrations ranges from 30% to 90% or more. An evidence for the influence of an electric field on the bone formation was given by Yasuda et al. (1955).

In order to provide means of standardization and interpretation of the electromechanical properties of bone, Gundjian and Chen (1974) proposed to transform measured values from a given sample to a reference standard sample with a well-defined idealized crystalline structure.

Various models of functional adaptation of tissues, including bones have been critically reviewed by Lekszycki and Telega (2002).

2.4. Amphoteric behaviour of collagen molecules

A collagen molecule consists of sequences of amino acids. Each amino acid is connected to another by a peptide bond. Most amino acids have side chains (R). The (R) groups may contain a negatively charged carboxylic group (COO^- - H^+) or positively charged amino group (NH_2+H^+). The side chains of a collagen molecule behave similar to a simple amino acid. The cathodic migration (towards the cathode) and anodic migration are observed, while in

neighbourhood of the isoelectric point no migration is observed, cf. Pethig (1979), Güzelsu and Saha (1984).

From behaviour of the amino acid substance between charged electrodes we infer that it can function both as an acid and as a base; thus it is an amphoteric substance.

Collagen molecules are organized into the tropocollagen macromolecules, cf. Herring (1971). The macromolecules consist of three α-polypeptide chains, each chain being twisted into a left-hand helix, and three chains being wound around a common axis in the form of right-handed superhelix. It forms a long thin rod of about 2800 Å in length and 14 Å in diameter. The tropocollagen molecules create heterogeneous centers for crystallization of the bone minerals, cf. Kummer (1972).

X-ray photographs of natural (biological) fibres show that the fibre substance is in most cases certainly crystalline, though often very imperfectly. It was found that X-ray photograph of the stretched hair is quite analogous to that of silk, and could be explained as arising from the fully extended polypeptide chians. Similar results apply to natural silk, and to collagen, muscle, etc., which also give rise each to a characteristic diffraction pattern. This observation due to Astbury (1933), was the point of departure for searching of piezoelectric properties of collagen.

At present, diffraction of X-rays can provide the following information on the fibrous protein collagen: its molecular structure, the axial arrangement of rod-like collagen molecules in a fibril, lateral arrangement of molecules within the fibril, and orientation of fibrils within a biological structure, cf. Wilkinson and Hukins (1999).

3. Piezoelectric effect

Some crystals, called piezoelectric, have the property of developing an electric dipole moment $\boldsymbol{P}$ under an applied stress $\boldsymbol{\sigma}$ even in the absence of an electric field $\boldsymbol{E}$. The most known examples of such crystals are quartz, Rochelle salt and some ferroelectrics. The polarization $\boldsymbol{P}$ is a polar vector and goes to $-\boldsymbol{P}$ under inversion. However, the mechanical stress is invariant under the operation of inversion so that the linear relationship between $\boldsymbol{P}$ and $\boldsymbol{\sigma}$ implies that $\boldsymbol{P}$ must identically vanish for crystals having a centre of symmetry. Thus we infer that crystals with inversion symmetry cannot be piezoelectric. This excludes 12 point groups and leaves only 20 point groups as the possible point groups of piezoelectric crystals.

A complete list of the nonvanishing piezoelectric strain coefficients for all the 20 allowed point groups can be found in the book by Bhagavantam (1996, p. 161).

The direct and inverse piezoelectric effects can be described on the phenomenological level using the Einstein sum convention by the relations

$$D_i = d_{imn}\sigma_{mn} + \varepsilon_{ik}E_k$$

$$e_{ij} = s_{ijmn}\sigma_{mn} + d_{kij}E_k$$

In the first equation the piezoelectric tensor d_{imn} and dielectric tensor ε_{ik} join the electric field E_i and the stress σ_{ij} to the dielectric displacement D_i, while in the second – the elastic compliance s_{ijmn} and piezoelectric tensor join them to the strain e_{ij}. We recall that $D_i \equiv E_i + P_i$. We recall also that the two systems of representation of the piezoelectric tensor are commonly used

index notation	d_{111}	d_{222}	d_{333}	d_{123}	d_{113}	d_{112}
	d_{223}	d_{213}	d_{212}	d_{323}	d_{313}	d_{312}
Voigt notation	d_{11}	d_{22}	d_{33}	d_{14}	d_{15}	d_{16}
	d_{24}	d_{25}	d_{26}	d_{34}	d_{35}	d_{36}

Apparently, Martin (1941) was the first who pointed out that suggested by Astbury (1933) and the commonly accepted picture of the peptide chain certainly leads to piezoelectricity (unless the chains are arranged alternately in opposite directions).

After the piezoelectric effect was observed in wool and hair (Martin, 1941), in wood (Shubnikov, 1946; Bazhenov and Konstantinova, 1950), cf. also Bazhenov (1961), Fukada (1968b,c), it was next discovered in a dry bone by Fukada and Yasuda in 1957. According to Demiray and Güzelsu (1977) the piezoelectricity in a dry bone was already discovered in 1953 by Yasuda, while according to Güzelsu and Demiray (1979) the effect was discovered by Yasuda in 1954.

The piezoelectric effect in wood materials is due to the crystalline properties of cellulose micelle which are highly oriented in the direction of the fibre axis.

The bone, on its part is (as we have mentioned above) mainly composed of highly oriented collagen fibres. The most part of the bone (except both ends) looks like a hollow cylinder. The axis of the bone is designated as the direction of length of the cylinder. The collagen fibres make the spiral structure along the axis. The direction of winding of the spiral is reversed to right and left by

each successive layer which compose the outer cylinder. Thus, the properties of the bone are assumed to be symmetrical with respect to this axis.

The collagen fibril is an excellent example of *pyroelectric structure* in biology. On account of this molecular order the pyroelectric behaviour is to be found in collagen structures, cf. Lang (1966) and Athenstaedt (1970). Therefore, the bones of the skeleton, which are mostly subjected to mechanical forces, are made of a tissue exhibiting pyroelectric behaviour, thus suggesting that the remodelling and regeneration of the bone are due to the interaction of both the mechanical and electrical forces. Under constant temperature a pyroelectric material does exhibit a piezoelectric property, see Cady (1964), Güzelsu (1978).

Recent analysis performed by Warner and Terentev (1999) proves that chiral chains (polymer chains made of chiral monomers) posses piezoelectric properties. Applying Rayleigh's method of random flights those authors show that the induced (piezoelectric) polarization of chiral nematic elastomers is given by

$$P_i = \frac{1}{2} n_s d\Big(\frac{b}{a}\Big)\epsilon_{ijk}\Big(\lambda_{ij}\ell^{(0)}_{ln}\lambda^{\top}_{nm}\ell^{-1}_{mk}\Big)$$

where n_s denotes the number of chains per unit volume, a and b are projected lengths of the monomer along its long axis $\boldsymbol{u}$ and the perpendicular axis $\boldsymbol{v}$, respectively, d is dipole component pointing in the $\boldsymbol{u} \times \boldsymbol{v}$ direction, $\boldsymbol{\ell}$ is the persistence tensor (being an effective tensor for bent monomers), while λ_{ij} is the deformation tensor.

4. Discovery of the piezoelectric effect in bones

The experiment of Fukada and Yasuda (1957) was the following. Small square plates of dry bovine and human bones, shown in Fig. 9, were cut from the outer layer of the femur. The length of the edge of a square specimen was 15 to 90 mm, the thickness was 2 to 3 mm. Very thin silver foils, used as electrodes were attached to the square planes by means of an alcoholic solution of shellac.

The measurements were performed on a dried specimen using the apparatus shown in Fig. 10. The pressure was applied to the side face of the specimen by a lever mechanism and the electrical charge appeared on the square face was led to the grid of a vacuume tube and the deflection of a galvanometr inserted in the plate circuit was measured. The direct and converse piezoelectric effects are shown in Fig. 11 and Fig. 12.

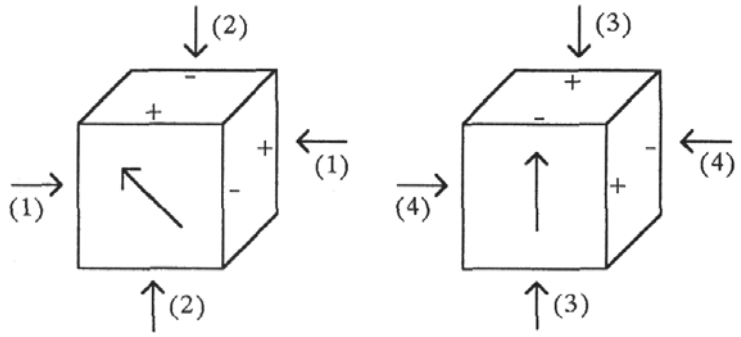

Fig. 9. Bone specimens. Signs represent those of electric polarizations which appear in square planes when the pressure is applied to each side plane, after Fukada and Yasuda (1957)

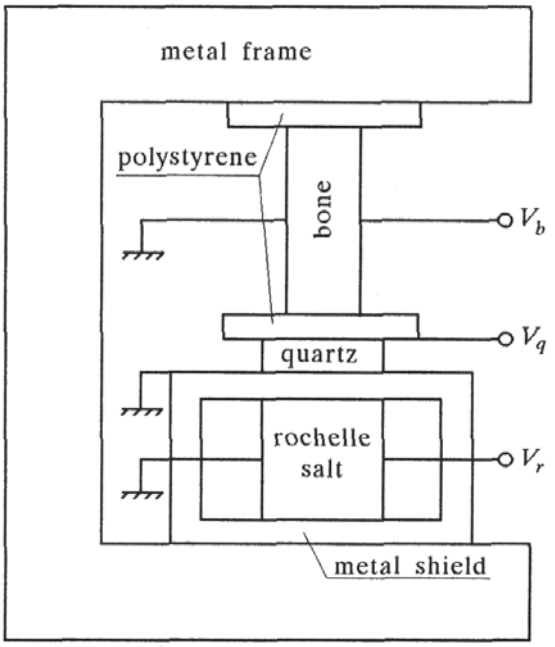

Fig. 10. Schematic diagram of the measuring device, after Fukada and Yasuda (1957)

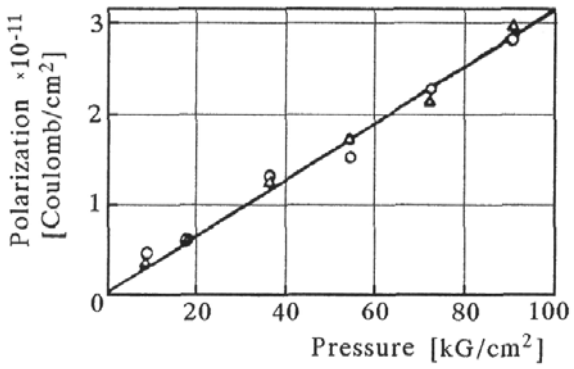

Fig. 11. Direct piezoelectric effect of the bone, after Fukada and Yasuda (1957)

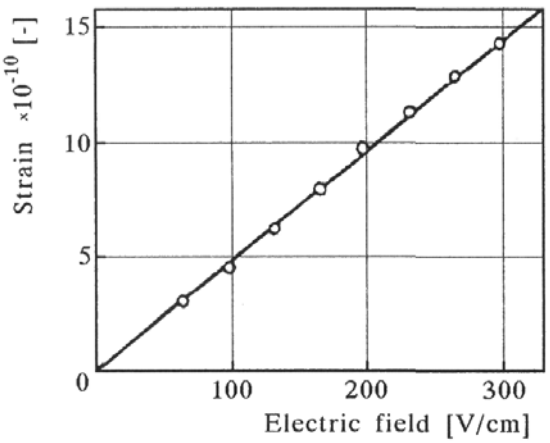

Fig. 12. Converse piezoelectric effect of the bone, after Fukada and Yasuda (1957)

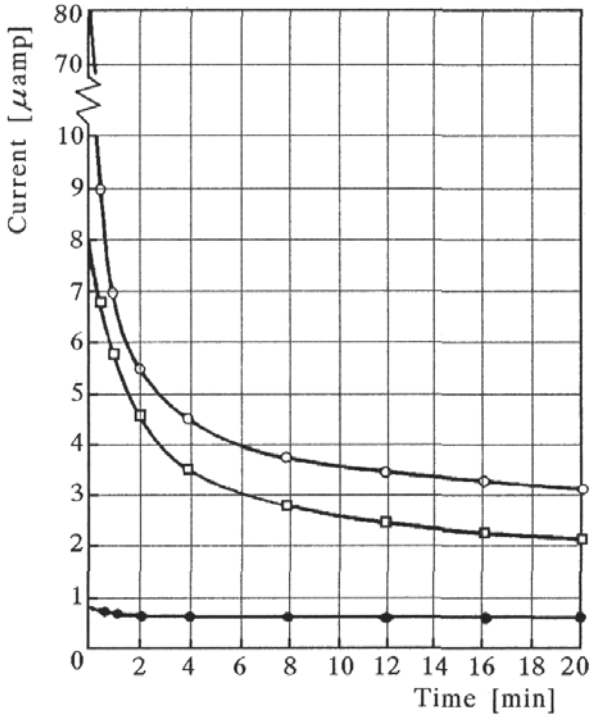

Fig. 13. Graph of current flowing in a living bone after various intervals. Initial values were 0.8, 8.0 and 80 μamperes. White circles ○ correspond to 100 μA pack, white squares □ to 10 μA pack, black circles • to 1 μA pack, after Basset et al. (1964)

As a result of this first experiment two elements of the matrix (in the Voigt notation) of the piezoelectric coefficient were found

$$\begin{bmatrix} 0 & 0 & 0 & d_{14} & 0 & 0 \\ 0 & 0 & 0 & 0 & -d_{14} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \tag{4.1}$$

This matrix is the same as that of wood and ramie bundle. The Z-axis assigned to this matrix is inclined by about 10 degrees with respect to the long axis of femur.

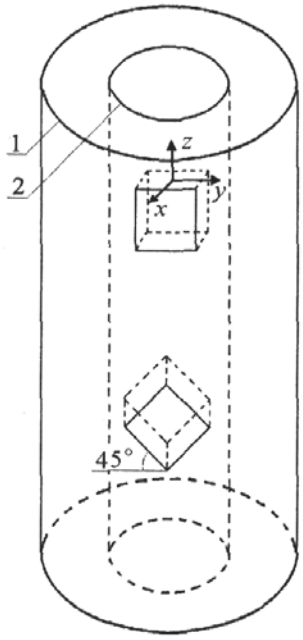

Fig. 14. Location of the bone specimen with respect to femur: 1 – periosteal surface, 2 – endosteal surface, after Reinish and Nowick (1975)

A small difference was observed between boiled and unboiled specimens, which (according to authors) means that the effect is not entirely of biological origin (and this interpretation was false!). However, Yasuda observed that the piezoelectric effect still remains after immersion of bone specimen in acid for 3 weeks; then the apatite crystals lying between the collagen fibres have completely dissolved. The values reported for d_{14} were compared with d_{11} of quartz: the highest value of the piezoelectric coefficient of the bovine bone is about one-tenth of the piezoelectric coefficient d_{11} in quartz.

The matrix of a piezoelectric crystal possesing hexagonal symmetry D_6 is represented by the above formula, and it is in agreement with the results obtained by Ramachandran and Kartha (1954) who proposed the hexagonal symmetry in the unit cell of collagen.

These investigations were further developed by Shamos et al. (1963 and 1964) who observed the same stress-induced electrical effect in a number of bones from different anatomical sites and species. The bones were cleaned and dried before an experiment. These authors claim that the local electric fields resulting from the surface charges (which are generated in the stressed bone *via* piezoelectric phenomenon) influence the deposition of ions and polarizable molecules.

Dainora (1964) proved that electrical signals can be obtained from the bone and demineralized bone. This author investigated small samples of the human femur.

Later investigations performed by Fukada and Yasuda (1964), Fukada (1968) and Yoon and Katz (1976) established point group 6 as the piezoelectric class for the bovine bone. These authors gave the values of piezoelectric coefficients. Compared with the α-quartz, whose permittivities are about the same as those of bone, the piezoelectric coefficients of the bone are approximately 10^{-2} times of those in α-quartz.

After investigations by Fukada and Yasuda (1957), Bassett and Becker (1962), Shamos et al. (1963), and Fukada and Yasuda (1959, 1964), the piezoelectric matrix for the bone and tendon was completed as follows

$$\begin{bmatrix} 0 & 0 & 0 & d_{14} & d_{15} & 0 \\ 0 & 0 & 0 & d_{15} & -d_{14} & 0 \\ d_{31} & d_{31} & d_{33} & 0 & 0 & 0 \end{bmatrix} \tag{4.2}$$

Consequently, the symmetry of the crystal lattice of collagen molecules coincides with the hexagonal class C_6, cf. Fukada (1968c). This author provided also a table of numerical values of the coefficients.

Fukada and Hara (1969) observed the piezoelectric effect in various kinds of tissues such as trachea, intestines, ligament, aorta and vena. Thus, the universal existence of the piezoelectric effect in biological tissues has been demonstrated. The anisotropy of the piezoelectric modulus may be regarded as an indicator of preferred orientation of piezoelectric protein fibers including collagen and elastin.

In samples with dimensions of a few mm, the compact bone is approximately isotropic in a transverse direction and has an infinite-fold rotational axis approximately parallel to the bone axis. The pyroelectric coefficient at a constant stress p_i^σ can be expressed by the pyroelectric coefficient measured at the constant strain p_i^ε and a contribution from the thermal expansion in the following form

$$p_i^\sigma = p_i^\varepsilon + d_{ijk} C_{jkmn} \alpha_{mn} \tag{4.3}$$

where d_{ijk} are the piezoelectric coefficients, C_{jkmn} are the elastic stiffnesses, and α_{mn} are the thermal expansion coefficients, cf. Lang (1969).

Liboff et al. (1973) supported the view that matrix (2.2) characterizes piezoelectric properties of bone. These authors indicated that the bone should produce electrical response to hydrostatic pressure and be pyroelectric, thus conforming the earlier works by Lang (1966, 1969).

Pfeiffer (1977) described experiments on the frequency response of the piezoelectric coefficients of the mineralized collagen fibril, including the ground substance of an adult human's cortical bone.

Ashero et al. (1999) pointed out that the piezoelectric coefficients cannot be assumed as an inherent property of the bone, constant for various animals; for instance values of the coefficient d_{23} differed by factor 2 on samples of five two-years old cows bred in the same conditions.

5. Electrical behaviour of the dry bone in bending

Williams and Breger (1974, 1975) confirmed that electrical signals produced by samples of the dried bone and tendon bent in the cantilever mode are direct current square waves in response to a step loading. The signals are similar to those produced by piezoelectric mineral crystals, but with an important difference: the sign of the voltage reverses on end-for-end rotation of the mineral samples but does not change for the bone and tendon. When the linear theory of piezoelectricity is extended by introducing the term f_{2332} relating the polarization to the gradient of the stress *via* a tensor of rank four, an agreement with experiment is achieved.

The challenging interpretation was undertaken by Johnson et al. (1980b), who showed that the anomalous piezoelectric properties of the dry bone and tendon can be viewed as a consequence of variation of the piezoelectric modulus along the stress gradient. Such behaviour of the dry bone can be duplicated by piezoelectric ceramic models if they are fabricated to exhibit the appropriate spatial variations.

6. Electromechanical behaviour of the wet bone

Bone is a vascular, constantly remodelling connective tissue which consists of a solid bone matrix but also of a fluid phase which contains blood and extracellular fluid.

The dielectric properties of biological materials have long been studied at radio and microwave frequencies, starting with the work of Cook (1941), including extensive studies by Schwan (1957) and his associates, cf. also Stuchly and Stuchly (1980), Dawson et al. (2000).

Freeman (1967) studied electrical properties of mineralized tissues. More precisely, he examined capacitive and resistive responses to frequency, sample orientation and temperature variations.

Independently of Japanese researchers, Basset et al. (1964) seemed to indicate that there exist a relationship between electric currents and bone cell activities.

As we have already mentioned in Section 1.1, Cole et al. (1969) investigated a possibility of application of the Rayleigh and Maxwell formulae for electrical resistance of suspensions of cylinders and spheres to measure the volume concentration of nonconducting cells in several tissues.

The first observations of piezoelectricity in the wet and living bone were made by Basset and Becker (1962), cf. also Basset (1968). Their measurements were of qualitative nature and they contributed a working hypothesis that the piezoelectricity leads to the physical explanation of physiological Wolff's law.

6.1. Criticism of the first interpretation

Anderson and Eriksson (1968) established that, although dry collagen is strongly piezoelectric, fully hydrated tendon collagen is not piezoelectric because of the structured water it contains. The electrical signals generated when fully wet collagen was stressed were shown to be streaming potentials, cf. Fig. 15 and Fig. 16, and our second paper in this issue.

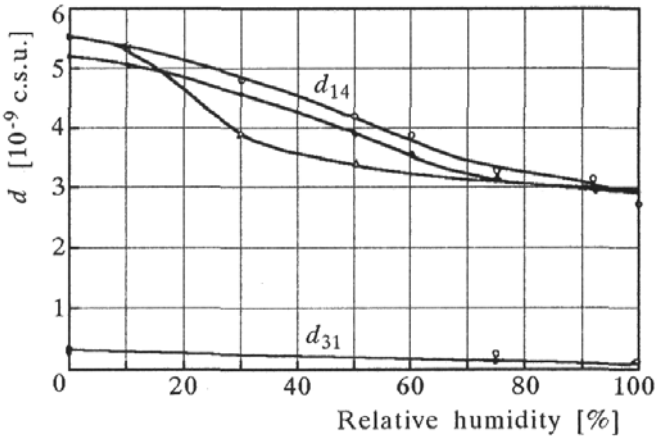

Fig. 15. Piezoelectric coefficients of the bone as functions of relative humidity measured at 5600 Hz:
d_{14}: converse effect, dry to wet – white circles ○; direct effect, dry to wet – black circles ●; converse effect, wet to dry – white triangles △;
d_{31}: converse effect, dry to wet – white circles ○; direct effect, dry to wet – black circles ●, after Anderson and Eriksson (1975)

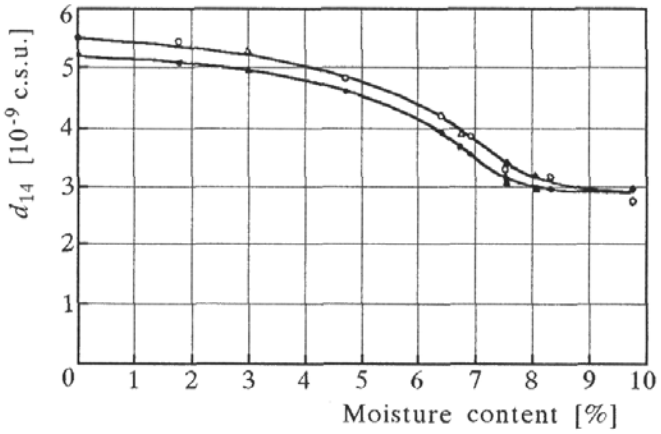

Fig. 16. Piezoelectric coefficient d_{14} of the bone as a function of moisture content: converse effect, dry to wet – white circles ○; direct effect, dry to wet – black circles ●; converse effect, wet to dry – white triangles △; direct effect, wet to dry – black triangles ▲, after Anderson and Eriksson (1975)

Anderson and Eriksson (1968) indicated drawbacks of the Fukada and Yasuda (1957) reasoning. The piezoelectric coefficients found by them correspond to the non-polar, polycrystalline point group $\infty : 2$ ($\infty/2$). For dry Achilles tendon Fukada and Yasuda (1964) found the coeffficients corresponding to the point group ∞. From seven possible point groups for polycrystalline materials, Shubnikov and Belov (1964) showed that only the groups ∞, $\infty : 2$ and $\infty \cdot m$ can exhibit piezoelectricity. These corespond to point groups 6, 6 mm and 622, and 4, 4 mm and 422, respectively, in matrix notation. Anderson and Eriksson found it intriguing that the bone should belong to a point group having a higher symmetry than that of its main constituent collagen, especially as its other constituent, hydroxyapatite, as centro-symmetrical is not piezoelectric. Accordingly, they decided to check the Fukada and Yasuda method. Unlike Fukada and Yasuda, the English researchers applied no heat in drying the specimen; silica gel was used instead. The silica gel is a form of amorphous silica SiO_2 obtained by the action of heat on silicic acid; it acts as a powerful dessicant due to its extreme porosity, the capillary pores accounting for over 50 per cent of its volume. Anderson and Eriksson (1968) found that the results could be explained purely in terms of those coefficients found for tendon collagen if one took into account the orientation of the collagen fibres relative to the long axis of the bone femur, which was used in the study. In a statistical approach, the collagen fibres exhibit the conical symmetry with respect to the long direction of the femur. Thus, both dry bone and dry collagen were allocated to the point group ∞.

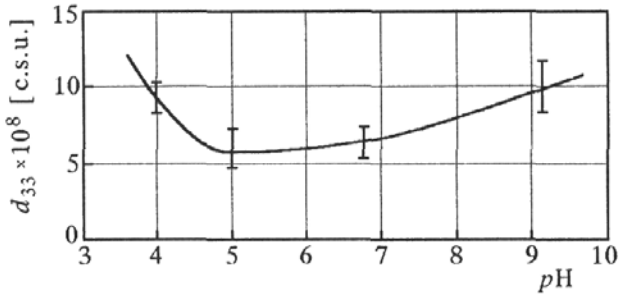

Fig. 17. Variation of the piezoelectric coefficient d_{33} with pH of the solution in which the specimen is saturated, after Reinish and Nowick (1975)

Fukada and Hara (1969) measured the temperature dependence of the piezoelectric modulus in the bone and they observed a decrease in the modulus with rise of temperature, in the range $(-150, 150)°$C. The decrease at around $-150°$C is attributed to the onset of some thermal motion of collagen molecules, and decrease at room temperature is assumed to be caused by the leakage current due to water.

Using a similar apparatus to that described by Fukada and Yasuda (1957), Anderson and Eriksson (1970) measured the piezoelectric properties of the dry bone and related them to the bone structure in terms of orientation of the bone collagen fibres relative to the bone long axis. Anderson and Eriksson (1970) observed also that the piezoelectric coefficients varied with the state of hydration of the bone and all decreased as the specimen was dried by evaporation of water in the apparatus. Because the 3-direction coincides with the long axis of the femur, and because the pores between fibres are also generally aligned in this direction, these authors concluded that the liquid held in the pores makes a large contribution to the apparent values of the coefficients. Such a contribution arises from streaming potentials which would vary with pH value of the solution. Figure 17 depicts the variation of d_{33} with pH; it passes through the minimum value at pH=5. This supports the view that the streaming potentials are important, but their contribution decreases to zero at the isoelectric pH-value.

Since up to one-third of the lengths of collagen fibrils are occupied by hydroxyapatite crystals, collagen cannot take up the full amount of water that pure tendon collagen is capable to absorbe. The loss of piezoelectricity in fully hydrated tendon collagen is associated with the attainment of a more symmetrical, non-piezoelectric structure by absorption of water.

In view of the confusion about the nature of the voltage developed when the wet bone is stressed, Reinish and Nowick (1975) performed an experiment

that, according to these authors, showed unambiguously that the wet bone behaves also as a piezoelectric material, cf. Fig. 14 and Fig. 17.

Their test apparatus was a modification of the apparatus used by Fukada and Yasuda (1957), however with a sinusoidally variable drive. These authors, although aware of the result of Fukada and Yasuda (1964) that the piezoelectricity of wet collagen decreases to zero, interpreed the results obtained that piezoelectricity at 100% humidity decreases only to *ca.* 50% of the value for dry bone. Consistent with these findings seemed to be the results of Marino and Becker (1975) who demonstrated that both bone and tendon are piezoelectric in a hydrated, though frozen, state.

Chakkalakal et al. (1980) determined dielectric properties of a fluid saturated bone (bovine femoral compact bone). The results obtained indicated that the dielectric behaviour of the fluid saturated bone *in vitro*, and, hence, also possibly *in vivo*, is determined mostly by the fluid-filled pores. This contradicts some of the commonly accepted views on the electromechanical effects in the bone. More precisely, these measurements imply that it would be difficult to view the piezoelectric effect in the wet bone at low frequencies in open circuit voltage measurements. This suggests that the electromechanical effect observed in the fluid saturated bone may not be the piezoelectric effect.

6.2. Feedback mechanism in the bone tissue

The feedback mechanism by which the bone tissue senses the change in load environment and initiates the deposition or resorption of the bone tissue is not understood. That is to say, the transduction of mechanical signals to chemical processes, the process underlying the functional adaptation of the bone tissue has not yet been established, see Cowin et al. (1991).

Dainora (1964), and Shamos and Lavine (1964) suggest that the origin of bioelectrical signals are either the piezoelectric effect or streaming potential or both.

As defined by Becker et al. (1964) $p - n$ junctions are functional units formed in the bone matrix between the collagen fibrils and apatite crystals; thus, semiconductor behaviour was attributed to the bone tissue, cf. Fig. 13.

Eriksson (1976) associates the feedback mechanism with the streaming potentials. This involves the ion containing fluid motion with respect to a solid which carries an immobilized ionic volume electrical charge. The bone matrix is piezoelectric, therefore it has the bound charges. Furthermore, it is amphoteric having the immobilized ionic volume charge which depends upon pH of the fluid phase.

Gjelsvik (1973) investigated the connection between piezoelectric properties of the bone tissue and remodeling of unloaded surfaces of bones. Applying the classical theory of piezoelectricity for determining the electrical polarizations in the bone tissue due to stress caused by external loads, he tried to explain the feedback mechanism of remodelling of the bone tissue. McElhaney (1967) indicated that the charge distribution on the bone surface is responsible for controlling the collagen fiber orientation and also for the bone remodeling. However, later experiments conducted on the wet bone samples by Anderson and Eriksson (1970) suggested that the streaming potentials at the interface may be another bio-electric signaling circuit for bone remodelling.

Ascenzi and Benvenuti (1977) showed the existence of the initial stress state in collageneous osteonic lamellae of cortical bone. The idea of Uklejewski (1993, 1994) was that because collagen is a piezopolymer, and the initial piezolectric polarization of collageneous osteonic lamellae must be associated with their initial stress state. In this manner, piezoelectricity is the initial whereas streaming potential – the direct generating mechanism. The weak side of this hypothesis is that the wet collagen tissue does not reveal any significant piezoelectric effect cf. Anderson and Eriksson (1968, 1970), Johnson et al. (1980a).

Hung et al. (1996a,b) performed experiments intended to explain whether there is a requirement for calcium entry from the extracellular space as well as calcium from intracellular stores to produce intracellular calcium response in cultured bone cells subjected to fluid flow. Understanding the calcium cell signaling is important for elucidation of the biophysical transduction mechanism mediating the conversion of the fluid flow to a cellular signal.

7. Difficulties with theoretical interpretation

Petrov (1975) introduced a mixture model for wet bones by taking into account electromagnetic interactions.

Additional evidence against the idea that the electromechanical effects in the wet bone are due to piezoelectric properties of collagen was brought by Johnson et al. (1980a). Especially, it was aimed against interpretations by Reinish and Nowick (1975), and Bur (1975, 1976) Date (1972), Zheludev (1974) and Güzelsu (1978).

Bur (1975, 1976) presented measurements of the dynamic piezoelectric coefficients as a function of temperature $20 - 60°C$, relative humidity (r.h.) $33 - 98\%$, frequency of applied stresses and sample orientation. For 98% r.h.,

the low frequency data are dominated by a polariaztion which varies as the reciprocal of the frequency; this polarization is attributed to the interfacial or Maxwell-Wagner polarization which occurs as a result of ionic conduction under the influence of the piezoelectric polarization field, cf. also Sillars (1937).

Johnson et al. (1980a) made observations on the electromechanical effect in the bone under cantilever bending subject to nonuniform stress. The results obtained for the dry and wet bone suggest that different mechanisms may be responsible for the signals in the wet and dry bone.

Comparison of the electromechanical effects in the wet and dry bone was performed by Johnson et al. (1980a), who treated the bone as a piezoelectric composite, following the earlier papers by Date (1972) and Zheludev (1974). Johnson et al. (1980a) presented an experimental evidence that the magnitude of signals observed in a bent wet bone is several orders larger than one would expect from the piezoelectric effect. This result, along with other experimental observations, implies that the electromechanical effect observed in the wet bone is not the piezoelectric effect. These authors indicated that there is a difference between electrical properties of the bone at 98% relative humidity and those of the wet bone, see Reinish and Nowick (1975), and Bur (1975, 1976). Bur (1975, 1976) also indicated the possible source of the errors. He claimed again that the electromechanical effect is due to piezoelectricity.

Now, we are going to describe this model following Güzelsu and Saha (1984), cf. also Anderson and Eriksson (1968), Maroudas (1968), Bourne (1971), Pethig (1979). The following points are essential in the formulation of the Güzelsu and Saha (1984) mixture model for wet bone:

- The bone matrix, which is made of collagen, hydroxyapatite crystals and ground substances (mostly mucupolysaccharides) has an immobilized electrical charge. This is because collagen molecules which are made of sequences of amino acids, are amphoteric (see Section 1.2) and are capable to act as acids as well as bases, depending upon the pH of the fluid phase.
- Bone salts (Ca and PO_4 ions in the fluid phase) which are the main constituents in the remodelling process of the bone tissue, can be deposited on the bone matrix or released by the bone matrix into the extracellular fluid by chemical reactions leading to the mass transfer.
- Hydrogen ions define the pH level of the fluid, and hence the net immobilized ionic volume charge of the collagen molecules. The streaming potentials in the bone tissue are generated by the net immobilized volume charges. Mechanical deformation of the bone tissue force ions carrying the fluid phase to move with respect to the immobilized charge.

- Electrolytes of the plasma and the interstial fluid, mainly Na, Cl, HCO_3 are responsible for the electrical conduction.

Among different phenomemena admitted by Güzelsu and Saha (1984) in the processes occurring in the wet bone, the piezoelectricity was also included. The theory is not clear in some points; for instance, there is no evident reason for the assumption on positivity of local entropy production. The entropy inequality principle applies only to the whole system: living body + its environment in adiabatic isolation.

In earlier publications by Williams et al. (1975) piezoelectric properties of the dry bone and tendon subjected to cantilever bending were shown to be anomalous in comparison with the expected behaviour. The piezoelectric moduli d_{ij} evaluated in the uniaxial compression by Fukada and Yasuda (1957), Liboff and Shamos (1971) combined with an expression derived by Williams and Breger (1974, 1975) for the voltage expected across a homogeneous piezoelectric beam under cantilever bending did not describe the response of biological tissues, neither quantitatively nor qualitatively.

Johnson et al. (1980b) proposed a model the central idea of which is that a nouniformly polarized solid will contain a bound charge, which can be elastically displaced by applied non-uniform stresses, leading to a change in the potential. Qualitative predications of the adopted mathematical models were confirmed by experiments.

Weinbaum et al. (1994) have advanced a new experimentally testable hypothesis for the mechanosensory transduction system, by which osteocytes sense very small *in vivo* strains in the calcified matrix components of the bone. These authors presumed that the osteocytes, also not responsive to substantial fluid pressures, can be stimulated by small fluid shear stresses acting on the membranes of their osteocytic processes. Biot's theory of porous media was used to relate the combined axial and bending loads applied to the whole bone to the flow past the osteocytic processes in their canaliculi, cf. also Tsay and Weinbaum (1991).

8. Bone tissue as a porous piezoelectric material

The trials to built a theory which compromises both hypotheses start with Avdeev and Regirer (1979) who proposed a model of the wet bone as a porous piezoelectric material, saturated with a viscous electrolyte fluid. The mass exchange between the solid and fluid phase was also admitted.

Alternative considerations were performed by us using the homogenisation method (1996, 1997, 1998), cf. also Bielski and Telega (1997). In these papers both the piezoelectric effect and the streaming potentials were allowed to occur. The fluid was treated as an electrolyte.

Zhang et al. (1998) applying the results due to Biot (1955), Rice and Cleary (1976), and Rudnicki (1985) calculated the bone pore water pressure due to mechanical loading, without the assumption of fluid incompressibility.

9. Final comments

The results of experiments on wet bones demonstrate that in contrast to dry bones the piezoelectric effect in wet bones is insignificant and irrelevant, cf. Anderson and Eriksson (1968), Salzstein and Pollack (1987), Johnson and Katz (1987), Scott and Korostoff (1990). In spite of the fact that the earlier hopes linking the piezoelectric effects with the bone remodelling failed, the significant scientific efforts were not wasted, since deeper understanding of some basic physical and physiological properties was achieved. Also, it became clear that the remodelling of tissues is a combination of mechanical and biological factors, cf. Lekszycki and Telega(2002).

Despite these seemingly convincing arguments an objection was raised. Marino et al. (1988) admit that the streaming potentials are the physical basis of the electrical signals observed in the bone, tendon and cartilage subjected to mechanical forces under physiological conditions. However, the piezoelectric signal is not usually measured under such conditions because the induced polarization is rapidly neutralized by ions in the bulk fluid. To determine whether the piezoelectric polarization could alter the bone-cell physiological function a study was undertaken by Marino et al. (1988). Piezoelectric and non-piezoelectric forms of the polymer polyvinylidene fluoride (PVDF) were implanted to rats, and the histological effect on the periosteum and bone was studied. More bone formation and periosteal reaction occurred in association with the piezoelectric form of PVDF. The effects were significant at 1-6 and 1-2 weeks postoperatively for the bone and periosteum, respectively. Neither mechanical nor chemical factors could account for the results, which therefore must have been recognized to be due to the quasi-static piezoelectric polarization (about $90\,\mathrm{pC/cm^2}$). The osteoprogenitor cells, being the immediate precursors of osteoblasts were regarded to be capable of responding to the polarization.

The last paper was an expression of new applications of the piezoelectric effect in biomechanics: as sensors and manipulators.

Goes et al. (1999) studied the piezoelectric and dielectric properties of collagen films, considering the development of new biomaterials which have potential applications to coating of cardioviscular prostheses, support of cellular growth to systems for controlled drug delivery. The resonance technique was used to measure the piezoelectric coefficient d_{14}, the elastic constant s_{55}, and the dielectric permittivity ε_{11}.

Kietis et al. (1998) studied saturation of proton e.m.f. in bacteriorhodopsin using experimental data on photoelectric response to simultaneous pulsed and constant illumination. On the basis of piezoelectric activity of strained conformation of the molecule, two related cycles in the transformation process of the electronic excitation energy was considered. Theoretical results derived from the Langmuir relationship and principles of the piezoelectric mechanism were compared with the experimental data.

The study on biomimetic growth of fluorapatite in gelatin matrices at ambient temperature was performed by Busch et al. (1999). The study starts with elongated hexagonal-prismatic seeds followed by self-similar branching (fractal growth) and ends up with anisotropic spherical aggregates. The chemical system fluorapatite/gelatin is closely related to *in vivo* conditions for bone or tooth formation and is well suited to a detailed investigation of the formation of an inorganic solid with complex morphology (morphogenesis). The fractal stage of the morphogenesis leads to the formation of closed spheres with diameters of up to 150 μm. The self-assembled hierarchical growth thereby shows immediate parallels to the topological branching criteria of the macromolecular starburst dendrimers. A second growth stage around the closed spheres of the first stage is characterized by the formation of concentric shells consisting of elongated prismatic fluorapatite units with nearly parallel orientation (maximum diameter of the complete core/shell spheres of 1 mm). The specific structure of the core/shell assembly is similar to the dentin/enamel structure in teeth. Together with the idea of the biological significance of electric fields (pyro-, piezo-electricity) during apatite formation under *in vivo* or biomimetic conditions the composite character of the material and the mechanisms of fractal growth (branching criteria and architecture, the influence of intrinsic electric fields etc.) are considered.

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Zjawisko piezoelektryczne w biologii tkanek

Streszczenie

Analizujemy różne prace i różne punkty widzenia dotyczące zjawiska piezoelektrycznego w kości i jego znaczenia dla biologii tej tkanki. Zjawisko to obserwowane wyraźnie w kości suchej, odgrywa według ostatnich badań mniejszą niż sądzono początkowo rolę w procesach adaptacyjnych kości żywej. Tym niemniej własności piezoelektryczne są istotne dla różnych procesów biologicznych.

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