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The Stimulation of Bone Growth by Ultrasound

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Summary. The ultrasonic stimulation of bone growth was investigated in a experimental work in which a set of 45 rabbits were studied according to the following procedure:

a) bilateral osteotomy of fibula (23 animals) and bilateral drilled holes on the cortex of femur (22 animals);

b) exposure of ultrasound for 15 min per day;

c) Radiological and histological evaluations of the progress of the callus;

d) photography and measurements of the area of the callus;

e) graphical comparisons using the results between controls and stimulated limbs. Pulsed ultrasound, in the form of short bursts, was used at low intensities (below cavitation threshold) so that the temperature variation, at the osteotomy site, was of the order of 0.01° C (constant) a fact that reinforces the assumption that the stimulation mechanism due to the appearence of electric potentials is of non-thermal origin such as that caused by piezoelectricity.

Zusammenfassung. In dieser Arbeit wurde die Stimulierung des Knochenwachstums bei Kaninchen durch Ultraschallbehandlung untersucht. Insgesamt dienten 45 Kaninchen den Experimenten, die wie folgt durchgeführt wurden:

a) 23 Tiere bei der zweiseitigen Osteotomie der Fibula und 22 bei bilateralen Öffnungen der Cortex des Femur.

b) Beahndlungsdauer mit Ultraschall täglich 15 min.

c) Die Kallusbildung wurde durch Röntgenaufnahmen und Gewebeuntersuchungen ausgewertet.

d) Es wurden Aufnahmen und Messungen des Kallusgebietes gemacht und

e) graphisch die nicht behandelten und stimulierten Glieder verglichen.

Es wurde pulsierender Ultraschall angewendet, kurz dauernde „bursts" und zwar so, daß die Temperaturschwankung an der Stelle der Osteotomie 0,01°C (konstant) betrug. Dieses stärkt die Hypothese, daß der stimulierende Mechanismus (elektrische Potentiale) nicht thermischen Ursprungs ist, wie durch Piezoelektrizität.

Introduction

The stimulation of bone growth by physical means has been investigated for many years. In the beginning of last century it was observed that small direct currents acting in the periosteum could provide induction of bone formation, and this was probably the first scientific approach to this fascinating field. However this previous effort was not enough to elucidate the factors behind the observation, and the phenomenon was little or not used until 1957 when the piezoelectricity of bone was discovered by Fukada [1]. After that a great deal of research has been done and dramatic efforts have been made, both in theoretical and experimental levels, to clarify the understanding of the matter and to achive acceptable techniques for clinical use. Nevertheless, the fundamental process through which the deformed wet bone create potentials is not fully understood and more efforts are necessary in the search of a consistent biophysical model.

In the clinical field some processes have been proposed and in spite of their degree of success, they present certain disadvantages such as:

a) the electrical stimulation by electrodes is invasive exposing thereby the patient to the risk of infection [2-4].

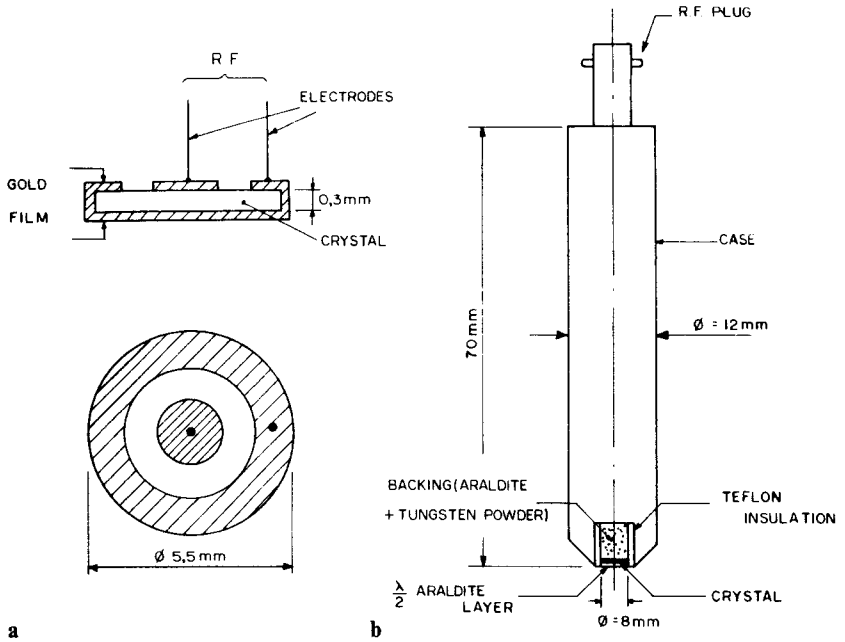

Fig. 1. a Quartz crystal transducer ($f_o = 4.93$ MHz) gold plated in both faces with a concentric ring to separate the electrodes. **b** Quartz transducer encapsulated in a metal case

b) the electromagnetic induction, employing coils, is a non-invasive process but takes a long period of time for the treatment (12–16 h a day during 4–8 months) [5–7].

The purpose of this work is to present the ultrasonic stimulation of bone growth which can be an alternative process to heal fractures. Being a non-invasive technique it belongs to the same class of the process pointed out on (b) and, moreover, because of its nature, it can be related to the piezoelectric effect of bone as can be seen on the Discussion.

Materials and Methods

In this experiment, ultrasonic energy was applied in osteotomized bones of 45 rabbits for which two kinds of bones were selected: a) fibula, b) femur. In the case of fibula, a bilateral osteotomy was performed 1 cm below the popliteous nerve, with the aid of a saw, the osteotomy trace (less tham a 1 mm) thus appearing at the middle shaft of the bone. This bone was chosen because it does not support load directly, and as a consequence, can be cut without the need of a cast after the operation.

In the case of femur, bilateral holes were drilled on the middle shaft of the bone. The hole diameters were 1.5 mm and the drill did not trespass the whole bone, being stoped at a depth equal to the thickness of the lateral cortex. No post-operative fracture was observed.

In all cases the surgeries followed standard asseptic conditions, and under general anesthesia (intra-venous Nembutal at a dosage about 1 ml per kilogram of animal weight).

The first used transducer was a quartz disc, 8 mm in diameter, gold plated on both faces with a garded central ring as shown on Fig. 1a. This disc was encapsulated in a metal case, and care was taken to insulate the crystal from the case. A half wavelength layer of Araldite was put on the radiation face of the transducer to protect it against moisture and mechanical shock. Fig. 1b shows schematically the whole unit.

The fundamental frequency of the quartz transducer was $fo = 4.93$ MHz and it was of the x-cut type.

Later, during the course of experiments, another transducer was used. This transducer was a lead zirconate titanate type PZT-4 which could be produced to work within the frequency range of 1 MHz to 2 MHz, and aside its larger diameter (20 mm), the rest followed the same aspects of that of Fig. 1b.

The PZT-4 ceramic disc offered advantages as compared to the quartz one, as far as mechanical strength is concerned, and also because of its low Q factor which facilitated the tunning through the electrical coupling unit.

The fundamental frequency of such a transducer was $fo = 1.65$ MHz and it worked in the thickness mode producing, therefore the same kind of wave of that of the quartz transducer. As can be seen later, there were no significative differences between the results produced by the two different transducers ($fo_{quartz} = 4.93$ MHz and $fo_{PZT} = 1.65$ MHz). In a previous work, the author [8] proposed a implanted transducer (10 MHz) attached to the bone diaphysis, but this method was found to be unnecessary since the transcutaneous technique, now described, proved to be, satisfactory, practical, and most of all, a non-invassive process which is always desirable.

Both transducer were driven by a MATEC electronic unit, model 9000 with plug-in model 755 operating from 1 to 20 MHz in which only the transmitter part of the apparatus was used. In this way short "burts" of RF energy were delivered to the osteotomized sites with the following characteristics:

Amplitude: 70 V peak to peak

Pulse width: 5 μs

Repetition pulse rate: 1000 Hz

The intensity corresponding to the PZT-4 transducer was 49.6 mW/cm^2 and that of the quartz transducer was 57 mW/cm^2. The acoustical powers were measured in a device described by [9] after a calibration with the aid of potentiometer recorder type K-5 (sensibility of 0.5 μV).

The stimulations, in all cases, started 24 h after the operations by applying the transducer to the skin (after tricotomy)

using glycerin as a coupling medium, right over the osteotomized site. The acoustical impedance of glycerin was found to be adequated since it is similar to that of skin (1.5×10^{-9} g · cm^{-2} sec^{-1}). The general experimental procedure for each animals was the following:

1) Stimulation on the left leg (or thig) during 15 min per day.
2) The right leg (or thig) received no ultrasound at all and was considered as the control. The extension of the treatment varied from 4 to 18 days ending with the sacrifice of the animals.

Evaluations of the progress of the callus were performed by X-rays, histological examinations and by measurements of the area of callus, comparing stimulated bones with the controls. To measure the area of the callus, the bones were excised from the animal bodies, cleaned up from soft tissues, photographed, magnified 50 times and measured with a planimeter around the callus irregularities.

With a collection of data and a subroutine program fed to the IBM 1130 computer, it was possible to obtain graphs both from stimulated and control areas of callus versus the number of days of treatment with the possibility of choice of the best fit of polynomy related to cloud of points, as can be seen on the Section of Results.

Temperature at the osteotomized site was measured during the stimulations, by using copper-constantan thermocouple, calibrated and electrically isolated from the medium. This procedure was made in the first 10 rabbits but it was found useless because the local average temperature increase, as a consequence of ultrasound energy, was less than 0.01°C for the intensities used in this work.

Results

Among the 45 animals studied in this work, 23 were separated for the treatment of fibula and 22 for the femur. In a previous work [8], the author presented results for 18 animals treated with the procedure of implantation of the transducer and the data so obtained showed the same average differences between stimulated and control osteotomies.

In the present work two kinds of evaluation were chosen: a) Qualitative (radiological and histological examinations); b) quantitative (measurement of the area of callus).

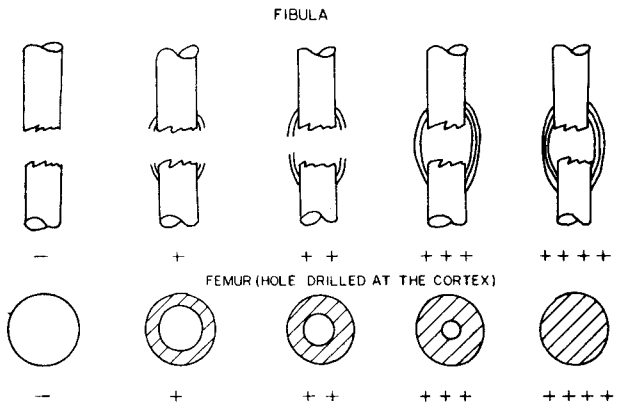

Fig. 2. Qualitative evaluation for both fibula and femur

The adopted criterion for the qualitative evaluation is illustrated in Fig. 2.

The area of the callus, taken with a planimeter, is shown in Table 1 for the cortical holes of femur and in Table 2 for the sheared fibula. The numbers represent the mean values among 5 measurements.

The curves of Figs. 3 and 4 were plotted by the computer after a sub-routine program for the choice of the best fit among data of Tables 1 and 2 respectively. The curves depicted the growth of the area of callus as a function of time both for stimulated and control bones.

The pictures of Figs. 5 and 6 represent the control and stimulated callus of the osteotomized fibula of a rabbit treated 13 days.

The pictures of Figs. 7 and 8 were taken from histological cuts of cortical holes of the femur prepared in accordance to the routine standard procedures. The control picture appearing in Fig. 7 shows the clot and a complete absence of bone trabecula. The stimulated one shows the invasion of the clot by normal trabecula. There are no evidences of abnormal neoformation, a fact that was observed in all histological cuts.

Table 1. Femur - (evaluation of bone growth inside the cortex hole)

Type of evaluation	Extension of treatment (days)										
	5	5	6	7	8	8	10	10	12	14	15
Control											
Qualitative	–	+	+	+	+	++	+	+	++	++	++
Callus area (mm^2)	0.0650	0.068	0.080	0.074	0.111	0.145	0.100	0.132	0.300	0.910	1.026
Stimulated											
Qualitative	+	++	++	++	++	++	++	+++	++	++++	++++
Callus area (mm^2)	0.163	0.233	0.465	0.567	0.698	0.975	1.275	1.304	1.572	1.601	1.599

Table 2. Fibula - (evaluation of bone growth in the site of osteotomy)

Type of evaluation	Extension of treatment (days)												
	5	6	7	9	11	11	12	13	13	13	15	17	17	18
Control														
Qualitative	–	–	–	–	+	+	+	++	+	+	++	++	++	+++
Callus area (mm^2)	3.622	3.453	3.200	3.673	4.022	3.862	4.100	4.127	4.000	5.008	6.104	6.777	7.410	9.911
Stimulated														
Qualitative	–	–	+	++	+++	+++	+++	+++	+++	++++	++++	++++	++++	++++
Callus area (mm^2)	3.218	3.985	3.988	9.857	12.270	12.882	12.690	13.109	13.321	13.550	13.373	13.401	13.998	13.654

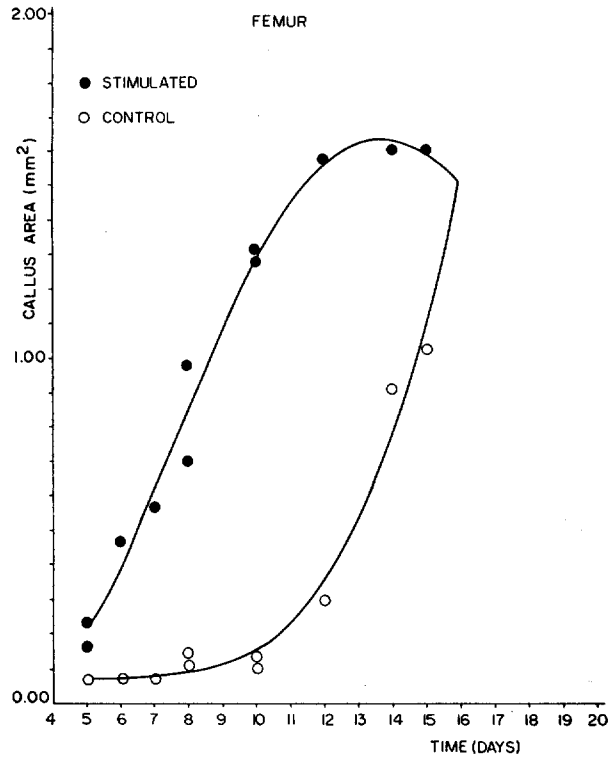

Fig. 3. Area of callus of drilled holes in femur plottet versus number of treatment days, by the computer, as a best fit among data. The points represent the mean value of 5 measurements

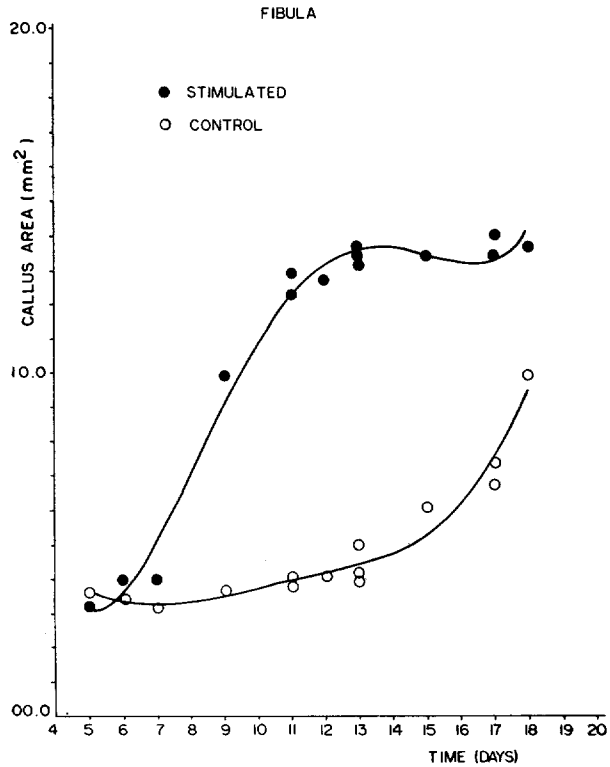

Fig. 4. Area of callus of osteotomized fibula plotted versus number of treatment days, by the computer, as a best fit among data. The points represent the mean value of 5 measurements

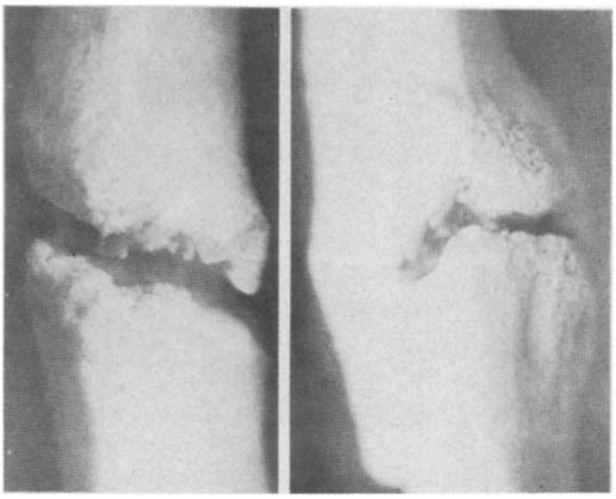

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Fig. 5. Control osteotomized fibula of rabbit no. 33 after 13 days. Periosteal callus beginning the growth. Endosteal callus stell in the cartilage phase. The union showed no bending strength

Fig. 6. Stimulated osteotomized fibula of rabbit no. 33 after 13 days. Periosteal and endosteal callus both in the final osseous phase. Bending strength was present when the union was under test

Discussion

The data in Tables 1 and 2 and respective curves (Figs. 3 and 4) show that ultrasonic induced changes on osteotomized bones increase fast in the first 10–12 days of stimulation, stabilizing after that period. The control osteotomies, on the contrary, show a slow rate process during the first 10–12 days, increasing rapidly the growing process after that. Recalling that the osteotomies were made bilateraly in each animal, therefore avoiding individual differences, it is possible to predict that ultrasonic energy, with appropriate parameters, can accelerate the healing of a fracture. Nevertheless some coments are necessary to help the discussion about what factors can influence the effect.

Frequency

Is was mentioned on the section—Materials and Methods—that because of experimental circumstances it was necessary to change the transducer, therefore to change the frequency from 4.93 MHz to 1.65 MHz, and that there were no significative differences in the results. Even applying ultrasound energy directly to the bone surface at a frequency of 10 MHz, as was previously presented by the author [8], yet there were no marked influences whem the results are compared to those of the present work. The only aspect that should be mentioned is that of the absorption of energy which depends on the frequency, the loses increasing with the penetration depth due to skin, fat, muscle and blood that are traversed by the ultrasonic beam as pointed out by Wells [9, 10].

However for limbs of rabbits, for which the bones are very close to the skin, the penetration depths are well within the calculated range for minimum absorption losses for them are within the Fresnel zone of the experimental transducers. For human beings, nevertheless, the change of frequency from ~1 MHz to ~5 MHz, can be a important factor and therefore must be considered taking into account the depth of different bones (tibia or femur for example). As a con-

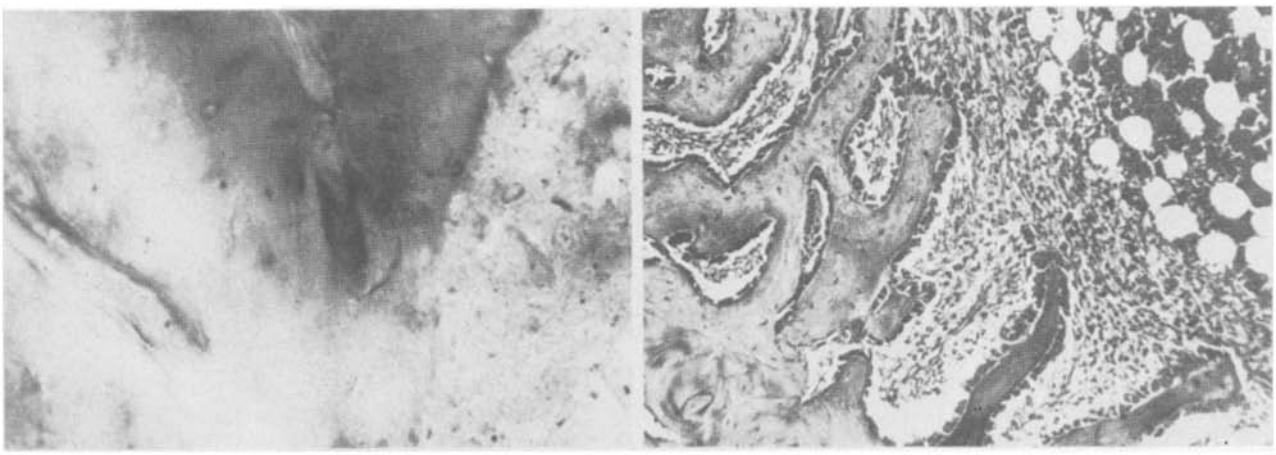

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Fig. 7. Histological cut of a control hole of the cortex of femur of rabbit no. 8 after 7 days. Dead bone is appearing in the sides and only a clot in the center (Hematoxylin and Eosin, 200×)

Fig. 8. Histological cut of the stimulated hole of the cortex of femur of rabbit no. 8 after 7 days. Trabeculae depicting numerous osteoblasts. On the upper right, part of the marrow (Hematoxylin and Eosin, 200×)

clusion there is no experimental evidence, in this work, whether frequency can influence the rate of bone growth or the magnitude of the induced bone callus.

Cavitation

Although cavitation can be generally hazardous for tissues, some investigators suceed to demonstrate that the collagen synthesis can be stimulated under its action [11]. They irradiated in vitro human fibroblasts at a intensity of 500 mW/cm^2 and noticed a enhancement of protein synthesis and did not report harmfull effects on the cells. In the present research the maximum intensity was 57 mW/cm^2 which is roughly 10% of their dosage and about half of the cavitation threshold pointed out by Hill [12] who worked in the frequency range of 1 MHz to 5 MHz in air-equilibrated water.

Moreover, the duty cycle used in this work (0.005), is far below the value observed by Hill [14] for positive indication of cavitation. Therefore, none significant cavitation effect, whether stimulatory or harmfull, should be expected from the ultrasonic parameters of the present work.

Temperature

The variation of temperature due to absorption of energy was negligible ($0.01°C \pm 0.005$) and insufficient to create pyroelectric potentials [13] since the temperature differences, besides small, were constant during the stimulations, which enables the conclusion that, for a pyroelectric and piezoelectric material as bone is [14] the major expected electric effect is probably of non-thermal origin.

Non-thermal Effects

Wet bone, definitely, presents an electrical response under stress [15–18]. The true mechanism through which bone develops this electrical signal is a problem that remains unsolved. Nevertheless attempts have been made by many investigators in order to present acceptable assumptions following experimental work. Some of those assumptions shall now be revised for discussion. Accounting for the separation of electrical charges there are two possible phenomena associated whith it: existence of streaming potentials and piezoelectric direct effect, both resulting from mechanical action on the bone. When bone is dry, piezoelectric effect as described by Fukada [1] is clear and understandable, but when bone is wet the magnitude of the signal is majored as indicated by Anderson and Erikson [17].

In this last case the problem is unclear and most of the investigators claim that the main dominant electromechanical effect is related to streaming potentials. In their assumption they are based on the existence of fluids fullfilling the bone vessels. The fluids may carry charge of one type when the vessels are squeezed by external pressure. If Poisson's bone coefficient is taken, $\nu = 0.20$, a small transverse deformation of 0.3% can be reached caused by an axial deformation $\varepsilon_Z = 1.5\%$.

This deformation corresponds to the ultimate strength of long bones. Therefore fracture may occur at any instant. So it is difficult to see how low intensity ultrasound energy can reach those local deformations on bone capable to cause a sudden shock wave of liquid inside the vessels thus generating drift of electrical charges. Higher electrical signals detected in wet bone may therefore proceed from different source rather than that of streaming potentials. One interesting fact is that the measurement technique (inserted electrodes) for the in vivo experiments may create muscle injury potentials as described by Lokietek et al. [19]. Those potentials, added to others caused by external means, may increase the amplitude of the measured signal in wet tissue.

Under ultrasonic field, soft tissues and fluids are subjected to unidirectional streaming as suggested by Dyson et al. [20]. In their work ultrasound was used as stimulus to enhance the growth of soft tissue. They worked at a intensity of 500 mW/cm^2 and the field force acting on the elements free to move was estimated to be 50 mg/ml for pulsed ultrasound in the miliseconds range. Their conclusion was favorable to the streaming potentials assumption. In the present case, however this assumption is unlikely firstly because bone is almost incompressible as compared to soft tissues and secondly because the intensity used in this work is roughly 10 times smaller than that employed by Dyson [20].

The piezoelectric effect appears to be more likely since bone exibits this particular physical property. Even when bone is wet and under stress the effect is present. In a recent work, Behari and Singh [21] have detected an electrical signal of 64 μV as the in vivo bone response to ultrasound energy produced by a transducer excited by a small voltage (1.15 V) at a low intenstiy of 3.83 mW/cm^2.

Accordingly the author, using variational principle, had calculated a expected signal of 1.5 mV generated by bone for the in vivo application of a transducer excited by 35 V at a dosage of 57 mW/cm^2 [22]. The physical model employed by the author was entirely grounded on the piezoelectric property of bone, and data concerning elastic and dielectric constants

and those of electrical conductivity of wet bone were thoroughly used.

Therefore, in this particular subject, theory and experiment are not very appart one from another, although the true mechanism accounting for the piezoelectric effect for the in vivo experiments still remains a challenge.

One important assumption that should draw attention is that of the reorientation of O-H dipoles of the periodic peptide unit of collagen molecule induced by stress. Piezoelectric mechanisms of polymers that present similar dipoles have been succesfully explained by that means [23, 24]. Nevertheless more basic investigation is necessary and both, electrical and stress relaxation can play a fundamental role in the understanding of stress induced potentials of collagenous tissues. In this way a experimental technique can be suggested for further investigation—the internal friction technique—. This procedure, commonly used in the study of relaxation mechanism of crystalline structures, can open a new research line by stablishing frequency and temperature dependence, relaxation times and activation energies associated with rotation of electrical dipoles in biopolymers. Finally it should be investigated wheter the piezoelectric effect for the in vivo bone is due only to the material as a transducer or if the cells also behave like a transducer in a cooperative phenomenon.

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