CHAPTER I

INTRODUCTION

I.1 Physiology of Bone

Observations on various physical properties of bone show that there may be a probable relationship between biophysical properties and physiological processes involved in bone-growth and remodelling (Cochran et al., 1968; Abendschein and Hyatt, 1970; 1972; Gjelsvik, 1973). From the standpoint of material study the current investigation of bone which consists of collagen fibres and hydroxyapatite crystal is expected to offer an explanation for mineralization along with suggestions to design new composite materials. The attempts to artificially control the growth pattern of bone by applying mechanical stress or electric field provide another reason for basic studies on its structure and properties.

Bone is biphasic composite material. The extracellular components of bone consist of inorganic crystals of calcium phosphate disposed within the collagen fibril of the organic matrix (Glimcher and Krane, 1968). The mineral phase constitutes approximately 65 per cent and the organic matrix 35 per cent of the tissue by weight (Eastoe and Eastoe, 1954; Eastoe, 1968). Interest in the organic matrix of bone stems partially from the awareness of its role as a structural component of the
tissue. The organic matrix consists of mainly collagen in addition to two types of carbohydrates, e.g. gluco-protein and mucopolysaccharides (Meyer et al. 1956; Herring, 1968; William and Peacocke, 1965) and a small amount of lipids (Irving and Wuthier, 1968). The exact anatomical location of these components at molecular level is not certain, and their state of aggregation and polymerization is not well known (Glimcher, 1959).

Collagen molecules perform in the soft connective tissues of molecules somewhat the same function as cellulose molecules perform in plants. The collagen molecule which has length of 3000 Å, diameter of 14 Å and molecular weight of 300,000 daltons, possesses a triple helix structure. A full consideration of intra-and inter-molecular forces which promote the stability of collagen has been made by Cooper and Russell (1969). The main functional groups on the collagen molecules which can take part in interaction are: (i) polar chains, (ii) the neutral sides, and (iii) the hydroxyamino acid side chains. Interaction with small ions would be expected to have effects on the properties of the collagen molecules. Water molecules are thought to have a function in maintaining the stability of collagen structures besides the obvious solvation effects (Fraser and Macrae, 1959). The inorganic part of bone consists mostly of hydroxyapatite crystalline material and possibly an amorphous calcium phosphate salt, similar to tricalcium
phosphate. It was shown by X-ray diffraction that the structure of bone mineral resembled the geological apatite (Posner et al. 1958). Apatite forms a class of compound which contains: (i) divalent cation; (ii) tetravalent trivalent anions; and (iii) monovalent anions. Bone salt in particular contains a calcium cation and orthophosphate and hydroxyl anions. The basic formula is represented as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and it crystallises in the centrosymmetric hexagonal class $\text{P6}_3\overline{m}$ (Eanes and Posner, 1970). These crystals are extremely small, possessing a typical dimensions as $80 \times 150 \times 500 \, \AA$ (Glimcher, 1959). The structure of the crystal is such that a large percentage of the atoms are found in surface or near-surface positions and this results in rapid exchange of ions between the crystals and the interstitial fluids (Glimcher, 1959).

The inorganic crystals and organic matrix form an integrated unit and the relationship between the two is important to understand the bone structure and function. 'In vitro', studies have shown that collagen from various sources can act as nucleators for apatite crystallization (Glimcher, 1959; 1960; Katz, 1969). However, the exact mechanisms underlying the mineralization has been a subject of dispute. The dominance of calcium over phosphate has been indicated in providing nucleation sites during the mineralization process (Höhling et al. 1968). Similarly, the study of the binding of
metal ions to collagen have suggested that coordination-complex formation may be involved in the calcification of bone matrix (Spadaro et al. 1970). Electron microscopic study has shown that there is often a close relationship between the collagen structure and the form and orientation of the apatite crystals (Glimcher, 1959). In conclusion, both collagen and mineral part along-with polysaccharides are embedded in such an organised pattern in bone that from both microscopic and macroscopic stand point, it sufficiently adopts and responds to all mechanical and chemical changes such as its biological function becomes superb and excellent. The response of bone structure to internally or externally applied stress and perturbations are mediated through specialized cells. Two types of cells are observed during active stage of bone formation and destruction and can, therefore, be termed as 'transient' elements of bone tissues as distinct from its permanent elements. The cells which are active in 'osteogenesis' are known as 'osteoblast' while those causing osteoporosis are known as 'osteoclast'. The osteoblast together with osteoclast provide the tissues with a large measure of plasticity through remodelling processes. These processes adopt to the structural elements of bone to mechanical demands with great precision. The third kind of cells called 'osteocyte' are associated with the maintenance of the tissue.
I.2 **Wolff's Law (Mechanical Stimulation)**

Bone responds to external mechanical forces (or their absence) by adoptive changes in its normal architecture. The immobilization produces a bone loss and is resorbed at the rate of 100 $\mu$ of a day (Ulthoff and Jaworski, 1978; Johnson, 1964). The self remodelling of long bone in child with midshaft fracture during angulation in response to mechanical stress is an example of the effects of environmental (epigenetic) factors on the genetically determined processes, development and tissue remodelling and may be explained with the help of Wolff's law. Wolff's law states that when a bone is bent under a mechanical load, it modifies its structure as to resist external pressure by bony apposition in the concavity and by resorption in the convexity. A modification to the Wolff's law was pronounced by Bassett in 1968 and is stated under:

The form of the bone given, the bone elements place or displace themselves in the direction of functional forces and increase or decrease their mass to reflect the amount of functional forces.

Furthermore, observations show that bone is anisotropic and viscoelastic material (Katz, 1971; 1980; Currey, 1969; Yoon and Katz, 1971; Abendschein and Hyatt, 1970). In general, bone is found to behave according to Hook's law for low values of strain. For higher values of strain, dry bone as well as wet bone cease to follow Hook's law
and behaves as viscous material. When it is subjected to mechanical strain, a part of the energy is converted to other forms and ultimately dissipated into heat.

What happens to this lost energy in living bone can be of considerable biological importance. In this connection, it may be mentioned that a correlation appears to exist between healing quality and power dissipation (Hassler et al. 1977). There is a rapid increase in bone growth with increase in power dissipation reaching an optimal somewhere in the range of 35 µW. This is manifestation of the fact that dissipated power is involved in bone growth and repair. It is further supported by the fact that the osteogenetic mechanical and electrical effects on organs and tissues had been previously demonstrated (Hamburg et al. 1971; Becker, 1972; Yasuda, 1955; 1974; 1977). It is quite clear that bone transduces mechanical to electrical energy and that changes in a cell's electrical environment can control its behaviour. From the data now available, the stimulus for bone formation and destruction appears to be highly electrical in nature. Presently two main possibilities have been suggested to account the response of bone to external stimuli. First hypothesis assumes that the pressure transmitted through the tissue to the cell surface or its extension produces a deformation which is received by the cell (Bassett, 1968). The second widely accepted hypothesis states that mechanical stress generates
an electrical signal which sets in motion the subsequent events. This hypothesis is based on facts that (A) in bone, exposed to mechanical forces, electrical signals are produced by piezoelectric effect/or streaming potential or electret (Bassett, 1972; Fukada and Yasuda, 1957), (B) electrical current affects bone-growth and remodelling (Bassett, 1972; Becker, 1967; Becker and Murray, 1967; Fridenberg et al. 1970; Lavine et al. 1974; Marino and Becker, 1971; Mascarenhas, 1974). The second hypothesis implies a transduction mechanism which may be attributed to the piezoelectric effects in bone. However, there is only speculation about mechanisms of actions and nature of command signals responsible for bone-growth and remodelling. Very little is known about the forces which govern bone-growth, repair and remodelling. If one considers that primary function of bone is to bear load, which is a physical function, then it does not seem unreasonable that the signal which directs bone-growth, repair and remodelling may be a physical signal. Of the several physical signals, which act upon or within a cell, electricity is one, has been studied extensively as described in the section to follow.

1.3 Electrical Stimulation

The earliest report of the use of electrical energy to directly stimulate bone healing seems to be from England in 1852 and from Boston in 1860. In both the cases, the technique involved was the introduction of
direct current (dc) into the non-united fracture site percutaneously via metallic needles, with subsequent healing of the defect. More attempts, though only slightly more sophisticated, have been made in the last twenty years to use electric current and electromagnetic field to stimulate bone-growth in animals and more recently in men. The reports in recent times are apparently from Yasuda et al. (1955) who observed periosteal bone-growth predominantly at the cathode using microampere direct current in rabbits and from Percy and Wilson (1955) who reported bone-growth in response to bimetallic epiphyseal stimulation. During the 1960's many investigators demonstrated that application of a small amount of electric current, be it a constant current or pulse current stimulated osteogenesis at the negative electrode or cathode (Bassett et al. 1964; Granberry and Janes, 1963). The techniques used in electrical stimulation, implantation and evaluation vary widely among all experimental reports. Electricity can be applied to bone using an invasive technique, wherein electrode leads and power. pack all are implanted in non-union extremity, a non-invasive technique wherein current is induced in non-union but all of the electrical apparatus remains exterior to the skin, and a semi-invasive technique wherein only the cathode is inserted into the non-union site. Furthermore, cathodic stimulation at the test site was more common than the bipolar or anodic stimulation and where
the both poles were used, more bone-growth was generally reported near the cathode. In the majority of reports either stainless steel, platinum-iridium or platinum were chosen as the active electrodes.

Clinical trials using electricity in various forms in the treatment of delayed union, non-union and congenital pseudoarthrosis began in the early 1970. Constant direct current, pulse current and electromagnetically induced current have all been used clinically to heal bone defects with varying degree of success. To this date it is not known which form of electricity is most efficient in stimulating osteogenesis. There is scarcity of data on various physical, electrical and electromechanical properties of bone. This leads to an idea to examine different solid state properties as discussed in the following section.

I.4 Solid State Properties

Since the work of Szent-Györgyi (1941), the relevant solid state properties (resistivity, Hall-effect, semiconduction, electret and piezoelectricity in relation to bone subsequently have been looked in the following years as described below.

I.4(i) Resistivity

Dry bone has been viewed as a semi-insulator with resistivity in the range $10^{10} - 10^{12}$ ohm-cm and an activation energy of greater than 6.7 eV (Shamos and Lavine, 1964). Wet bone on the other hand has been reported to
have a conductivity greater than $10^{-8}$ mho/cm with an activation energy of less than 1 eV (Becker et al. 1964). It may be pointed out that the role of water is significant in biomaterials, such as bone. Becker et al. (1965) concluded that the bone samples exposed to room humidity, revealed a thin film of free water on their surface which cannot be removed by heating beyond 100°C since it produces irreversible damages to collagen fibres. Resistivity increases with the proportion of hydration in bone (Becker, 1965). All these data show the importance of bound and free water in controlling the solid state properties. Another parameter which governs bone resistivity is the temperature (Behari et al. 1974). These studies offer some data relating to the charge-transport phenomena in bone.

I.4(ii) **Charge Transport in Bone**

The existence of bioelectrical activities in bone is closely related to the presence of charge carrier and the mechanism and the nature of charge transport. Conductivity is the measure of charge transport in the material and therefore the data on electrical conductivity will be useful in explaining the charge-transport phenomena in bone. To explain the electrical conductivity in bone, an indirect approach was proposed and followed by Behari et al. (1975) on the assumption that charge carriers (during conduction) are sensitive to radiation. Further, bone materials have hydrogen bonded structures.
(Ramachandran, 1967; Berendsen, 1962; Hamilton, 1968) and hydrogen bonds in these are thought to be affected by the exposure of UVL and result in a decrease in the electrical conductivity (Behari et al. 1975; 1977; Behari and Guha, 1976). This was attributed to the breaking of hydrogen bonds which then hampers the charge transport. This further leads to propound that protonic conduction may be possible in such a solid. Recently some more data have been reported on parameters related to charge transport phenomena in bone. These include activation energy (Andrabi, 1978), Hall-effect, Hall-mobility and drift mobility (Behari and Andrabi, 1978), and Hall-effect under magnetic field (Andrabi and Behari, 1979). The decrease in conductivity with temperature (Andrabi, 1978) and the small value of Hall-mobility (Behari and Andrabi, 1978) suggest the inapplicability of the simple band model to explain the charge transport phenomena in such biological materials. Also, as mentioned above, a decrease in conductivity along with diminution of the Hall-voltage is observed on exposure to UVL and is suggestive of an important role of ionic-transport in the biological systems. The low value of mobility and the observed no change in the Hall-voltage under the reversal of magnetic field may probably because of the presence of dipoles. However, there are some noteworthy observations in Hall-coefficient ($R_H$) in case of apatite. Hall-voltage increases by an order of magnitude when the magnetic field
increases from 10 K-gauss to 16 K-gauss. This may be attributed to the fact that in apatite bonding is comparatively weak (Hamilton, 1968) so that the atoms or (groups of atoms) are more mobile and hence they are more prone to the magnetic-field variation. This is supported by the fact that mobility (drift and Hall) is consistently higher in case of apatite as compared with those for bone and collagen.

I.4(iii) Semiconduction

Several studies have established that bone behaves in some aspects like a semiconductor (Becker et al. 1964; Becker and Brown, 1965; Becker and Marino, 1966; Becker et al. 1968). The basic functional unit in bone appears to be a PN junction diode formed by a precise molecular association between the collagen fibres (N-type material) and mineral apatite crystal (P-type material). In addition to semiconductor effects, photovoltaic and photoelectric effects have also been reported (Becker and Brown, 1965). Recently some more data have been reported on PN junction characteristics and photoelectric effects in bone (Andrabi and Behari, 1980; 1981). However, Shamos and Lavine (1964) expressed doubts concerning the semiconductor like behaviour of bone.

I.4(iv) Electret

Another important phenomenon of bone 'in vitro' as well as 'in vivo' is its charge retention capacity. The charge retention 'in vitro' bone was first studied by
Mascarenhas (1974) and the effect is termed as electret. The dried bone has been shown to be able to store charge of the order of $10^{-8}$ C/cm$^2$. Recently some more data have been reported on thermo-electro- and magneto-electret effect in bone and its two major constituents, e.g. collagen and apatite (Andrabi and Behari, 1981). All the three bone materials, e.g. bone, collagen and apatite show the electret effect and are able to store large amount of polarization charge ($10^{-8}$ C/cm$^2$ - $10^{-6}$ C/cm$^2$). It has been further observed that the samples have been found to retain charge even after 5 months. The efficiency of retaining charge for apatite was found to be comparable with bone and collagen. The electret effect in bone materials has been observed to be governed by the temperature of the specimen. Charge is lost if the specimen is put for about twentyfour hours at freezing temperature. This is suggestive of the fact that at lower temperature the mobile-charge are in a state of 'freeze' in an ordered manner such that they produce no net electric field. At higher temperature, the packed arrangement is broken resulting into a net polarization charge.

Results on apatite are at variance with the findings of other workers (Mascarenhas, 1974) in the sense that authors observed electret effect in apatite also (Andrabi and Behari, 1981). These authors have suggested that electret-state in apatite may be attributed to the asymmetry in the crystal structure produced during its
extraction from full bone and some inherent lattice-defects.

1.5 Nature of Ultrasound-Tissues Interaction

The study on ultrasound-tissues interaction dates back to the work of Wood and Loomis (1927) wherein they published results on the mechanical, thermal, chemical and biological effects of ultrasound. Since then many investigators have reported on different aspects of ultrasound-tissues interaction mechanisms (Pohlam, 1939; Bradfield, 1950; Buchtala, 1952). Observations show that biological effects of ultrasound-tissues interaction may be divided as primary and secondary. These effects may be thermal or non-thermal in origin depending upon intensity, frequency and exposure time of ultrasound. The primary effect of ultrasound irradiation can be cyclic and cycle-averaging effects. Cyclic effect is the imposition of an oscillatory movement on the tissues, accompanied by waves of pressure. The movements tend to be bulk movements in which the whole of the irradiated tissue is involved. The effects of such movements on either bond formation or on the enhancement of diffusion rates would thus be presumably very small. It seems unlikely, in the light of available data, that they are of much importance in introducing any physical or chemical changes in tissues. In cycle-averaging effects, the response of the tissue is not linear to mechanical pressure. This is because that the
irradiated medium (tissue) is compressible and there is a component of mechanical force which does not time-average to zero over a cycle. This component causes steady, unidirectional streaming to be set up in tissue elements, which are free to move and result into microstreaming, Bernoulli-forces, Viscous-drag and radiation pressure etc. (Dyson et al. 1968).

Among the secondary cause of various physical and chemical effects in tissues under ultrasound irradiation, cavitation is the most important. By cavitation is meant that empty spaces or gas bubbles are formed during the phase of rarefaction. When these cavities in tissues collapse during the phase of compression, a hydraulic pressure is created (Lehmann and Herrick, 1953) and results into variety of changes in medium.

All these observations established a link between ultrasound and its impact on various biological phenomena in tissues and particularly in bone. However, the quantum of extent still remains a vital issue to be discussed and investigated. Inherent ultrasound bone behaviour is an aspect which seems to be of great importance 'in vivo' of already existing results regarding piezoelectricity.

I.6 **Piezoelectricity in Bone**

Piezoelectricity may be defined as electricity resulting from stress on crystal. Classically, the phenomenon is described for a single inorganic crystal which
lacks centre of symmetry (Cady, 1946). The observations that stress-induced electrical signal play an important role in bone-growth and remodelling (Fukada and Yasuda, 1957; Bassett, 1971; Marino et al. 1971; Gjelsvik, 1973) has given an impetus to studies on the electrical and electromechanical properties of bone. Many investigators have reported on piezoelectricity in bone (Fukada, 1960; Shamos et al. 1963; Shamos and Lavine, 1964; 1967; Bassett and Becker, 1962; Braden et al. 1966; Cochran, 1966). Furthermore, Bassett and Becker (1962) reported that piezoelectric effects in bone may be attributed to the semiconduction properties. Subsequently, Becker et al. (1963) proposed a semiconductor rectifier theory to account for generated electrical unidirectional current flow rather than an alternating potential upon repeated application and release of stress. However, Shamos and Lavine (1965) expressed doubts about the semiconduction phenomenon as the possible explanation of piezoelectricity in bone since collagen is piezoelectric even without possessing PN rectifying junction. The existence of piezoelectric effects in dry bone and collagen have been reported (Fukada and Yasuda, 1957). At present, there is no experimental data to indicate that inorganic part of bone has piezoelectric properties, although Posner (1969) has considered this possibility at the theoretical level in a positive manner, while Bassett (1968) and Lang (1966) have negated the same. These results indicate
that the controversy exists over the origin of the electric response in bone, not its presence.

From the above sections, it is clear that bone materials possess variety of physical, solid and electromechanical properties. These properties are characteristics of bone and are modified under the actions of external stimuli. However, the origin of mechanisms at work and their biological significance is not completely understood. Further, it is clear that bioelectrical response in bone of mechanical deformation is biologically active and plays an important role in bone-growth. The piezoelectrical effect in bone is now generally accepted as possible mechanisms by which mechanical energy is converted into electrical energy and is used in bone-growth (Marino and Becker, 1972; Reinish and Nowick, 1975; Lakes et al. 1975). Apart from it, the increasing use of ultrasound in diagnosis, therapy and tissue regeneration provides an independent support to the necessity of physical, acoustic and electromechanical characterization of bone materials in high-frequency range (greater than 100 KHz). However, not much work has been reported regarding the role of high-frequency ultrasound in the control of biological system. There is scarcity of various electrical and electromechanical data in the high-frequency range. It thus seems imperative to study physical, electromechanical and related parameters in bone materials in high-frequency. With a view to identify
some and connect the other phenomena, a number of observations have been taken both 'in vivo' and 'in vitro' bone as described below. The latter being examined from a purely material point of view.

The present work consists of two parts. In the first phase, ultrasound propagation 'in vivo' bone has been examined under different conditions while the second part concerns with the inherent ultrasound behaviour of bone and its constituents.

In Chapter II, various electrical parameters, e.g. biopotential, impedance, loss-factor and related quality factor 'Q' have been determined in unstressed 'in vivo' bone. These data have been further examined 'in vivo' bone under dc and ultrasound stimulation. Electret-effect is another phenomenon of interest that has been studied 'in vivo' bone under constant direct current (μA) stimulation. It is hoped that this will shed light on the nature of interaction of ultrasound with biological systems in general and bone in particular as well as its effects on bioelectrical activities 'in vivo' bone.

While data on acoustic and electromechanical parameters are existing on bone 'in vitro' conditions, those 'in vivo' are either non-existent or scanty. In order to bridge this lacuna 'in vivo' bone characterization is undertaken. Chapter III consists of demonstration of piezoelectricity and determination of certain acoustic parameters 'in vivo' bone at 1.27 MHz. These acoustic parameters include ultrasound propagation velocity and
attenuation constant 'in vivo' bone and an estimate of average ultrasound propagation velocity in skin-fat-muscle-blood-bone layered system.

Chapter IV concerns measurements of various physical (resistivity, density, Curie-temperature), dielectric (dielectric constant, dielectric-loss), piezoelectric (charge constant, voltage constant) and electromechanical (frequency-constant, coupling-coefficient) properties of bone and its two major constituents, e.g. collagen and apatite in addition to characterization of electrical properties of bone materials in high-frequency (1-70 MHz). To have an assessment of this material as an ultrasonic transducer, it has been obtained in standard transducer configuration with proper backing and loading media. To study the behaviour of bone transducer in high-frequency range (0.5-108 MHz), the variation of electrical impedance, phase-angle and relative voltage has been examined. From the impedance and phase-angle measurements and the information regarding the frequency response curve of bone transducer, it has been observed that it has sufficient transduction capacity of electrical power in the frequency range (greater than 10 MHz). The peaks in the frequency response curve are found to fall around 56, 112 and 168 MHz, indicating the presence of first, second and third harmonics respectively. Furthermore, velocity dispersion has been examined in bone materials in frequency range (1-108 MHz), indicating
that bone materials are viscoelastic. In order to study the quantitative comparison of electric and mechanical parameters of bone transducer with other known piezoelectric ceramics and Quartz transducers, an equivalent circuit has been proposed for bone materials. With the knowledge of resonant frequency, charge constant and density, the parameters of equivalent electrical circuit have been calculated. It is hoped that these data will be useful in explaining the mechanism contributing to bone piezoelectric behaviour and related phenomena in bone-growth and remodelling.

This further led to the idea of examining various, dielectric, piezoelectric and electromechanical properties of bone materials when they are subjected to various manipulations in chemical compositions. The observations that mineral content and trace elements present in bone, may influence electrical properties of bone materials, has created a considerable amount of interest in the material characterization of doped bone with varying proportions of mineral-contents and different trace elements. In Chapter V, data on different parameters as mentioned above are measured on test pieces of bone compositions having different proportions of mineral-content and various trace elements, e.g. AlBr₃, Na₂CO₃, Li₂CO₃, SrCO₃, Sb₂O₃, ZnO, Nb₂O₅, PZT and Pb(NO₃)₂. In addition ultrasound propagation velocity has also been measured.
Conceiving piezoelectric like effects in bone and the observed role of ultrasound on bioelectrical activities have generated interest in understanding the mechanisms involved in biological-effects of ultrasound.

Chapter VI consists of data on temperature-rise in skin, fat, muscle, bone and blood of (live and dead) intact rabbit femur under ultrasound exposure of intensities at 2.0, 2.5, 3.0 and 3.5 W/cm² at 800 KHz for 5, 10 and 15 minutes. It is expected that such data on temperature profile in different layers in intact rabbit femur will be useful in deciding the guidelines in ultrasound medical applications.

These data as mentioned above are supposed to contribute to better understanding of well recognized piezoelectricity and related phenomena in bone. These will be discussed, explained and is hoped that these data in totality may contribute to the understanding of possible mechanism of bone-growth and remodelling under external stimuli.