CHAPTER - III

BONE BEHAVIOUR AND THE EFFECT OF ELECTRICAL STIMULATIONS

III.1 INTRODUCTION

In recent years the use of electrical and electromagnetic fields stimulations of bone have opened new horizons to attain its growth and repair. Bassett's pioneer work in this field have opened up new possibilities to treat clinically the disordered function in bone. There have been a number of recent reports, (Bassett, 1982, Sharrard et al. 1982, Bassett et al. 1981, Saha et al. 1982, 1981, Sevitt 1981, Brighton et al. 1981, Paterson et al. 1980, Simmons 1980, Bassett 1978, Paterson et al. 1977, Bassett et al. 1977, Harris et al. 1977, Becker et al. 1977, De Haas et al. 1980, Piekariski et al. 1978, Jaffee et al. 1977, Connolly et al. 1977, Jorgensen 1977, Lavine 1977), indicating that electromagnetic field applied to various tissues have a significant effect on its osteogenesis, and fracture healing. Various techniques can be used for applying the fields in bone; surgical implantation (a totally invasive method), current delivering electrodes, capacitive coupling of electrical field of tissue, inductive coupling of electromagnetic field etc. A biological potential in stressed bone arises mainly from organic bone matrix as was first observed in 1957 by Fukada et al.
Non-stressed potential, known as bioelectric potential are not stress dependent but are dependent on cellular viability (Friedenberg et al. 1966, 1973). Contemporary research has investigated the stress bioelectrical potential as the possible stimulus for bone formation, partial limb regeneration, bone regeneration in artificial defects. Healing in fracture sites have been enhanced by using artificially created negative electric potentials (Norton 1974, Becker 1972, Friedenberg et al. 1971). The production of electricity by mechanical deformation is a unique and inherent property of long chain polymers such as collagen, cellulose, keratin, nucleic acids, protein polysaccharides and other structurally similar materials (William et al. 1974, Fukada et al. 1964, 1974, Black et al. 1974, Bassett et al. 1972). Bone may be described as a transducer, converting mechanical energy into surface electrical potentials (Bassett 1971, Shamos and Lavine 1967). Small amount of electric current produces a measurable biological effects on various systems. Bone formation is an active cellular process that require optimum concentration of various enzymes, minerals and organic compounds in addition to a suitable source of energy. It can thus be conceived that changes in microenvironment of bone forming cells may well alter
the osteogenic response of such cells. In conformity with this idea a number of workers attempted bone regeneration by choosing a few out of a variety of possible combinations. Out of these the D.C. stimulation (Constant Current) and low frequencies stimulations have been widely attempted for obvious reasons. Low frequencies electromagnetic field, falling close to that of physiological interest have particularly found favour. These set up also do not involve any complicated electrical circuit design considerations.

The observations that electrically negative regions are associated with bone formation and positive regions with bone resorption appears to correlate with other laboratory's and clinical findings. For example, it is known that osteoblastic activity occurs on concave surface of bone surface will become electronegative on bending (Black 1971, Bassett and Becker 1962).

Becker et al. (1967) suggested that generation of very low amperage on the surface of bone in the presence of tropocollagen initiates bone deposition. Subsequently it was used over a small area to enhance and speed up fracture healing in amphibians (Becker and Murrey 1970). There is evidence that low current level oscillating negative voltage is locally antibiotic and generates
new apposition (Norton 1975). Application of similar voltage levels on pathological bony defects should enhance their ability to heal. Becker (1972) achieved blastema formation and multicellular differentiation with currents on nano-amperes range. Smith (1974, 1967) suggested that in non-regenerative animals this minute level of current is the most conducive to normal physiologic regeneration. Bassett (1968) stated that this osteogenic electrical energy is likely to be undirectional signals, otherwise it might mediate both apposition and absorption alternatively to have a net lack of yield effect. It is suggested that it might be possible to increase the bone growth by means of slowly alternating electrical potential similar in magnitude and frequency to the natural occurring deformation in active animals.

Hassler et al. (1977) implanted brush type electrode within the osteotomy to effectively distribute the current throughout the osteotomy and provide a relatively large amount of healed bone for analysis. The electrodes were inserted in the rabbit calvaria using aseptic surgical techniques. The two osteotomies were cut with a hand-held jeweller's saw. The bone growth was studied, after stimulation, by densitometry measurements of the post-encephalomy. It was found that there is a rapid increase in bone growth with increase in power dissipations reaching
on optimum somewhere in the range of 35 µwatts. A number of workers reported bony periosteal callus in femur of rats wrapped by the electret made from teflon film. The use of electret film on bone surface to induce callus formation (Yasuda 1977, Inoue et al. 1977) has acquired importance in bone growth and healing of fracture. It was also found that electric fields can alter growth in long bone of newborn leg horn rooster chick (Norton 1974). The ability to control facial bone growth improve alveolar contour, close cleft plates, and enhancement of healing following orthognathic surgery are among the many implications of bioelectric phenomena (Norton 1975). Periodontal disease usually results in bone loss and correlative thereby should ideally regenerate bone loss as a result of such pathology.

Galvanic and thermocouple generators were designed to deliver a current in nano-amperes range. The galvanic unit produces a direct current. Thermocouple unit was designed to deliver an oscillating d.c. currents. Neither thermocouple nor galvanic device has produced any significant change clinically or radiographically (Jacobs and Norton 1977). The demonstration of osteogenesis by direct current stimulation, however, suggest that it might be possible to speed up fracture healing by electrical
stimulation. This clinically attractive idea faces two major problems. First, bone formation is limited to discrete region around the cathode. It is a region in which possibly the deleterious effect is due to electrolysis may exist. Secondly, in large bones of man, multiple electrodes are required to produce clinically significant bulk of callus in a reasonable time. This makes the method surgically invasive.

Point application technique has been utilized to hasten bone fracture healing (Shamos et al. 1971, Friedenberg et al. 1971) to change the existing bone structure (Lavine et al. 1972) and enhance primordial limb regeneration (Becker 1971, Smith 1967). There have been attempts to theoretically define mechanism of piezoelectric effect on osteogenesis.

It is clear that the electrode material is one of the determinants of the optimum current magnitude required for electrically induced osteogenesis. Materials ranging from platinum, stainless steel, silver, titanium, and vitallium (Yasuda 1977) in intimate contact with cells, extra-cellular matrix and fluids, are invariably used. Various electrolysis effects are always present (Pilla 1974). At sufficient high d.c. current both anodic and cathodic electrolysis products are deleterious. Under
pulsating and a.c. conditions, it becomes easier to avoid net electrolysis by careful choice of duty cycle and potential control (Pilla 1973, 1972). In spite of these advances, the mechanism of electrical and electromagnetic field stimulation still remain a matter of conjecture. However, several workers have reported about the change of electrical environment of tissue under electrically stimulated conditions. The ionic products have been observed in the tissue surrounding both the anode and the cathode. The cations and anions move through the tissue in the presence of an electrical current. The cellular environment will remain unchanged if there is a balance of positive and negative ions. However, there is a high probability that in the path of ions there exists selective adsorption of some ions and possible semi-conductive properties of tissue and differences in the ionic mobility would produce (Digby 1974) local changes in $p_H$ and thus affect precipitation of calcium salts. It is known that cellular environment would control its metabolic activity (Parseyian 1974).

It has been stated in earlier reports that electricity whether it is constant direct current, pulsed direct current, or magnetically induced current can stimulate osteogenesis, but it is not known which form of electrical energy is the most efficient stimulator of osteogenesis.
An attempt has been made to fill in some of the gaps in the existing lacuna. As a first step, a scanning of relatively high frequencies field in affecting the bone electrical activity is attempted. The success of these efforts and rapidly expanding base of fundamental data suggest that many important advances can be made as new research endeavour characterize effects of electromagnetic field. This includes dc excitation, low frequencies fields, 1.2 MHz frequencies etc and pulsating electric currents on bioelectric bone behaviour. Taking into account the piezoelectricity in bone surrounded by biological fields a system is supposed to exist which controls/affects the bone electrical activity and enhance its growth. All the observations reported though quite diversified seems to focus attention to dynamic approach to an in vivo situation wherein interfacial electrochemical phenomena that occurs between the cells and its environment demonstrates that the effects are caused by a change in electrical environment. With a view to open up new dimensions of research in this area, it has been decided to scan the electromagnetic field in MHz frequency range, alongwith low frequency stimulations. This has been compared with dc stimulation. Invasive and non-invasive methods of bone excitation have been attempted and compared. While the seemingly useful clinical data appears
to exist yet little attempt is made to understand and underline the biological phenomena at work. In the present work is attempted the field stimulations and possible other combinations which would produce optimum effects. It is hoped that these results will be useful for clinical purposes.

III.2 MATERIALS AND METHODS

III.2.1 Electrode implantation:

The experiments are performed on adult rabbits weighing from 1.5 kg to 2.0 kg. The method consists of measuring biopotential and applying stimulation by implanting high speed stainless electrodes inside the tibia and femur bone under general anaesthesia. Five electrodes (of 1 mm diameter) insulated all along their length except the tips are drilled through bone with their ends touching its inner portion and clamped at their positions with dental cement. The position of electrodes (Fig. III.1) on tibia and femur are kept 1 cm away between consecutive electrodes respectively. The thin sheet of perspex is used for holding the electrodes on tibia and femur respectively. Usual surgical techniques for implantation of electrodes in bone is adopted (Bahari and Singh 1981a).

III.2.2 Control data

Biopotential has been measured by high input
IMPLANTED ELECTRODES (dia=1mm) POSITIONS IN LIVE INTACT RABBIT FEMUR AND TIBIA.
impedance digital electrometer (Keithley model 616). The reference electrode is taken according to the convenience. However, the lowest electrode '1' is used as reference electrode in the most of the measurements, while the biological potentials are recorded between any two electrodes. The care is taken to stabilise the system by shorting the two electrodes. The data on biopotential are measured on fifteen rabbits. Biopotential has also been measured after stimulation of electrical and electromagnetic fields which are applied under various combinations.

III.2.3 Pulsating fields

The pulse electromagnetic field is generated by applying the pulse by a pulse generator in the range of 1V to 10V through the Copper Coil (22 gauge, 15 turns) implanted along the tibia of an adult rabbit. The signals at different frequencies (0.1 - 10 MHz), pulse delay (0.1 μsec - 100 msec), and pulse width (0.1 μsec - 100 msec) are applied.

The biopotentials are recorded after and during the exposure of fields. Instantaneous rise is noted in all the cases. The procedure is repeated for seven days and the enhanced values of biopotentials are recorded. Before each stimulation, the potential between 1,2 ele-
Implanted electrodes (dia. 1 mm) position and coiling in live intact Rabbit Tibia under pulsating stimulation.

Fig. III. 2
ctrodes (Fig. III.2) is measured so as to have comparison with the previous set observations.

III.2.4 dc stimulation

Constant current source of 100 μA is used for dc stimulation. The excitation are given through the implanted electrodes in tibia and femur.

In order to know the formation of charge, a constant direct current of 25 μA and 50 μA is chosen and applied between the two electrodes (1,5). The instant rise of biopotential is recorded.

The excitation of 1.27 MHz is also applied after dc stimulation. The cross stimulations of two excitations being examined and fall of biopotentials recorded. In order to confirm the magnitude and sign of biopotentials the polarity of basal value voltages is reversed and the biopotentials noted again. Usual precautions were taken in making these measurements.

III.2.5 Electromagnetic field (50 Hz) stimulation

The control value of biopotential is measured between electrodes (1,2), using Keithley digital electrometer. The data are recorded till the time, observations were found reproducible and consistent. In order to examine the field exposure effect on bone biopotential the current is passed through the solenoid and biopoten-
tial recorded as discussed in previous sections. The solenoid coil is having 200 number of turns and 8 cm diameter (field = 1.8 gauss at the centre). The tibia of the rabbit with implanted electrodes is invariably placed in the solenoid field for the purpose of recording biopotentials under stimulated conditions. The biopotential is recorded with the field in "on" and "off" positions. The combinations of AC (1.27 MHz) excitation and this 50 Hz electromagnetic field applied in that order, is examined on bone bioelectrical activity. The procedure being repeated for seven days on six animals and the changed values of biopotentials recorded in each case.

III.2.6 Continuous fields

In another experiment a frequency of 1.27 MHz (45V) is used for introducing oscillating field through the implanted electrodes by the signal generator. The surface biopotentials are measured by 26 gauge needle electrodes on tibia and femur, by removing all skin, muscles and fats from the surface of bone. The needle electrodes of identical material (stainless steel) and having tip sizes are used. The insulax (E-33, USA) is used for insulation on the needle electrodes except the tip. The painted electrodes being dried in incubator at a temperature of 30°C for 24 hours. The immediate
rise in biopotentials in longitudinal and transverse direction are measured all along the bone by Keithley electrometer (616 B). A change in surface potentials due to stimulation is considered as streaming potential.

III.3 RESULTS AND DISCUSSION

The effect of various stimulations on the bio-electric potential of bone are presented here. The basal biopotentials have been reported earlier (Behari and Singh 1981B) and these fall in the range (60-100 mv), and are measured by a stainless steel electrode. It is found that there is a definite trend in the variation in potential from tibia to femur. The control data (basal potential) are measured before each set of excitations. The effect of direct current, and low frequencies pulsating field stimulations on bone behaviour in vivo, have been examined before (Becker et al. 1977, Bassett 1982, Spadaro 1977, Ilfeld et al. 1974, Levy et al. 1972, Bassett et al. 1964), though the high frequency effect has not been attempted. The present observations confirm the established view that the area of the active growth have higher electrical activity (Klapper and Stellard 1974, Friedenber and Brighton 1966, Assima 1968, Bassett et al. 1964). The bioelectrical state is measured with
the full length of the electrode (Fig. III.1 and Fig. III.2) which is insulated except at the tip, suggesting that potentials are picked up from the bone's inner surface. The choice of the electrode material has been made so as to reduce the polarization effect. Also, since both the electrodes are made of identical materials, the polarization due to the galvanic cell is identical in the vicinity of both the electrodes. This helps reducing the polarization effect. In view of our earlier findings regarding in vivo bone sensitivity to frequency in MHz range (Behari and Singh 1981a), a more thorough search is carried out to identify the corresponding pulse width and time delay. The range of frequencies has been fairly broadened (0.1 MHz, 1 MHz, 10 MHz) in order to identify the spectrum in this repetition frequency range.

Fig. III.3 shows the effect of biopotential rise at various frequencies 0.1 MHz, 1 MHz and 10 MHz having pulse width 0.1 μsec and pulse delay in the range of 0.1 μsec to 100 msec. It is seen that the effect of 1 MHz at 0.1 μsec pulse delay is most prominent followed by pulse delay of 10 μsec at the same frequency. At 10 MHz (pulse delay = 0.1 μsec) the effect is same as at 1 MHz (pulse delay = 1 μsec). The increase in biopotential as produced
Biopotential under pulsating wave stimulation at frequencies of 0.1 MHz, 1 MHz & 10 MHz.

Pulse width = 0.1 μ sec
Pulse delay
1. 0.1 μ sec
2. 1 μ sec
3. 10 μ sec
4. 0.1 m sec
5. 1 m sec
6. 10 m sec
7. 100 m sec

Fig. III.3
Biopotential under pulsating wave stimulation at frequencies of 0.1 MHz, 1 MHz & 10 MHz.

Fig. III.4
by 0.1 MHz stimulation is consistently less for all pulse delay applied (0.1 \text{ usec} to 100 \text{ msec}). This seems to suggest that the pulse delay in combination with given frequency would optimize the biopotential bone response.

Similarly, Fig. III.4 is drawn by keeping the pulse delay 0.1 \text{ usec} and changing the pulse width (0.1 \text{ usec} to 100 \text{ msec}). It may be seen here that 10 MHz pulse delay of 100 msec has the maximum effect, closely followed by 1 MHz frequency having the same pulse delay. It is seen from Fig. III.3 and Fig. III.4 that the effect of pulse delay and pulse width is symmetrically distributed with respect to various repetition frequencies. The results support the view (Bassett 1982, 1978) that pulse induced currents depend on the characteristics of the pulse, e.g. pulse design, shape, duration and repetition frequency. The pulse excitation is made more effective by choosing the pulse design to exert selective influences on ionic species in the vicinity of the cell and its membranes, and produces frequency matching and response effects (Bassett 1978). The selective action is not possible with dc. This may be suggested that stimulus generated is modified by dose response as also observed by Friedenberg et al. 1974. Guided by these observations, it is further suggested that the rise in biopotential is dependent upon the pulse width (pulse delay constant,
Fig. III.4). Also the increasing pulse width produces higher stimulus. Similar conclusions are drawn from Fig. III.3 also. Fig. III.5 shows the effect of dc stimulation and a consequent superposition of 1.27 MHz. The DC and the AC (1.27 MHz) have been applied between the electrodes position (1,5) and (1,4) respectively. It may be mentioned that these electrodes are implanted longitudinally in cortical region of the bone as mentioned earlier. The dc stimulation of 25 μA and 50 μA (constant current) by constant current source are used for excitations which fall in the stimulation range of physiological interest (Friedenberg et al. 1970). D.C. stimulation of 50 μA applied for a period of 15 minutes raises the potential from +50 mv to about -500 mv, and an application of 1.27 MHz A.C. produces a diminution in the potential, which recovers almost immediately to original value once the A.C. stimulation is put off. There is a usual sharp decline in the biopotential value in a period of about 20 minutes (after putting off the dc stimulation), the potential falling close to the basal value. As could be anticipated a smaller dc source stimulation leads to a smaller rise in bone basal potential values. It is seen that due to 1.27 MHz excitation there is almost a sudden rise which tends to reach to a saturation value once the stimulation is put off the bone potential returns to the
BONE POTENTIAL (3, 2)
(1, 5) D.C.
(1, 4) A.C.
A Constant current 50 µA
B Constant current 25 µA
↓ 1.2 MHz A.C. put on
↑ 1.2 MHz A.C. put off
← D.C. stimulation put off

Fig. III. 5
original value. In subsequent Fig. III.6, a set of observations are plotted to underline the fact that the change in potential due to A.C. excitation is independent of the polarity to the basal value voltages and the rise due to D.C. stimulation as shown in Fig. III.5. It may be seen from Fig. III.6 that a change in bone potential produced by A.C. stimulation is about 70 mv which is close to that produced when the polarity of basal voltages is reversed. The effect produced by A.C. stimulation remains the same, even when it is preceded by dc stimulation. This is clear from above figures where the rise in potential is in reverse direction due to polarity of the basal voltages. Fig. III.7 reflects the effect of coupling of electromagnetic field with the possible ultrasonic wave propagation generated by excitation of 1.27 MHz. It is seen that due to 1.27 MHz excitation there is almost a sudden rise in bone potential followed by slow rise, which tends to reach a saturation value. At this point applied electromagnetic field (50 Hz) through solenoid tends to increase the biopotential further. Switching off the 1.27 MHz excitation brings about a fall in bone potential which tends to fall further with switching off low frequency (50 Hz), field. Fig. III.8 indicates the effect of low frequency electromagnetic field alone. Application of this field brings
ELECTRODE POSITION
BONE POTENTIAL (5,1)
EXCITATION ON (3,4)
Fig. III.7

BONE POTENTIAL (1,3) EXCITATION ON (2,3)

ELECTROMAGNETIC FIELD ON

ELECTROMAGNETIC FIELD OFF

EXCITATION (1.2 MHz) OFF

EXCITATION (1.2 MHz) ON

POTENTIAL (+mV)

TIME (MINUTES)

30  40  50  60  70  80  90  100  110  120  130  140
Biopotential under electromagnetic field (50 Hz) stimulation

Bone Potential (1.3)

ELECTROMAGNETIC FIELD OFF

ELECTROMAGNETIC FIELD ON

Fig. III.8
Bone surface biopotential hypodermic needle electrode

- Normal
- Ultrasonic stimulation (127 MHZ)

**Fig. III.9**
Transvers surface Biopotential 26 gauge Hypodermic needle electrode

- Normal
- Ultrasonic stimulation (1.27 MHZ)

Fig. III.10

DISTANCE BETWEEN ELECTRODE (mm)
about an increase of bone potential which tends to return to original value, once the field is put off. This clearly indicates the effect of induced field in 'in vivo' bone.

The measurement of surface potential is shown in Fig. III.9 with changing distance between two electrodes in tibia and femur. It is seen from this figure that on the application of 1.27 MHz stimulation potential uniformly decreases in the region extending from tibia to femur. The excitations are given on electrode position (1,2 and 4,5). Transverse surface potential is shown in Fig.III.10 at various distances between the two electrodes at different location of bone. It is found that near the tibia and femur joint the electrical activity is more in comparison with other positions. It is also seen that the surface potential in longitudinal direction is more than the potential in transverse direction. It can be suggested by this observation that endochondrial ossification (Ogden 1980) is responsible for longitudinal growth is more as compared to appositional growth. The above observation indicate that the bone activity is very sensitive to the stimulations applied. However, the degree of effectiveness is significantly variable. As is well established an application of D.C.
stimulation causes polarization and hence the biopotential increase with time.

Biopotentials represent a summation of all electrical activities from the cells in the local area of bone (Friedenberg et al. 1973, Harlow et al. 1971). It is concluded that the electrical activity is an inherent property of bone and it varies along its length. Our results seem to support the earlier findings (Mohr et al. 1976, Bassett 1974) that inductively coupled, dynamic electromagnetic fields are able to efficiently guide the process of osteogenesis which is linked to the electrical activity of the tissue. The existence of permanent electrostatic field has been reported earlier (Athenstaedt 1974). It may be pointed out that biopotential plays a significant role in controlling the direction of biological growth processes. We suggest that the generation and reorientation of the permanent dipoles as a result of external stimulation is in a direction which controls the potential variation for the enhancement of bone growth. Sawyer et al. (1953) have also suggested that the direct current potential may be related to the tabular shape of the bone and charge accumulation may vary in different regions due to the flaring of the ends and variation in blood flow.
It is clear from above observations that electrical activity 'in vivo' bone is significantly enhanced by direct current and bone growth can possibly be enhanced. It may be postulated that precise origin of dc electrical activities may be ionic-gradient and ionic transport across membranes to the inhomogeneous structure of tissue that gives rise to polarized molecules. This is in agreement with the reported results (Behari et al. 1979, 1975, 1974, Andrabi 1978) wherein it has been mentioned that source of electrical activities are dipoles, ionic-species and the phenomena of proton-conduction ($\text{H}^+$ Vacancy migration).