Hydrocortisone effects on the dielectric properties and bone composition of rat leg tissues

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Abstract: hydrocortisone is an important mainstay drug of systemic connective tissues disorders such as rheumatoid. However, using high doses of hydrocortisone or normal doses for long time causes osteoporosis.

This work aims to study the effects of long time hydrocortisone treatment on the dielectric properties of the whole leg, skinned leg and tibia-rat bone.

The obtained results showed significant changes in dielectric parameters, minerals and water content. A good correlation was found between the obtained changes in bone tissues dielectric parameters and the changes in mineral and water content. The present work indicated the possibility of using dielectric parameters measurement for prediction of osteoporosis and decalcification of bone.

INTRODUCTION

Most whole bones consist of: diaphyses composed mainly of the cortical or compact bone outer- shell, metaphyseal ends composed of porous cancerous trabecular portion and marrow filling the pores of cancellous, **(Hncox, N.M.,1972).**

Several investigators have measured the electrical and dielectrical properties of cortical bone **(De Mecato G. and Garcia-Sanchezf J. 1988, 1992. Saha, S. and Williams, P.A. 1992).** Measurements have been made to the permittivity and conductivity of normal and wounded human skin material over the frequency range 10 MHz to 10 GHz. The permittivity of wounded tissue was found to be about 12 % higher than that of the normal tissue and a similar percentage increase was observed for the conductivity. Studies on the irradiated skin dielectric properties of human at radio frequencies for cellular changes **(Tamura, T. et al., 1994)** showed a decrease, especially at low frequencies, in control values of both dielectric constant and conductivity. Also, the dielectric relaxation of irradiated skin (in acute or late reaction) occurred at higher

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(Manuscript received 20 / 4 / 2003, Revised form 30 / 10 / 2003, accepted 3 / 11 / 2003)

frequencies than of normal skin. **Gabriel et al. (1996)** gave conductivity values for many different human living tissues, including intracranial tissues.

Ferree, T. C. and Tucker, D. M. (1999) calculated the average conductivity value of living intracranial tissues and resistivity values were measured in different living brain tissues **(Jula Latikka, et al. 2001)**.

Capacitance, conductance and dielectric loss spectra are obtained, in vivo, for a number of electrode separations in the gastrocenemius muscle of frog. At each frequency the reciprocals of these parameters are plotted versus electrode separation. From these resulting lines, the complex permittivity and the conductivity of the muscle can be determined, with electrode effects elimination. The electrical properties measured in vivo with needle electrodes are similar to those measured with surface electrodes for frequencies between 1 kHz and 1 MHz **(Hart, F. X. and Dunfee, W. R. 1993). Williams, P. A. and Saha, S. (1996)** examined the electrical properties of wet human cortical and cancellous bone tissue from distal tibia and their relationship to the wet, dry, and ash tissue densities.

Although glococoriticoids are used in many aims of therapy such as arthritis, but long term glococorticod treatment is associated with severe side effect such as osteoprosis **(Ima- Nirwana S. and Suhaniza S., 2004)**. Glococoriticoids stimulate protein and RNA synthesis in the livers and they have catabolic effects on lymphoid and connective tissues, mussels and skin. Supra-physiologic amount of gloococorticoids lead to decrease mass of muscle and weaken catabolic effects on bone which are the cause of osteoporosis in Cushing's syndrome and constitute major limitation in the long term therapeutic use of glococorticoids **(Betram G. K., 1989).**

The aim of present work is to study the effect of long time hydrocortisone treatment on the dielectric properties of the whole leg, skinned leg and tibia bone of the rat.

METHODS

Hundred female white rats were used with an average weight 120gm each. The animals were classified to six groups, a control group untreated rats, and the other five groups were treated with a suitable dose of 37.8 mg/day of hydrocortisone (by intramuscular injection) up to five weeks before investigation **(Paget and Barnes, 1964)**.

All groups were kept under the same condition of nutrition. The rat was anesthetized with ether; the hair of hind leg was removed from the area at which the electrodes were placed in contact with the leg. Metal disc Ag-AgCl electrodes are used with different contact areas according to the requirements of each experiment.

Three methods of measurements were carried out from the same place and area of the leg as follows:

1- Whole leg: the whole leg was sandwiched between the two disc electrodes.
2- Skinned leg: the leg was sandwiched between the two electrodes after removing the skin from the same contact area of the previous whole leg method.
3- Bone only: The two electrodes were placed on tibia after removing the muscles from the contact area of the previous skinned leg method.

Programmable automatic RCL meter bridge (Philips Mode /PM 6304) was used for measuring the resistance, capacitance, impedance and phase angle; hence dielectric constant ε' and dielectric loss ε" and electrical conductivity σ_t were derived.

The plot of **ε"** against **ε'** gives semicircles; with centers located below the x- axis with an angle $\frac{\pi}{2}\alpha$. Where **α** is the phase angle and was calculated form the plots. The macroscopic relaxation time τ_o was calculated by using the data obtained from the cole-cole semicircles and the following equation:

$$\frac{v}{u} = (\omega\tau_o)^{1-\alpha}$$

where; ***v*** is the distance between ε_s and the experimental point on the semicircle, ***u*** is the distance between ε_∞ and the experimental point on the semicircle, and **ω** is the angular frequency (according to the schematic illustrative diagram)

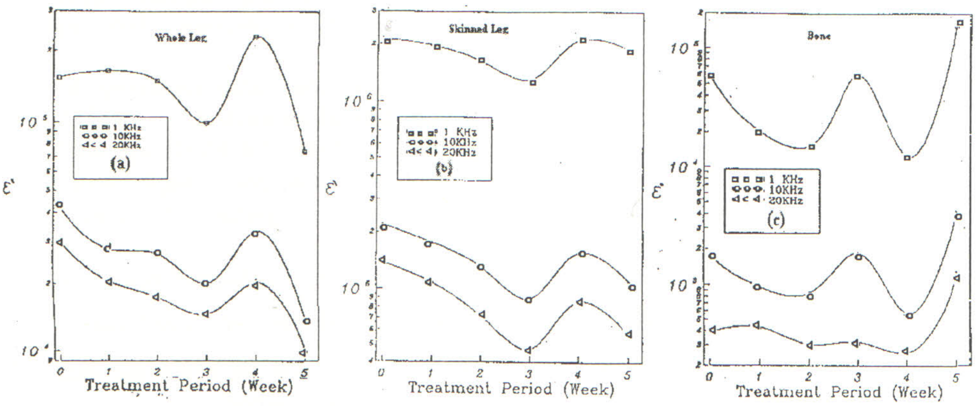
The microscopic relaxation time can be calculated from; $\tau = [2\varepsilon_s - \varepsilon_\infty / 3\varepsilon_s]\ \tau_o$.

Bone specimens were promptly weighted and placed in an oven at 105°C until attain constant weights. The dried samples were then ashed at 550°C in a muffle furnace for 5 hours. The water content (W_W) in the samples in addition to mineral (W_M)and organic material (W_o) contents were calculated in mg/gm wt according to the method used by **Bahnasaway, M. H. 1993**.

RESULTS

The dielectric properties measurements of whole, skinned and bone were carried before and after hydrocortisone treatment for hind leg of the rat. The obtained data are summarized in table (1) for the present methods.

Figures (1) and (2) represent the relations between the dielectric constant ε′ and the dielectric loss ε″ with the period of cortisone treatment at different frequencies. The plots showed attenuation in values of ε′ and ε″ during the first three weeks of treatment, while irregular changes were recorded during next weeks for the whole and skinned leg. Whereas, for bone measurements, the irregularity in ε′ and ε″ values appeared after two weeks of treatment.



Figures (1): The dielectric constant, ε′, variation with different treatment periods at frequencies for Whole hind-leg a; Skinned hind-leg; b and Bone hind-Leg measurements ;c.

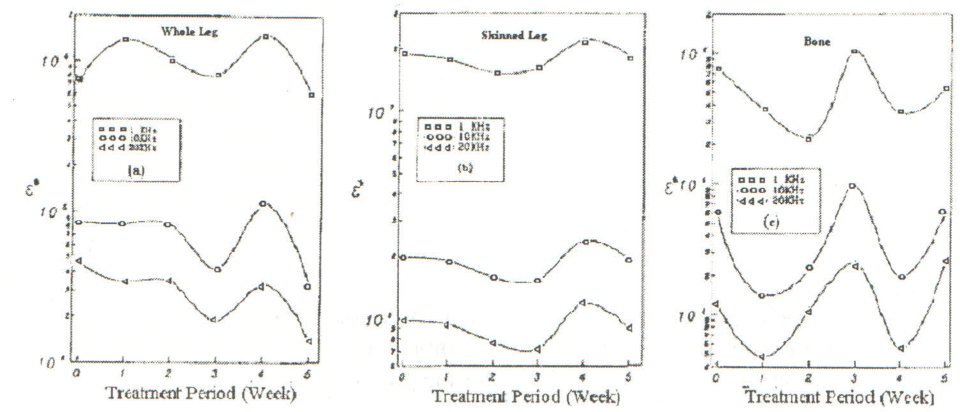


Figure (2): The variation of dielectric loss, ε′, with different treatment periods at different frequencies for Whole hind-leg (a), Skinned hind-leg (b) and Bone hind-leg measurements (c).

Figure (3) shows Cole-Cole diagram for ε'' against ε' at different periods of treatments. The derived data of ε_∞, ε_s and τ_o obtained from those figures are summarized in table (1), which showed an increase in ε_∞ value for bone during the five weeks of treatment, while the value of ε_∞ showed a decrease for whole and skinned leg tissues during the same period of treatment.

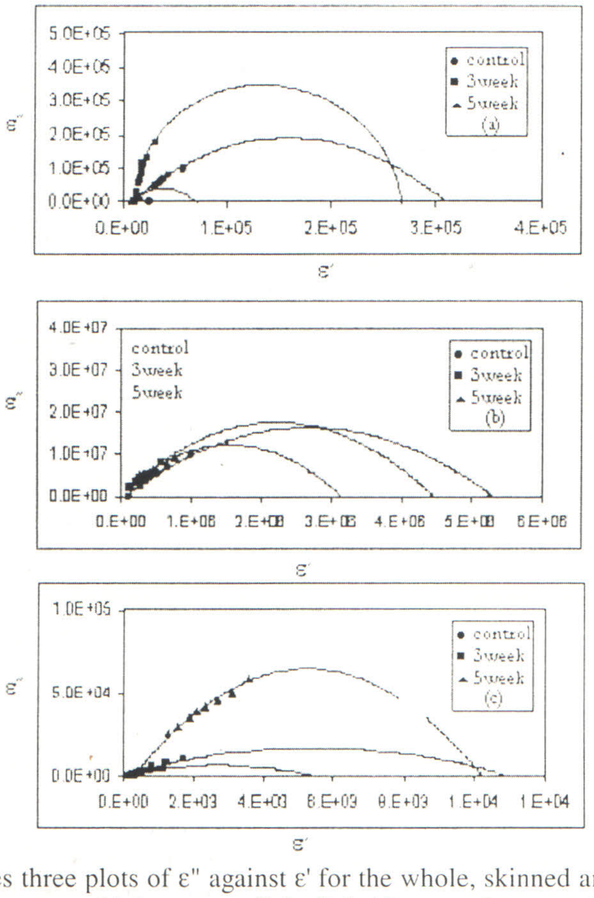


Figure (3): illustrates three plots of ε'' against ε' for the whole, skinned and bone measurements which suggest Cole-Cole diagram for control and at three and five weeks of treatment measurements.

Table(1): dielectric parameters at different periods of hydrocortisone treatment compared with the control group; for the whole leg, skinned leg and bone ($p<0.01$).

	Period (week)	ε_∞ $\times10^3$	ε_s $\times10^4$	α	τ_0 μs	τ μs
Whole leg	control	27.85 ± 0.63	29.78 ± 0.67	0.190	279.7 ± 0.45	195.2±0.15
	1	15.60 ± 0.35	27.08 ± 0.61	0.011	67.8 ± 0.021	46.5±0.066
	2	13.64 ± 0.36	45.61 ± 1.21	0.0104	98.4 ± 0.124	66.6±0.040
	3	11.09 ± 0.10	23.15 ± 0.21	0.053	95.9 ± 0.088	65.5±0.028
	4	16.36 ± 0.20	45.82 ± 0.55	0.018	85.6 ± 0.071	58.09±0.023
	5	9.326 ± 0.08	7.615 ± 0.07	0.046	76.0 ± 0.035	53.77±0.002
	Period (week)	ε'_∞ $\times10^4$	ε'_s $\times10^6$	α	τ_0 ms	τ ms
Skinned leg	control	9.091 ± 0.363	5.288 ± 0.212	0.463	2.440 ± 0.141	1.641 ± 0.078
	1	5.461 ± 0.190	5.162 ± 0.180	0.442	1.004 ± 0.068	0.672± 0.036
	2	3.636 ± 0.110	4.247 ± 0.127	0.428	2.007 ± 0.088	1.344± 0.048
	3	1.827 ± 0.077	4.461 ± 0.187	0.358	1.700 ± 0.084	1.138± 0.043
	4	2.906 ± 0.115	4.232 ± 0.169	0.354	0.785 ± 0.043	0.525± 0.024
	5	3.091 ± 0.093	3.220 ± 0.097	0.329	0.646 ± 0.026	0.433± 0.014
	Period (week)	ε_∞ $\times10^2$	ε_s $\times10^3$	α	τ_0 μs	τ μs
Bone	control	1.37 ± 0.03	5.3289 ± 0.079	0.125	82.17 ± 0.6	55.48 ±0.33
	1	5.25 ± 0.10	4.1393 ± 0.073	0.382	80.5 ± 0.42	57.07 ±0.25
	2	1.57 ± 0.002	2.731 ± 0.043	0.221	2.88 ± 0.037	19.3 ± 0.25
	3	2.000 ± 0.05	15.889 ± 0.30	0.1	67.3 ± 0.118	45.0 ± 0.6
	4	2.11 ± 0.032	3.046 ± 0.046	0.071	55.9 ± 0.66	38.6 ± 0.37
	5	3.33 ± 0.067	29.79 ± 0.60	0.025	45.3 ± 0.025	30.4 ± 0.14

The total conductivity σ_t variations against frequency, for whole, skinned and bone tissues of rat hind-leg, are shown in figure (4). The conductivity of whole hind-leg σ_t increases slightly, with increasing frequency. Also, the conductivity of skinned hind-leg increases slightly to asymptotic value with increasing frequency at the lower range of frequency and then decrease with increasing frequency. The bone hind-leg conductivity, σ_t, attenuated clearly wi h the frequency.

Figure (5) illustrates the variation of σ_t against $\mathbf{W_o}$, $\mathbf{W_M}$ and $\mathbf{W_W}$, which are the organic materials, minerals and water contents respectively of the bone. It was observed an increase in total conductivity of bone σ_t with increasing water and mineral contents, while it decreased with increasing the content of organic materials.

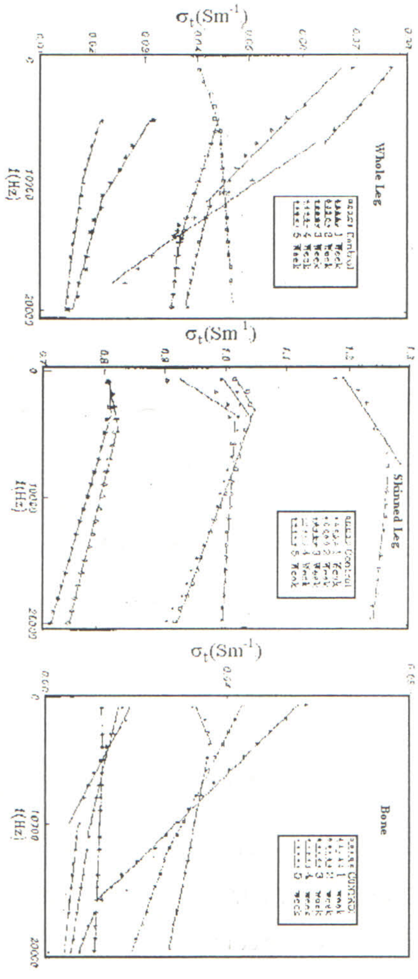


Figure (4): The frequency dependance of conductivity, σ_t, at different treatment periods for Whole hind-leg (a), Skinned hind-leg (b) and Bone hind-leg measurments (c).

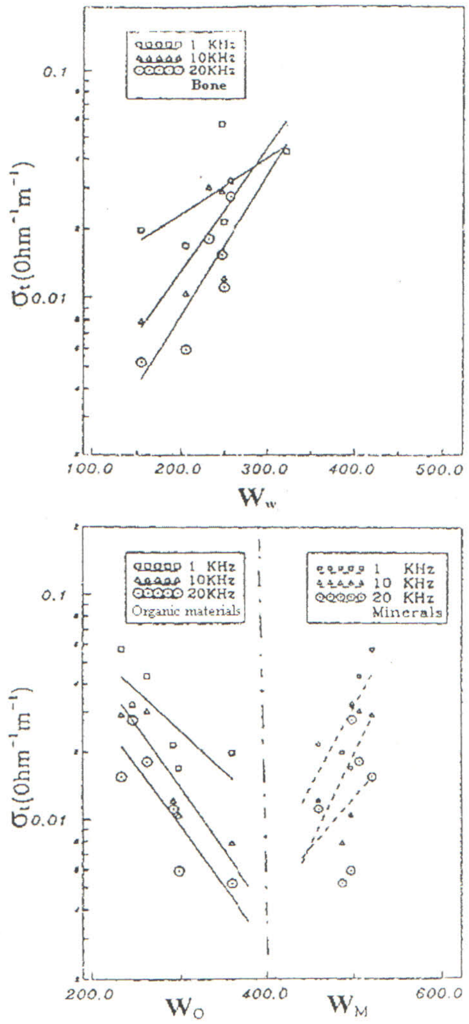


Figure (5): the variation with regression analysis of total conductivity, σ_{ac}, at different frequencies, versus the minerals and organic content's (W_o, W_M) a and water content (W_W); b, of bone.

DISCUSSION

It is evident from the obtained results that, hydrocortisone caused some noticeable and significant changes in the dielectric properties of the hind leg tissues of the rat and these changes were accompanied with changes in mineral and water contents. The variation of ε' and ε" with treatment periods appeared to be frequency dependant. The irregular changes in ε' and ε" appeared early one week of treatment in bone tissue than that in the whole and skinned leg tissues, this may possibly be due to the relative decrement of water content in bone than that in both skin and whole leg tissues. **Saha S. and Williams P. A. (1995)** have suggested that water content could be the main source for permittivity differences in cancellous and cortical bone.

During the first three weeks, the values of dielectric parameters ε_∞ & ε_s showed a decrease greater than that for next weeks in all maintained tissues, after hydrocortisone effects. Hence, the relaxation time "τ_o" increases during three weeks while it decreases at the fifth week in all tissues. This change in values of ε_∞, ε_s and τ_o of tissues tested indicate changes in the cellular structure and permeability of cellular membrane.

During the first three weeks, the values of dielectric parameters; ε' and ε" showed a decrease with treatment period in skinned and whole leg measurements. During next weeks, these parameters have an irregular behavior. In bone, ε' and ε" decreases during the first two weeks, after that they have irregular behavior. This change in ε' and ε" values of the tested tissues indicate changes in the cellular structure and permeability of cellular membrane. This may be due to the hydrocortisone effects. Hence, the intracellular water flow to extra-cellular region through the cell membrane, may lead to a decrease in membrane area causing the observed decrease in ε' and ε". In the last weeks, the redistribution of water depends on the type of cell tissues leads to the observed irregular values of ε' and ε". These behaviors were due to slow rate of ionic and water transfer across cell membranes **(Merae and Estrick, 1992)**, and perhaps also due to specific membrane types and higher water contents **(Surowiek et al, 1985).**

An irregular variations in σ_t values with the hydrocortisone treatment period is observed for all samples under investigation. In addition, the variation of σ_t for both whole and skinned hind-leg at different treatment periods showed attenuation in their values up to three weeks of treatment. After that, an anomalous behavior occurred in the relation between σ_t and treatment period. The variation in conductivity of all tissues may be due to variation in water content and structure, **(Saha, S. and Williams, P.A., 1995)** and **(Williams, P.A. and Saha, S. 1996)**. Also the total conductivity σ_t increased with the increase of water content W_w. The increase of W_W leads to an increase in water which facilitate ionic diffusion, while the increase of organic content means a decrease of water and minerals.

All present measurements showed that, the changes in the dielectric parameters of bone tissues occurred one week before changes occurred in whole and skinned tissues. This means that the side affects of the hydrocortisone occurs in bone earlier than in muscles and skin. Therefore the changes in whole leg dielectric parameters can be used as an indication to change in mineral composition of hind leg tissues and prediction to some disease such as osteoporosis. Finally, the present results indicate the increasing health risk of long period hydrocortisone treatment.

ACKNOWLEDGEMENT

Deep thanks to **Prof. Dr. M.K. El-Mansy** and **Prof. Dr. H. O. Nafie**, for facilities and helping during the different steps of this work.

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