The Effects of *In Vitro* Electrical Stimulation on Eukaryotic Cells: Suppression of Malignant Cell Proliferation

G. D. O'Clock, Ph.D. (E.E.), P.E.

Abstract

For more than 16 years an electrotherapeutic technique developed by Dr. Björn Nordenström has been effectively utilized in the treatment of a variety of cancerous conditions. This technique offers a relatively comfortable, low cost and minimally toxic approach toward the treatment of cancer, and it also provides an alternative treatment for malignancies that have developed a resistance to conventional therapeutic approaches. Many of the mechanisms that have been proposed for the tumor regression/remission results achieved with Nordenström's electrotherapeutic technique have been associated with the attraction of white blood cells to the tumor site, pH gradients, water starvation and gas formation. The in vitro *results reported in this paper strongly indicate that some of the mechanisms associated with electrotherapy may also be occurring at the cellular level.*

Introduction

An appreciable amount of electrical and electrochemical activity occurs in living cells. It may be proposed that electrical stimulation of cells can have harmful and/or therapeutic effects depending upon the method of stimulation and the type, magnitude and frequency of the stimulating source.[1-10]

From a Western medicine point of view, electrical stimulation of living systems for therapeutic applications dates back to the Franklin era (1745) with the development of the Leyden jar. [6] A variety of electrical techniques have been applied to the treatment of cardiovascular disease, skin problems and cancer since the mid-1800s.[6] Formal medical research dealing with the electrical stimulation of living cells has been reported since the early 1920s.[7-9] Much of this research involved the effect of electrical currents on cell properties and cellular multiplication. The effects of electric fields,[10,11] magnetic fields,[12,13] and electromagnetic fields[14,-16] on cell properties and cell growth have been studied since the early 1960s.

From an Oriental medicine point of view, stimulation of living systems (non-electrical and electrical) for therapeutic applications has roots in acupuncture, dating back almost 5,000 years. One of the first records of acupuncture treatment, the Nei Ching, was written around 2600 BC.[17,18]

A significant number of scientists and medical doctors have published the results of their research on the therapeutic potential of various forms of electro and magneto therapy. Albert Szent-Györgyi, who won the Nobel prize for his discovery of vitamin C, described semiconduction processes in proteins and proposed "putting electricity back into living matter." [19, 20] Dr. Robert Becker, an orthopedic surgeon, developed a number of theories on cell dedifferentiation, cell and organ regeneration, fracture healing in bone and electrical activation of repressed genes.[20] In the early 1980's, Dr. Björn Nordenström introduced the first systems approach to describe electrically driven life processes when he introduced the concept of biologically closed electric circuits (BCEC).[1]

Nordenström essentially "closed the loop" with respect to the electrical activity in living systems and described a closed system of adaptive electrical circulatory sys-

1. College of Science, Engineering and Technology, Mankato State University, Mankato, MN, 56002-8400 USA

tems that maintain and regulate various functions in living systems and promote healing processes as well. Nordenström utilized these concepts to develop an electrotherapeutic technique (electrochemical therapy) to treat cancer.[1, 2, 21, 22]

Nordenström's BCEC concept may extend into the cell as well. The BCEC conductive path in the human body utilizes combinations of blood, blood vessels, interstitial fluid and nerve fiber. At the cellular level, the BCEC conductive path could involve the cytoskeleton, cytoplasm and the membranes of the cell and nucleus.

The concept of biologically closed electric circuits provides a fundamental way to describe circulation of energy in living systems. BCEC also provides the self-regulating mechanisms that manipulate energy to activate the healing process. Nordenström's concepts appear to have formed the strong links and interconnections that will ultimately bind Oriental and Western medicine.

Background

Electrotherapeutic techniques utilized in the treatment of well defined breast and lung tumors have been quite successful over the past 16 years. A number of electrotherapeutic approaches were developed to destroy malignant tumors with localized high frequency alternating current spark techniques in the early 1900s.[23] Inducing tumor regression with the absorption of non-ionizing electromagnetic waves for frequencies in the range of 20 MHz to 3 GHz (hyperthermia) has also been modestly successful for carcinoma of the breast and cervix, especially for tumors that have not responded to x-ray radiation therapy. [5]

The application of direct current to needle electrodes (galvanopuncture) has been used to treat aneurysms as early as 1849. [24] An extension of galvanopuncture has been developed and successfully utilized by Nordenström with percutaneously applied platinum electrodes and direct current electrical stimulation to decrease the size of breast tumors.[1,2,25] Five year survival rates for breast cancer patients treated with Nordenström's electro-chemical therapy (ECT) approach have been in excess of 60%.[4] Also, combining herbal/nutritional therapy with ECT appears to add another 5% to 10% to the five year survival rate for cancer patients.

Nordenström's characterization of the electrical processes occurring *in vivo* include a variety of mechanisms that are driven by electrochemical polarization of tissue.[21,25] The driving force promotes the transport of charged material by dielectrophoresis.[1,21] Coupling these mechanisms with electroösmosis, Nordenström describes an additional circulatory system for electrogenous mass transport between blood and tissue, or a vascular-interstitial closed electric circuit.[21]

Figure 1. Nordenström's Electrochemical Therapy approach to tumors.

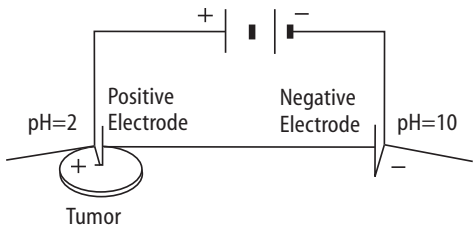


Figure 2. Schematic of the excitation/measurement approach to cell proliferation.

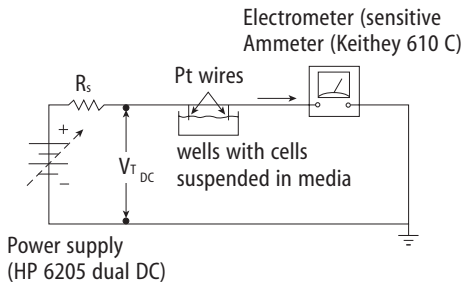


Table 1. Mechanisms associated with *in vivo* tumor size reduction or remission with the application of electrochemical therapy

•With a positive electrode inserted into the tumor, cancer-fighting white blood cells are attracted to the site of the tumor because white blood cells possess a negative charge on their membrane surface.
•Autolysis processes at the positively biased tumor site cause a significant decrease in pH, which help to promote necrosis in the tumor. Also, the increase in acidity at the tumor site appears to damage red blood cells, inhibiting delivery of oxygen to the tumor.
•The electric field at the tumor site draws water away from the tumor (electroösmosis). The water starvation stresses the poorly formed tumor vascular system and causes the tumor to shrink, thereby constricting tumor blood vessels and interfering with the tumor's blood supply.
•Cathodic and anodic gas formation (H_2, Cl_2 and O_2) elevates the pressure in the cancerous tissue and can produce further stress on the structure and blood supply of the tumor.

One of Nordenström's ECT techniques involves the insertion of a platinum electrode near the center of the tumor mass, and the insertion of another platinum electrode in healthy tissue several centimeters away from the tumor site. The electrodes are connected to a DC voltage source, with the tumor electrode connected to the positive terminal and the healthy tissue electrode connected to the negative terminal (**Figure 1**, p. 174). Over a treatment period of 6 weeks to 6 months, for most of the cancer patients, tumor reduction or remission occurred at direct current levels less than 40mA at DC voltages less than 20V. [1]

The mechanisms behind the tumor reduction or remission results observed using Nordenström's ECT technique are usually identified with large-scale effects associated with the attraction of white blood cells to the tumor site, pH gradients, water starvation and gas formation as shown in **Table I** (above). [22]

Our *in vitro* research results strongly indicate that some of the mechanisms associated with electrochemical therapy induced tumor necrosis may be occurring at the cellular level.[26-28]

Experimental Procedure

Normal and malignant cells were electrically stimulated with low-level direct currents in a microtiter plate (**Figure 2**, p. 174).

Normal splenocyte cell suspensions were obtained from DBA/2J male mice sacrificed by cervical dislocation. The cells were suspended in RPMI 1640 media with 10% fetal bovine serum. Initially, two different types of malignant cells were evaluated. Murine EL4 lymphoma cells were obtained from Dr. T. Whiteside of the Pittsburgh Cancer Institute, Pittsburgh, PA. The EL4 cells were suspended in RPMI 1640 media with 5% fetal bovine serum. An IL-6 hybridoma melanocyte line was purchased from American Type Culture Collection (ATCC), Rockville, MD. The hybridoma cell line was maintained in RPMI 1640 media, with 10% fetal bovine serum supplemented with 5μM 2-mercaptoethanol. One and one half milliliters of cell suspension were added to the appropriate wells of 24-well tissue cul-

Figure 3. Plot of an *in vitro* splenocyte cell proliferation (compared with control) vs. DC stimulation current using 2 mm diameter platinum wire electrodes in a mixture of RPMI 1640 media with 10% fetal bovine serum ($5 \cdot 10^6$ cells/ml initial concentration). Error bars for standard error of estimate are also shown.

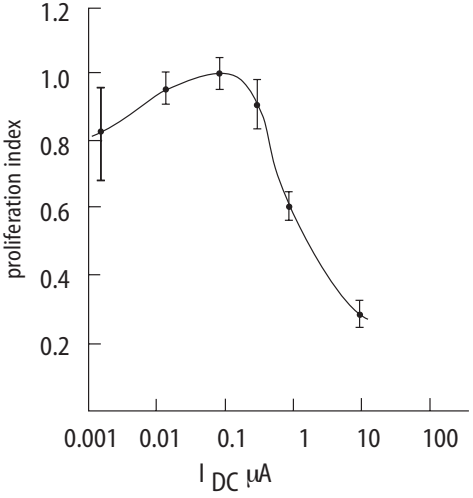


Figure 4. Proliferation of IL-6 hybridoma melanocyte cells (compared with control) vs. DC stimulation current in a mixture of RPMI 1640 media with 10% fetal bovine serum using 2 mm diameter platinum wire electrodes. Error bars for standard error of estimate are also shown.

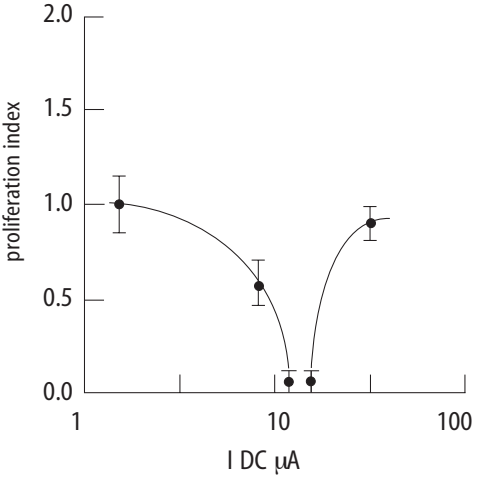


Figure 5. Plot of an *in vitro* EL4 lymphoma cell proliferation [16] (compared with a control) vs. DC stimulation current using 2 mm diameter platinum wire electrodes in a mixture of RPMI 1640 media with 5% fetal bovine serum (10^6 cells/ml initial concentration). Error bars for standard error of estimate are also shown. (with permissionfrom *J Nat Cancer Inst*, Vol 83,.

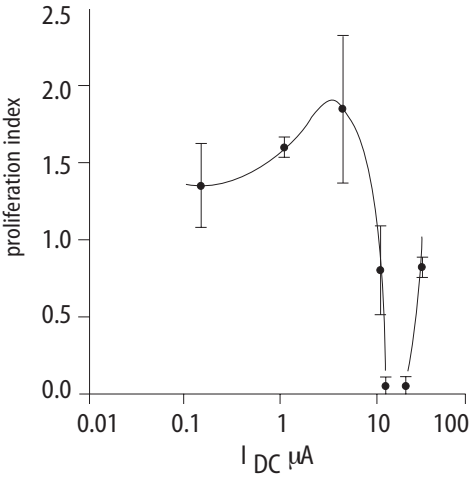
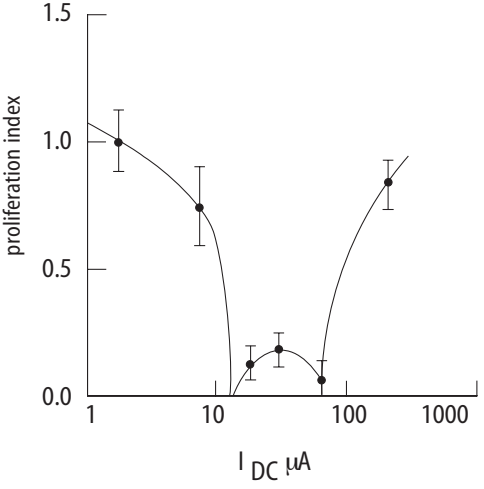


Figure 6. Plot of an *in vitro* EL4 lymphoma cell proliferation (compared with a control) vs. DC stimulation current using 2 mm diameter platinum wire electrodes in a mixture of RPMI 1640 media with 10% fetal bovine serum ($0.66 \cdot 10^6$ cells/ml initial concentration). Error bars for standard error of estimate are also shown.



ture cluster plates, and electrically stimulated in an incubation chamber at 37°C in an atmosphere of 95% air and 5% carbon dioxide for 16-24 hours.

Platinum wires, 2 mm in diameter, spaced approximately 0.8 cm apart and immersed in the cell growth medium were utilized as stimulating electrodes. After the incubation period, the cell proliferation characteristics were measured and compared with various controls. Cell proliferation was assayed using liquid scintillation counter measurements of radioactive thymidine uptake into newly synthesized DNA.

The cell proliferation index provides a method to compare proliferation characteristics for different stimulation currents and different cell species. The proliferation index is the ratio of the electrically stimulated cell population density over the non-stimulated cell population density (control).

$$\text{Proliferation Index} = \frac{\text{population density measurement for electrically stimulated cells using the liquid scintillation counter}}{\text{population density measurement for non-stimulated cells (control) using the liquid scintillation counter}}$$

Results

Data obtained for different kinds of eukaryotic cells indicate that stimulation with low-level direct currents can promote or inhibit cell proliferation. For instance, non-malignant murine splenocytes exhibit a region of promotion, reaching a maximum value at a certain direct current magnitude (**Figure 3** p. 176). Then, as the direct current is increased further, the data shows a steady increase in the amount of suppression for the cell proliferation characteristics. Electrical stimulation data obtained for a number of different types of malignant cells show significantly different cell proliferation characteristics compared with the non-malignant cell proliferation data.

Measurements obtained for the proliferation characteristics of the IL-6 hyridoma and EL4 lymphoma cell lines indicate that a window of suppression exists for low-level direct currents in the range of 10μA to 20μA as shown in **Figures 4 and 5**. (p. 176) The amount of proliferation suppression for the malignant cells (compared with controls) is approximately 95% to 99.9% within the 10μA wide windows of suppression.

The reverse transcriptase polymerase chain reaction (RT-PCR) technique was utilized in an attempt to measure the production of cytokines from immunologically responsive white blood cells that are also under the influence of the low-level direct currents. Macrophage cells were stimulated with different levels of direct current for approximately 24 hours. Some of the cells were stimulated with low current (approximately 0.55 μA), others were stimulated with a higher current (approximately 5μA). A current of 5μA appears to be close to the value of the maximum useful current because one of the high current samples did not register any housekeeping gene band at all (**Figure 7**, p.178). Apparently, the high current caused the cells to break apart (lyse).

In this case, the electrical stimulation can have an effect on cytoplasmic chemical processes, organelles and the nucleus. It has been proposed that electromagnetic fields associated with frequencies in the range of 10 Hz to 100 Hz could interact directly with DNA, producing intramolecular charge movements resulting in gene activation and transcription activity.[31] Higher frequencies (such as those associated with millimeter wave electromagnetic fields) can induce chromosomal damage.[32] As the frequency increases even further to the ultraviolet range, select regions of DNA strands can be broken and recombined. [33]

Therefore, as the frequency of the electrical of electromagnetic stimulating source increases, its effect tends to progress from the membrane to the cytoplasm to the nucleus as shown in **Table 2** (p. 178).

Table 2. Impact of electromagnetic waves on biological systems.

- At DC, the primary interaction appears to be at the receptor and cell membrane level.
- At ELF frequencies, the effects appear in the cytoplasm and nucleoplasm.
- Radio frequencies (RF) affect a variety of processes and systems at the cellular and organ level.
- At millimeter wave frequencies, the effects are noticed at the level of the organelle and chromosome.
- Electromagnetic waves at sub millimeter, infrared and visible wave lengths would tend to be absorbed at body surfaces.
- Photo dynamic therapy (at 630 nm - laser light) can be implemented by combining visible light frequencies and photosensitive drugs (hematoporphyrin or dihematoporphyrin)– which destroy the tumor's network of blood vessels by raising porphyrin levels. (The drugs mimic hemoglobin and attach to structures with rich blood supplies.) This is applied toward cancers of esophagus, colon, rectum and bronchial passages.
- Electromagnetic waves at U.V. wavelengths (~200 nm) can affect selected regions within a DNA strand causing thymine dimerization (through cross linking).
- Electromagnetic waves at X-ray and g ray wavelengths (<1 nm) can interact directly with a DNA molecule or indirectly (producing free radicals) which will damage a DNA strand. g rays can cause thymine dimerization, cleavage of ribose sugar - base bonds and cleavage of phosphodiester bonds.

Figure 7. Housekeeping gene bands for ß-actin from electrically stimulated macrophage cells. Starting from the left, the first two bright bands are the controls (cells not electrically stimulated); the second two bright bands are associated with the low current (approximately 0.55 μA) stimulated cells; the last bright band to the right is associated with one of the high current (approximately 5 μA) stimulated cell suspensions. The band in the sixth position (high current cell suspension) is missing. These cells appear to have lysed under the influence of the higher current.

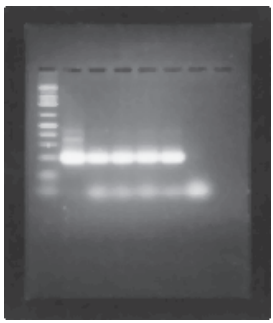


Table 3. Some of the cell membrane structures that can be affected by direct current stimulation.

- Cyclic AMP receptor - Impacts glycolysis, cell aggregation, cell differentiation, cell proliferation, inhibits tumor growth in mammalian cells.
- Glucocorticoid receptor - Regulates gene transcription, cell differentiation and proliferation.
- Na^+/H^+ antiporter - Regulates cell pH in virtually all cells.
- Ion channels - Control cell pH and polarity.
- Immunological system receptors - Assist in proliferation of T-cells, B-cells and antibody production. Serve as identification badges for immune system.

The pronounced windows of suppression for malignant cell proliferation (shown in **Figures 4-6**, p. 176) strongly indicate that ECT induced tumor size reduction and remission also involve mechanisms at the cellular level. A variety of cell structures and constituents could be contributing to the suppressed proliferation characteristics for the malignant cells including hormonal and immunological receptors and ion channels. Oncogene derived proteins could also be incorporated into malignant cell membrane structures initiating a variety of events that contribute to the suppressed proliferation characteristics of malignant cells under electrical stimulation conditions. Media pH variations produced electrically can have a significant and very different impact on malignant cell proliferation compared with normal cell proliferation. There are a variety of cell structures that an electrical stimulus can affect, as shown in **Table 3** (above).

In addition, a number of ECT practitioners have noticed increases in therapeutic effectiveness in the range of 5% to 10% when ECT is combined with herbal/nutritional therapy.[34] The effect of combining herbal/nutritional therapy with conventional radiation therapy yields even larger increases in cancer survival rates. One study involving 272 nasopharyngeal cancer patients reported a five year survival rate of 67% for the patients who received the combined therapies vs. 48% for those who received radiation therapy alone. [35,36]

Certain herbs (and herbal combinations), used in Traditional Chinese Medicine, appear to stimulate the immune system and inhibit metastasis. These include Rhizoma Atractylodis Macrophalae (*bai zhu*), Radix Ginseng (*ren shen*), Tuber Dioscoreae Bulbiferae (*huang quin*) and Radix Astragali (*hang qui*). [35] Herbal and non-herbal antioxidants such as Turmeric (*Curcuma Ionga*), Schizandra (*Schizandra chinensis*), vitamin C (10 g to 200+ g/day administered orally and injected), Vitamin E (d alpha tocopherol at 4000+ IU per day), coenzyme Q10 (300+ mg/day) and vitamin A (less than 250,000 IU per day to minimize liver toxicity problems) appear to complement therapeutic techniques in the treatment of cancer. [35,37-39]

Conclusions

A considerable amount of research done with cell stimulation indicates that for direct currents and voltages, the primary electrical effects associated with electrical

stimulation involve cell membranes and cell receptors.

Nordenström has discussed a number of mechanisms that can induce necrosis and inhibit the proliferation of cancer cells under the influence of low-level direct currents. Our results indicate that the proliferation of certain eukaryotic cell species can be controlled with low-level direct current stimulation. Also, it appears that the proliferation of certain malignant cells can be inhibited by low-level direct currents. The magnitude of malignant cell suppression compared with normal cell suppression produced within the same low-level direct current range suggests the possibility of optimizing and extending the therapeutic techniques pioneered by Nordenström in the mid 1970s.

The therapeutic value of the electrical stimulation approach will be dependent upon the ability to operate at current stimulation levels and proliferative states that will maximize malignant cell suppression while minimizing suppressive effects for normal cells. Our data indicates that for different cells of the same species, there can be a considerable amount of variability in the response to an electrotherapeutic approach, just as there is for chemotherapy and radiation therapy.

Acknowledgments

The author wishes to thank Dr. Mark Lyte, Director of Clinical Laboratory Sciences/Medical Technology, Mankato State University for his valuable assistance in this research effort. The author also wishes to express his appreciation to graduate students Kien T. Nguyen and Bernard Arul for their assistance. This work was supported by a Mankato State University Faculty Research Award.

References

1. Nordenström BEW: *Biologically Closed Electric Circuits.* Stockholm, Sweden: Nordic Medical Publications, 1983.
2. Nordenström BEW: Electrochemical treatment of cancer: variable response to anodic and cathodic fields. *Am J Clin Oncol*, 1989; 12: 530–536.
3. Xin, YL: Organization and spread of electrochemical therapy (ECT) in China. *Eur J Surg*, (Suppl.) 1994; 574: 25-29.
4. Xin, YL: Advances in the treatment of malignant tumors by electrochemical therapy (ECT), *Eur J Surg*, (Suppl.) 1994; 574: 31-36.
5. Hall EJ: *Radiology for the Radiologist, 3rd Ed.* Philadelphia, PA, J.B. Lippincott Co. 1988.
6. Geddes LA: The beginnings of electromedicine. IEEE *Engineering in Medicine and Biology Magazine*, 1984; 3: 8-23.
7. Ingvar S: Reaction of cells to the galvanic current in tissue cultures. *Soc Exper Biol Med Proc*, 1919-20: 17: 198–199.
8. Huzella T: Electrical phenomena in tissue cultures in relation to organization. *Arch Exper Zellforsch,* 1934: 15: 250–254.
9. Ingvar D: Experiments on the influence of electric current upon growing nerve cell processes in vitro,. *Acta Physiol Scand,* 1947; 13: 150–154.
10. Klee M, Plonsey R: Stimulation of spheroidal cells-the role of cell shape. *IEEE Transactions on Biomedical Engineering,* BME-23: 1976; 347–354.
11. Bernhardt J and Pauly H. On the generation of potential differences across the membranes of ellipsoidal cells in an alternating electric field. *Biophysik,* 1973; 10: 89–98.
12. Semm P, Schneider T and Vollrath L. The effects of an earth-strength magnetic field on the electrical activity of pineal cells. *Nature,* 1980; 288: 607–608.
13. Yuan Z, Huang S and Pan Z. Magnetic field and tumor. *IEEE Transactions on Magnetics,* 1980; MAG-16: 824–829.
14. Webb SJ and Booth AD. Microwave absorption by normal and tumor cells. *Science,* 1971; 174: 72–74.
15. Lyle DB, Schechter P, Adey WR and Lundak RL. Suppression of t-lymphocyte cytotoxicity following exposure to sinusoidally amplitude-modulated fields. *Bioelectro Mag*, 1983; 4: 281–292.
16. Colacicco G, Pilla AA: Electromagnetic modulation of biological processes: ATPase function and dna production by raji cancer cells in vitro,. *Z Naturforsch*, 1083; 38: 468–470.
17. Duke M: *Acupuncture.* New York. Pyramid House, 1972.
18. Manaka Y and Urquhart IA. *The layman's guide to acupuncture.* New York. Weatherhill, 1972.
19. Szent-Györgyi A: *Bioenergetics*. New York. Academic Press, Inc., 1957.
20. Becker RO , Selden G: *The body electric.* New York. William Morrow and Co. 1985.
21. Nordenström BEW: An additional circulatory system: vascular-interstitial closed electric circuits (VICC). *J Biol Phys,* 1987; 15: 43–55.

22. Nordenström BEW: Survey of mechanisms in electrochemical treatment (ECT) of cancer. *Europ J Surg, Suppl.* 1994; 574: 93-109.
23. Doyen E: Sur le destruction des tumeurs canceruses accessibles. *Arch Elect Med.et de Physiol*, 1909; 17: 791–795.
24. Petrequin JE: Traitement de certains aneurysmes. *Bull Gen de Therap,* 1849; 1-9.
25. Nordenström BEW: Biokinetic impacts on structure and imaging of the lung: the concept of biologically closed electric circuits. *Am J Roentgenol,* 1985; 145: 447–467.
26. O'Clock GD: *Studies of the effects of in vitro electrical stimulation on eukaryotic cell proliferation.* MA Thesis (Biological Sciences), Mankato State University, Mankato, MN, 1991.
27. Lyte M, Gannon JE, O'Clock Jr GD: Effects of in vitro electrical stimulation on enhancement and suppression of malignant lymphoma cell proliferation. *J Nat Cancer Inst,* 1991; 83: 116–119.
28. O'Clock Jr GD, Lyte M: Potential uses of low-level direct current electrotherapy for the treatment of cancer. *Proceedings of the 15th Annual International Conference of the IEEE Engineering in Medicine and Biology Society,* San Diego, CA, Part 3: 1993; 1515-1516.
29. Polk C, Postow E: *Handbook of biological effects of electromagnetic fields.* Boca Raton, FL, CRC Press, 1986.
30. Poo MM, Poo WJ, Lam JW: Lateral electrophoresis and diffusion of Concanavalan A receptors in the membrane of embryonic muscle cell. *J Cell Biol,* 1978; 76: 483–501.
31. Blank M, Goodman R: Do electromagnetic fields interact directly with DNA? *Bioelectromagnetics,* 1997; 18:111-115.
32. Lerner EJ: Biological effects of electromagnetic fields. *IEEE Spectrum*, 1984; 21: 57-69.
33. Russel PJ: *Genetics.* Boston. Brown and Co. 1986.
34. Zhou S: Treatment of cancers using ECT combined with Chinese herbs. Second International *Symposium for biologically closed electric circuits,* Jupiter, FL, October 29 -November 2, 1995.
35. Boik : Cancer & Natural Medicine. Oregon Medical Press, Princeton, MN 1996.
36. Li L, Chen X, Li J: Observations on the long-term effects of "yi gi yang yin decoction" combined with radiotherapy in the treatment of nasopharyngeal carcinoma. *1992; J Trad Chinese Med,* 12: 263-26.
37. Weiner MA: Herbal antioxidants in clinical practice. *J Orthomol Med,* 1994; 9: 167-176.
38. Zwelling M: Price, Prejudice and Vitamin C. *J Orthomol Med,* 9: 1994; 140-144.
39. Challem J: Nutritional therapy at the crossroads. *J Orthomol Med*, 9: 1994; 145-150.