Biochemical and Structural Approach to Collagen Synthesis Under Electric Fields

G. GULER[1], N. SEYHAN ATALAY[1], C. OZOĞUL[2] and D. ERDOĞAN[2]

1 *Gazi University, Faculty of Medicine Department of Biophysics, Ankara, Turkey,*
2 *Gazi University, Faculty of Medicine Department of Histology and Embryology, Ankara, Turkey*

Abstract. The effects of electric fields of different intensities and directions on the collagen synthesis of guinea pigs was studied. The effects of vertical and horizontal electric fields of 1.9 kV/m and 0.9 kV/m, generated with voltages of 300V and 150V DC, respectively, on collagen synthesis, was evaluated by assessing the amount of hydroxyproline in the liver tissue and by histological examination of tissue samples. The Stegemann-Stalder method was used to determine hydroxyproline content of the tissues. While the 0.9 kV/m vertical and horizontal electric fields substantially decreased the amount of hydroxyproline in the liver, the 1.9 kV/m electric fields in both directions increased the hydroxyproline content. The vertical electric field was more effective than the horizontal one both in the increases and the decreases. These findings were verified histologically.

Key words: Electric field — Tissue — Hydroxyproline — Fine structure — Histopathology

Introduction

The effects of electric fields (E) on living organisms have been studied by many researchers. The essence of these studies is that living cells are sensitive to electrical changes due to their electric properties. Membranes have a structure capable of changing their permeability levels to various ions when affected by an externally applied electric field. This may cause changes in tissue functions like EEG, Ca^{++} ion efflux behaviour etc. (Gavalas-Medici and Walter 1970; Gavalas-Medici et al. 1973; Kaczmarek and Adey 1974; Sheppard and Eisenbud 1977; Blackman and Benane 1982). Studies on the interaction of surrounding electric fields and tissue

Correspondence to: Goknur Guler, Gazi Universitesi Tip Fakultesi Biyofizik Abd. 06510 Beşevler, Ankara, TURKIYE. E-mail: gutfb-e@servis.net.tr

electric fields are quite new (Guler et al. 1994, 1995, 1996a,b,c,d; Guler and Atalay Seyhan 1995a,b, 1996a,b). Such studies will be very important for the understanding of the mechanisms involved.

How much is the electrical field of the cells in living organisms affected by the electric field in the atmosphere and the electric fields of all the electrical instruments in the environment. In this study, it was hypothesized that collagen synthesis would be affected from externally applied electric fields of different intensities and directions. For this purpose the effect of vertically and horizontally applied electric fields on collagen synthesis was assessed by determining the hydroxyproline content in tissue and by histological examinations.

Materials and Methods

Sixty male white guinea pigs weighing 350–400 g were used for the study. The animals were divided into four separate groups and subjected to various intensities and directions of electric fields. Ten guinea pigs were in each group. The E field of 1.9 kV/m obtained from a DC (Direct Current) power supply with 300 V was applied in vertical direction to 10 guinea pigs (Group I), and in horizontal direction to other 10 guinea pigs (Group II). In the same manner, 0.9 kV/m obtained from a DC power supply with 150 V was applied in vertical direction to 10 guinea pigs (Group III), and in horizontal directions to other 10 guinea pigs (Group IV). The remaining 20 guinea pigs were used as control without any E field exposure, but otherwise maintained under the same conditions. The guinea pigs were exposed to E fields for 3 days, 9 hours/day (between 8 a.m. and 5 p.m.) in wooden cages (50 cm × 50 cm × 14 cm) with copper plates mounted vertically or horizontally over them. Neither the control group guinea pigs nor those exposed to E fields were taken out of the wooden cages for 3 days. Taking into consideration that placing more than one animal per cage would create a stress factor, only one animal was placed per cage. The intensity of the current was kept constant and controlled continuously throughout the experiment with a multimeter connected to the circuit.

Vertical and horizontal electric field circuit. In experiments with vertical electric field circuits, two copper plates of the same dimensions as the surfaces of the cage were mounted on the top and the bottom of the cages. The circuit was completed by connecting the positive outlet of the power source to the copper plate on top, and the negative outlet to the one at the bottom. For the horizontal electric field application, the copper plates were mounted to both sides of the cage. This circuit was completed by always connecting the positive outlet of the power source to the plate on the right hand side, and the negative outlet to the one on the left. At the end of day 3 of E field application, liver tissue samples were taken from all guinea pigs after decapitation, and hydroxyproline content was determined and histological examination was performed.

The modified Stegemann-Stalder method was used to determine hydroxyproline levels in the liver tissues of guinea pigs exposed to vertical and horizontal electric fields and controls (Stegemann and Stalder 1967). The principle of this method is to get the hydroxyproline of the tissue by hydrolyzation of the sample after homogenization and measuring the optical density of the color formed by adding p dimethylaminobenzaldehyde, perchloric acid and propan-2-ol at pH 8 and at $\lambda = 560$ nm. Hydroxyproline contents of the tissue samples were determined using the standard curves for samples containing known concentrations of hydroxyproline (Sigma H 1637).

Two samples were taken from each homogenized tissue and the concentrations measured by spectrophotometry were averaged.

Five tissue samples from each group and ten from the control group were taken for histological examinations. These tissue samples were cut into pieces of 1 mm^3 each and subjected to electron microscopic method by first being assessed with gluteraldehyde buffered with phosphate and then with OsO_4. Semi thin sections cut and stained with toluidine blue and examined under photomicroscope.

Results

For each group hydroxyproline contents of tissues from groups exposed to electric field and their controls were compared with DUNCAN test (Table 1) and the results are shown in Figs. 1 and 2.

Horizontal and vertical application of electric field of 0.9 kV/m decreased the hydroxyproline levels as compared to controls ($p < 0.05$ and $p < 0.01$ respectively) whereas 1.9 kV/m electric field increased ($p < 0.01$) the level in both application directions (Fig. 1). Both the horizontal and the vertical application of electric field

Table 1 Liver tissue hydroxyproline levels (μg/g tissue) in groups exposed to vertical and horizontal electric field and control group. The values in the table represent the least squares means ± standard deviation

Electric Fields		Hydroxyproline (micrograms/g tissue)
Intensity	Direction	x±S.D.
1.9 kV/m	Vertical	0.56 ± 0.19 ($n = 10$)
	Horizontal	0.46 ± 0.18 ($n = 10$)
0.9 kV/m	Vertical	0.08 ± 0.02 ($n = 10$)
	Horizontal	0.12 ± 0.03 ($n = 10$)
Control		0.26 ± 0.15 ($n = 20$)

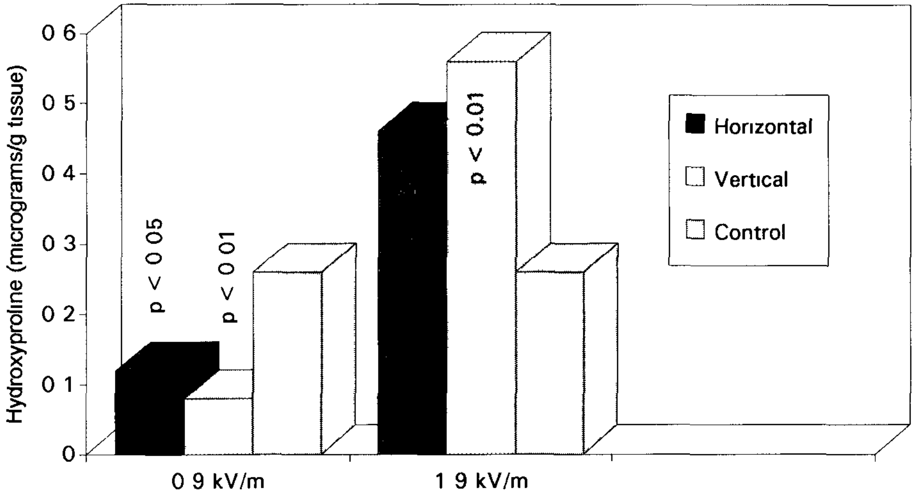

Figure 1. Changes in hydroxyproline levels upon the application of vertical and horizontal electric fields as compared to controls

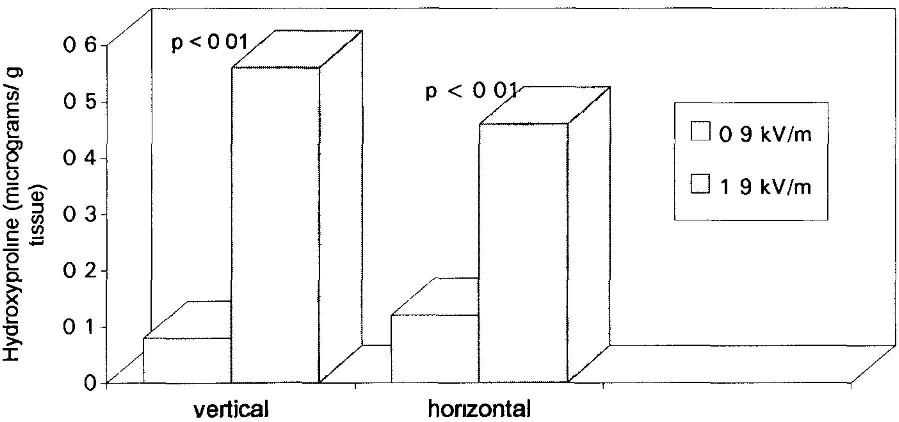

Figure 2. Differences in tissue hydroxyproline levels upon the application of electric fields in the same direction at two different intensities

of 1 9 kV/m increased ($p < 0\ 01$) hydroxyproline levels as compared to the effect of the electric field of 0 9 kV/m (Fig 2)

Vertical application of both electric fields was found more effective than the horizontal one, the differences were not statistically significant ($p > 0\ 05$) The result of the histological examinations of control tissues and those from animals

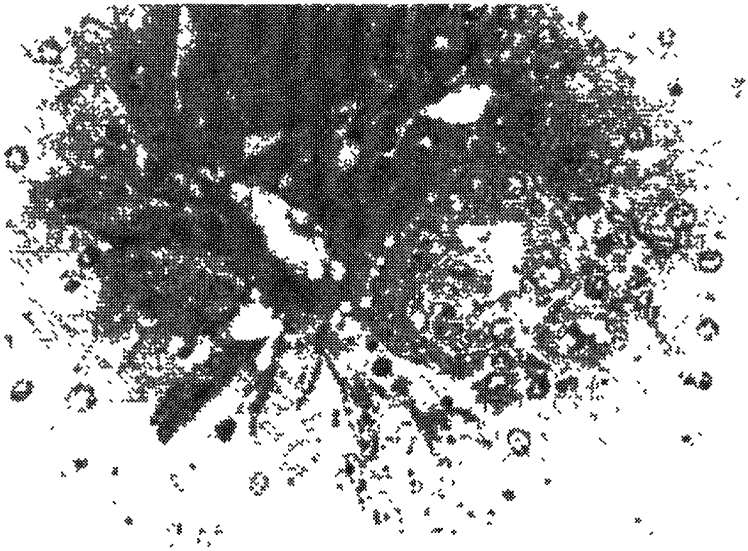

Figure 3. Control group, low magnification Toluidine blue ×400

Figure 4. Control group, high magnification Collagen fibers (arrows) Toluidine blue ×1000

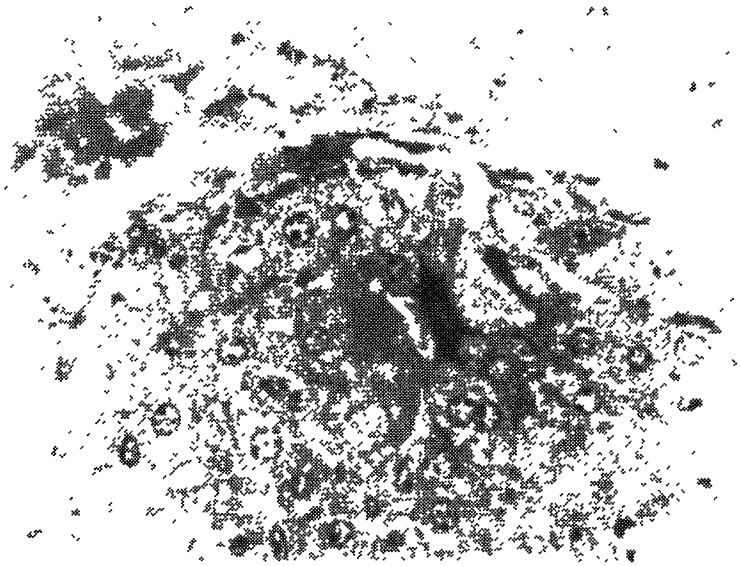

Figure 5. The group exposed to 0.9 kV/m vertical electric field (low magnification) Toluidine blue ×400

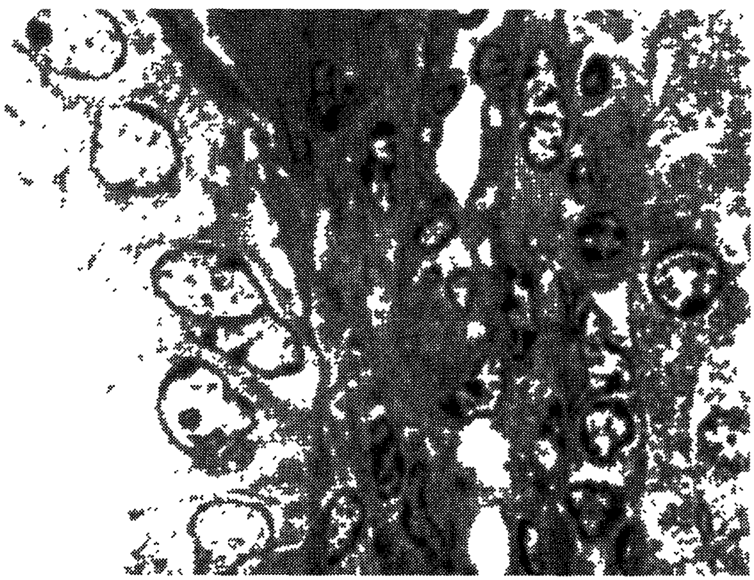

Figure 6. The group exposed to 0.9 kV/m vertical electric field (high magnification) The amounts of collagen fibers were decreased Toluidine blue ×1000

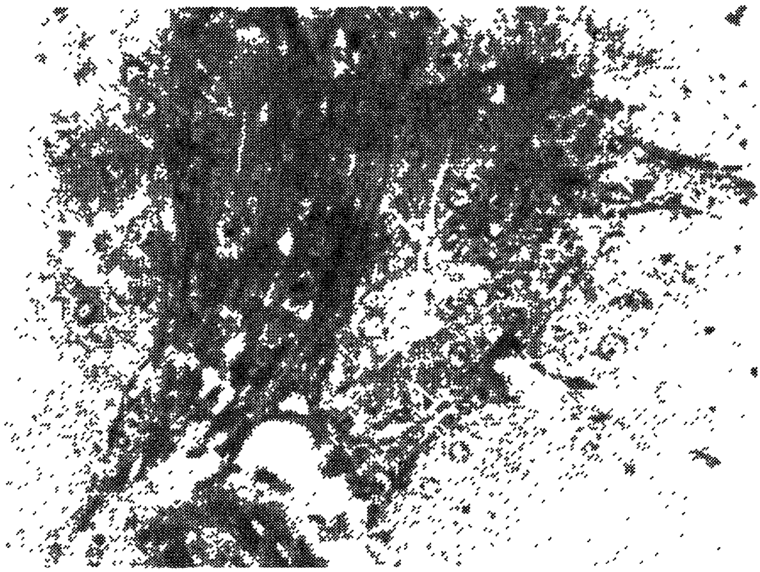

Figure 7. The group exposed to 1.9 kV/m vertical electric field (low magnification). Toluidine blue ×400

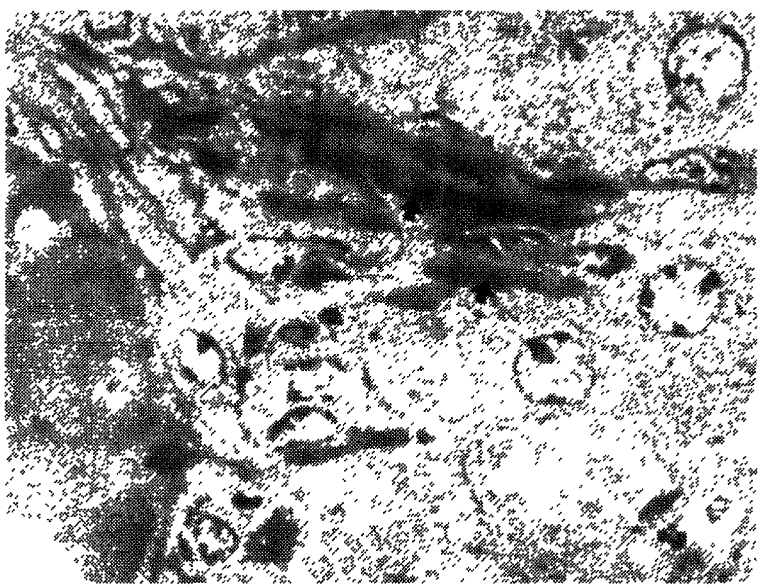

Figure 8. The group exposed to 1.9 kV/m vertical electric field (high magnification) The amounts of collagen fibers were increased (arrows). Toluidine blue ×1000

exposed to electric field supported our biochemical findings. In the control group the amount of collagen fibers was normal, particularly in the portal section where the connective tissue of the liver was dense (Figs. 3, 4). Under the effect of the vertical electric field of 0.9 kV/m a substantial decrease of collagen fibers was observed compared to controls (Figs. 5, 6), whereas an increase in collagen content of the connective tissue was evident under the effect of the vertical electric field of 1.9 kV/m (Figs. 7–8).

No histological difference was observed between the liver tissues of guinea pigs exposed to horizontal electric fields of 0.9 kV/m and 1.9 kV/m as compared to that of the control group.

Discussion

If 1 μA direct current is applied to a collagen solution soluble in acid, collagen forms a concave band near the cathode (Fukada 1983, Haupt 1984, Singh and Kartz 1986). This finding shows that collagen acts as a cation under E field.

Proteins with a net electrical charge move under the influence of electric field. Since they are of different molecular structure and shape they are subject of different frictional forces in a medium. For these reasons, different protein molecules move with different velocities in the same electric field. Amino and carboxyl groups are responsible for the net charge of a protein molecule. Secondly, proteins may gain positive or negative charge or they may have no charge according to the pH value of the medium. In the latter case proteins do not have the ability to move in an electric field. If the pH value of the medium is less than the isoelectric pH of the protein, then the protein posseses a net positive charge and moves in the direction of the electric field, whereas it gains a negative charge and moves in the opposite direction if the pH value of the medium is greater than the isoelectric pH of the protein.

The observed increase of hydroxyproline content under the influence of the electric field (1.9 kV/m) generated by applying 300 V DC may be explained by the above mentioned facts: since proteins have a net electric charge depending on the pH value of the medium, and therefore are mobile within the electric field, the fibroblast cells move along the electric field, thus synthesizing more collagen resulting in an increase in the HP content in the tissue. This increase in hydroxyproline content has also been substantiated with our histological findings. The dense appearance of the fibroblasts in the histological picture of the tissue from this group supports the increase in hydroxyproline content. In the livers of animals exposed to a vertical electric field of 1.9 kV/m, the increase in collagen fibers is quite obvious in the areas where the connective tissue is dense.

Studies have provided a clearer understanding of the mechanisms of absorption of electromagnetic energy by the tissues (Schwan 1957, Schwan and Piersol 1984).

Many major hormones including corticosterone, testosterone, and melatonin, have been examined under electric field in rats and mice (Phillips et al. 1979). The immunocompetence of animals exposed to electric field was observed in rats, mice, chicks and guinea pigs (Le Bars and Andre 1976, Morris and Phillips 1983). Changes in heart rate and blood pressure under electric field were also analyzed (Certelli and Malaguti 1976). Electric field intensity that will cause cell fusion and rotation has been reported to be in the range of 10–100 kV/m (Pohl 1978). A calcium efflux effect had also been reported from *in vivo* studies in cats (Adey 1980). Changes in EEG patterns (Blanchi et al. 1973), concentration of glial cell proteins and in blood cell populations have also been reported (Hansson 1981). Long term exposure to ELF and multi-generation studies revealed developmental defects (Phillips 1981, 1983). Changes in hydroxyproline level in chick embryonic tibia due to the effect of electric field were found by Fitzsimmons and Farley (1986). Nessler and Mass (1987) also found an increase in rabbit flexor tendon with the effect of *DC* microcurrent. Atalay reported enhanced collagen synthesis in wound tissue at *DC* current intensities of 200–400μA (Atalay Seyhan and Çelik 1985, Atalay Seyhan et al. 1986, 1988, 1992, Atalay Seyhan 1994, Canseven et al. 1994, Canseven and Atalay 1995, 1996). When low intensity direct current is applied to tissues, O_2 is consumed at cathode. Tissue O_2 tension decreases and an increase occurs in the number of hydroxyl radicals present (Brighton et al. 1977, Brighton 1981, Barker 1984). The increase in radicals can be traced with the variation in malondialdehyde (MDA) level. In our other study carried out in parallel to this one, contrary to 300 V both vertical and horizontal *E* fields of 150 V caused an increase in MDA levels in the liver and the medullar glands of guinea pigs, whereas a decrease in the hydroxyproline levels of the liver tissue was observed (Atalay Seyhan et al. 1994). Reassessing the results of this study from this point of wiev, we may say that as a result of energy transfer of electric field to the applied tissue area, molecular O_2 could have been transformed to free radicals. The increase in free radicals may decrease molecular O_2 which is required for hydroxyproline synthesis.

Also it has been reported that the direction the *E* field was important. Marino and Berger (1983) applied 19.7 V/m *E* field in vertical and horizontal directions to mice, and observed a big decrease in serum protein fractions in the group exposed to vertical *E* field and a slight change in the group exposed to horizontal *E* field. In his other study, Marino (1976) applied 15 kV/m vertical and 10 kV/m horizontal *E* fields to mice, and observed an increase in the mortality in the vertical *E* group as compared to the horizontal field group. Guler and Atalay found alterations in total blood protein levels, superoxide dismutase, ascorbic acid and hydroxyproline levels of the lung and kidney tissues (Atalay Seyhan 1994, 1995, Atalay Seyhan and Guler 1994, Atalay Seyhan et al. 1994, Guler et al. 1994, 1995, 1996a,b,c,d, Guler and Atalay Seyhan 1995a,b, 1996a,b). In our present study, the decrease and increase in tissue hydroxyproline levels under the influence of the vertical electric field were stronger than under the horizontal electric field

Since the liver hydroxyproline content increased under 1.9 kV/m and decreased under 0.9 kV/m there may be the threshold electric field value in the transition from decrease to increase between 1.9 0.9 kV/m Studies using small increases between 1.9 kV/m and 0.9 kV/m may reveal this threshold value

References

Adey W R (1980) Frequency and power windowing in tissue interactions with weak electromagnetic fields Proc IEE *E* **68** 119—125

Atalay Seyhan N (1994) What is the mechanism of wound healing with the effect of electric current? Gazi Med J **5** 99—103

Atalay Seyhan N (1995) Does electric field effect collagen synthesis in tissue? Gazi Med J **6** 1—6

Atalay Seyhan N Çelik S (1985) The effects of direct current on bacterial growth Med Biol Eng Comput **23** Suppl 1 100—101

Atalay Seyhan N Guler G (1994) The effect of horizontal and vertical electric fields on lung 6th National Biophysics Congress Silivri/Istanbul September 28—30 Proceedings p 64 (Turkish)

Atalay Seyhan N Aydin G Pamuk F (1986) The effects of alternating current on bacterial growth IEEE/VIII Annu Conf Eng Med Biol Soc Fort Worth Dallas November 7 10 Proceedings CH2368 9/86/00 pp 1398 1399

Atalay Seyhan N Aydın G Işık B Toktaş S (1988) Low intensity constant direct current effect on wound healing World Congr Med Phys Biomed Eng San Antonio Texas August 6-12 Proceedings p 312 Medical Physics May/June Vol **15** p 464

Atalay Seyhan N Aydın G Işık B Toktaş S (1992) Accelerating wound healing with electrical current and the effect of current on tissue hydroxyproline content J Biochem Special Issue Vol **XVII** Suppl B 15

Atalay Seyhan N Guler G Koz M Gonul B (1994) The effect of electric field on malondialdehyde (MDA) levels of the adrenal gland Medical J Turkiye **1** 161—167 (Turkish)

Barker A T (1984) Some biological effects of low frequency magnetic and electric fields In Proceedings of the II International Conference on Applications of Physics to Medicine and Biology (Eds Z Bajzer P Baxa C Franconi) pp 421- 434 World Scientific Publ Company Tokyo

Blackman C F Benane S G (1982) Effects of ELF fields on calcium ion efflux from brain tissue *in vitro* Radiat Res **9** 510—520

Bianchi C L Cedrini L Ceria F Meda E Re G (1973) Exposure of mammalians to strong 50 Hz electric fields 2 Effects on heart and brain electric activity Arch Fisiol **70** 30—34

Brighton C T (1981) The treatment of non unions with electricity J Bone Joint Surg Amer **63**, 847—851

Brighton C T Friedenberg Z B Mitchell E I Booth R E (1977) Treatment of nonunion with constant Direct Current Clin Orthop **124** 106—123

Canseven A Atalay Seyhan N (1995) The interaction of electric current - collagen synthesis in wound healing I, Medical J Turkiye **2** 71—77

Canseven A G , Atalay Seyhan, N (1996) Is it possible to trigger the collagen synthesis by electric current in skin wounds? Indian J Biochem Biophys **33**, 223—227 (Turkish)

Canseven A G , Guler G Atalay Seyhann N (1994) Biochemical changes in skin with the effect of low intensity direct current (LIDC) World Congress on Medical Physics and Biomedical Engineering, Rio de Janeiro Brazil, August 21—26, Phys Med Biol , **39a** p 767

Cerretelli P , Malaguti C (1976) Research carried out in Italy by ENEL on the effects of high-voltage electric fields Rev Gen Electr Numero special (juillet), 65—74

Fitzsimmons R J , Farley J (1986) Embryonic bone matrix formation is increased after exposure to a low-amplitude capacitively coupled electric field *in vitro* Biochem Biophys Acta **882** 51—56

Fukada E (1983) Piezoelectric properties of organic polymers Ann N Y Acad Sci **405**, 7—25

Gavalas-Medici R J , Walter D O (1970) Effect of low level low frequency electric fields on EEG and behavior in macaca nemestrina Brain Res **18**, 491—501

Gavalas-Medici R J Bawin S M Adev W R (1973) Effects of modulated very high frequency fields on specific brain rhythms in cats Brain Res **58** 365—384

Guler G Atalay Seyhan N (1995a) A new approach for altering superoxide dismutase activity Electric field exposure International Congress on Free Radicals in Health and Disease Istanbul September 6--10 Programme & Abstracts p 176

Guler G Atalay Seyhan N (1995b) Does surrounding electric fields effect protein synthesis 21st National Physiology Congress Ankara September 26 28 Proceedings p 125 (Turkish)

Guler G Atalay Seyhan N (1996a) The effect of vertical and horizontal electric fields on collagen synthesis Progress in Biophysics & Molecular Biology XII International Biophysics Congress, Amsterdam 11—16 August **65,** Supplement 1, p 215

Guler G Atalay Seyhan N (1996b) Changes in hydroxyproline levels in electric field tissue interaction Indian J Biochem Biophys **33** 531—533

Guler G , Canseven A G , Atalay Seyhan N (1994) *DC* Electric field effect on tissue hydroxyproline level World Congress on Medical Physics and Biomedical Engineering Rio de Janeiro Brazil 21—26 August Phys Med Biol **39a** p 767

Guler G Atalay Seyhan N Dinçer S Koz M Gonul B (1995) The effect of *DC* electric fields on protein synthesis, ascorbic acid level and radicals VII National Biophysics Congress Adana October 11—13 Proceedings p 16 (Turkish)

Guler G , Atalay Seyhan N Ozogul C Erdogan D (1996a) *DC* electric field effect on structure of tissues VIII National Biophysics Congress Gebze/Kocaeli September 26—28 Proceedings p 32 (Turkish)

Guler G Atalay Seyhan N Omeroglu S , Erdogan D (1996b) The effect of AC electric field on bone structure VIII National Biophysics Congress Gebze/Kocaeli September 26 –28 Proceedings, p 25 (Turkish)

Guler G Atalay Seyhan N , Bor V Çevik C (1996c) The effect of electric fields on blood Ca^{++} $Cl^{-}$and P^{+} levels VIII National Biophysics Congress Gebze/Kocaeli September 26—28 Proceedings p 23 (Turkish)

Guler G Atalay Seyhan N Altan N , Gonul B Çevik C (1996d) Tissue response to electric fields with different intensities and directions Progress in Biophysics & Molecular Biology, XII International Biophysics Congress Amsterdam 11—16 August, **65,** Supplement 1, p 215

Hansson H A (1981) Purkinje nerve cell changes caused by electric fields Ultrastructural studies on long term effects on rabbits Med Biol 59 103—110
Haupt H A (1984) Electrical stimulation of osteogenesis South Med J 77 56 64
Kaczmarek L K Adey W R (1974) Weak electric gradients change ionic and transmitter fluxes in cortex Brain Res 66 537—540
LeBars H Andre G (1976) Biological effects of an electric field on rats and rabbits Rev Gen Electr Numero special (juillet) 91—97
Marino A A Berger T J (1983) Electric field effects in selected biological systems Ann N Y Acad Sci 405 136—144
Marino A A Becker R O Ullrich B (1976) The effect of continuous exposure to low frequency electric fields on three generations of mice A pilot study Experientia 32 565—566
Morris J L Phillips R D (1983) Effects of electric fields on specific humoral and cellular components of the immune systems Bioelectromagnetics 4 294
Nessler J P Mass D P (1987) Direct current electrical stimulation of tendon healing *in vitro* Clin Orthop 217 303—312
Phillips R D (1981) Biological effects of 60 Hz electric fields on small and large animals In Biological effects of static and low frequency electromagnetic fields Symposium of the US/USSR Scientific Exchange Program on Physical Factors Kiev USSR May 1—8 Kiev
Phillips R D (1983) Biological effects of electric fields on miniature pigs In Proceedings of the Fourth Workshop of the US/USSR Scientific Exchange on Physical Factors in the Environment June 21—24 Research Triangle Park North Carolina National Institute of Environmental Health Sciences
Phillips R D Gillis M F Kaune W T Mahlum D D (1979) Extremely Low Frequency (ELF) Fields Environmental Health Criteria 35 World Health Organization Geneva
Pohl H A (1978) Dielectrophoresis The Behavior of Matter in Non-Uniform Electric Fields London Cambridge University Press
Schwan H P (1957) Electrical properties of tissue and cell suspensions In Advances in Biological and Medical Physics (Eds J H Lawrence C A Tobias) pp 147 209 Vol 5 Academic Press New York
Schwan H P Piersol G M (1984) The absorption of electromagnetic energy in body tissues In Biological Effects of Electromagnetic Radiation (Ed J M Osepchuk) pp 6—22 IEEE Press New York
Sheppard A R Eisenbud M (1977) Biological Effects of Electric and Magnetic Fields of Extremely Low Frequency New York University New York
Singh S Katz J L (1986) Scientific basis of electro-stimulation J Bioelectricity 5 285 327
Stegemann H Stalder K (1967) Determination of hydroxyproline Clin Chim Acta 18 267—273

Final version accepted December 13 1996