Introduction: Former studies have given conclusive indications for effects of extremely low frequency (ELF) combined static and alternate electromagnetic fields (ELF) tuned for several ions according to the Ion Cyclotron Resonance (ICR) formula [1,2,3]. ICR orthopedic procedures have been developed using the alternate terminology combined magnetic field. From classical physics, one can determine for any ion a cyclotron resonance (CR) frequency when that ion is subjected to combined AC and DC magnetic fields applied in the same direction. Simply stated, the resonance frequency is:

$$f = \frac{q}{2 \pi m} \times B$$

where \(f\) is the resonance frequency (Hz), \(q\) is the charge on the ion, \(m\) is the mass of the ion (kg) and \(B\) is the static magnetic field (T; 1 Tesla = 1 x 10^8 Gauss).

In order to prove and verify the effects of ICR conditions on the development and growth of complex biological systems we have chosen to extend the approach of S.D. Smith et al [1,7] examining the effects of combined static and alternate magnetic fields tuned for calcium, potassium and magnesium ions as per the ICR condition [4] on embryonic chick femora.

Methods: Femoral rudiments of 8 ½ day old white leghorn chick embryos were removed by blunt dissection. Paired femora were placed separately into numbered wells of 12-well culture plates containing Fittion-Jackson BGJ medium containing Gentamicin and Fungizone.

One femur of each pair was used as control, while the other was exposed for 30 min/day for 7 days to 16 Hz sinusoidal magnetic fields that were applied in a specially constructed Helmholtz coil system simultaneously with various parallel static magnetic fields tuned to calcium (20.9 x 10^{-6} T), magnesium (12.7 x 10^{-6} T), and potassium (40.7 x 10^{-6} T), as per the ICR condition. The peak value of the 16 Hz fields in each case corresponded to an AC/DC ratio of one (20.9 x 10^{-6} T, 12.7 x 10^{-6} T, 40.7 x 10^{-6} T peak).

At the end of the experiment, all bones were fixed in neutral buffered formalin. Using a vernier caliper, the length and the central diaphyseal diameter were measured. The femoral rudiments were then randomly assigned to three groups for assay. The bones of the first group were embedded in paraffin, serial sections were cut longitudinally at 8 µm and stained with hematoxylin/eosin (HE). The sections were examined for their histological appearance and photographed. Measurements of the diaphyseal collar length were made. The rudiments of the second group were stained for calcium with alizarin red-S (AR-S) and a quantitative extraction procedure was carried out using cetylpyridiniumchloride (CPC) [5]. The solubilized AR-S was qualified by absorbance at 570 nm. Each femur of the third group was digested with papain to solubilize the glycosaminoglycans, which were then quantified by dimethylmethylene blue (DMB) absorbance at 525 nm [6].

Results: ICR tuning for calcium ions increased significantly (p < 0.001), the mid-shaft diameter (+10.6%), the length of the rudiments (+4.1%), as well as the diaphyseal collar length (+28.3%), as compared with controls. The histological picture was markedly altered. Besides a considerably advanced maturation, all chondrocytes except those at the ends seemed to be hypertrophied. The central diaphyseal region showed a markedly thickened diaphyseal collar, more chondrocytes with pyknotic nuclei and a slight basophilia of the cartilage matrix. The treatment with calcium-ion-selecting ICR fields appeared to increase the AR-S content (+26.0%, p < 0.01) and the GAG content (+67.4%, p < 0.001), as compared with controls. When the fields were tuned to magnesium ions, the rudiment diameter (+13.5%) and length (+3.7%) and the diaphyseal collar length (+25.7%) were significantly (p < 0.001) increased. The histological picture presented an advanced maturation and a considerably thickened diaphyseal collar. The central diaphyseal region showed considerably more hypertrophied chondrocytes with more pyknotic nuclei and marked basophilia of the matrix. The AR-S content was increased (+23.4%, p < 0.01) and the increase of GAG content was especially great (+89.5%, p < 0.001).

Discussion: Although the microscopic observations suggest a different response to calcium- and magnesium-tuned ICR fields, both the reported gross measurements and the biochemical assays indicate that calcium and magnesium ion tuning are similarly stimulatory to growth and development of embryonic chick bones. Conversely, the effect of potassium-resonant fields was substantially opposite to that of calcium and magnesium ions, namely gross measurements, biochemical assays as well as histological appearance indicate an essentially inhibitory effect. The most impressive and meaningful observation of the present experiment is that, when the AC frequency is maintained, but the DC field is altered, both stimulatory and inhibitory effects occur with relatively small changes in magnetic field strength.

Conclusion: The statistically highly significant results of the present experiments show clearly that for in vitro situations complex biological tissue systems can be influenced by ICR conditions in the same way as simple cell systems. There was a remarkable cartilage response. The present as well as previous experiments have provided much valuable information about the effects of ELF resonant electromagnetic fields on chondrogenesis and osteogenesis [1,2,7]. However, further experiments are needed in order to investigate and to understand the origin of this bi-directional effect and its mechanism. We are currently preparing additional experiments that will probe the molecular biological response to ion cyclotron resonance conditions.


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**EVIDENCE FOR ICR MAGNETIC FIELD EFFECTS ON CARTILAGE AND BONE DEVELOPMENT IN EMBRYONIC CHICK BONE EXPLANTS**

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