

ELECTRICAL CAPACITANCE METHOD APPLIED TO THE STUDY OF ACUTE EDEMAS

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Abstract. *The aim of this study is to show that electrical capacitance can be used to determine the presence and time evolution of an acute edema in biological tissue. Measurements were done using a LCR meter, model HP LCR-815B, which measured the electrical capacitance between the electrodes localized on the rat skin across an edema induced by an inflammatory process. The technique was undertaken in rats, which received an injection of physiologic serum or carrageenin. The electrical capacitance was measured each 15min. Results for the physiologic serum group showed that there was an increment on the electrical capacitance with the rise of the water content and ion concentrations in the region between electrodes. Subsequently, capacitance decreased because of the absorption by the organism of the injected serum. Likewise, for rats with a carrageenin injection occurred first an increase in the electrical capacitance values, followed by a capacitance decrease accompanying the inflammation reduction. It was also studied the effect on the electrical capacitance when an anesthetic was injected into the rats. On the basis of our results it is possible to conclude that electrical capacitance measurements may be an adequate method to determinate the edema evolution in vivo.*

Keywords: *edema, inflammation, carrageenin, electrical capacitance.*

1. Introduction

Edema, a clinical sign of the inflammation, is a result of the alterations of the vessel caliber, increases of the blood flow and vascular permeability, the liquid extravasation to the interstitial tissue, the emigration of inflammatory cells of the blood towards the place of the lesion, and consequently, the liberation of painful agents induced by chemical mediators (Chandrasoma and Taylor, 1993; Contran *et al.*, 1995).

Several methods are being used for edema evaluation, for instance: the use of an hydrorethysmometer, in this case the edema volume is calculated by the difference between the values before and after the edema is formed (Honmura *et al.*, 1992; Honmura *et al.*, 1993); by fluorescence techniques (Honmura *et al.*, 1993) and by histopathology (Lespi, 2000). The edema to be studied, can be induced in the rat skin by a subcutaneous injection containing carrageenin, that is a sulfated polysaccharide extracted of an alga, known as Irish Moss (Di Rosa, 1972).

The historical and clinical exam of the skin allows specialists to make an immediate evaluation of the edema; however, the subjectivity is the main disadvantage of that method. The biopsy and the microscopy, that can supply detailed information regarding the skin layers and their alterations, are also subjective, since it is based only on the pathologist observation. Between the clinical evaluation and the most sophisticated techniques, we have the bioengineering methods that are non invasive and that can supply objective information in vivo and in real time (Serup, 1992).

Among the methods used in bioengineering, stands out the measure of the electrical impedance, that is the resistance to the electric current flow when an ac voltage is applied between two electrodes. The human skin, as well as the animal one, presents a resistance to the flow of the electric current (electrical impedance). Electrical impedance is composed by the electrical resistance (real part) and by the inductance and capacitance (both form the imaginary part) (Halliday and Resnick, 1965).

According to Ollmar *et al.* (1994) the first studies involving electrical impedance of skin were on 1919. Thereafter, the electrical impedance method have been extensively used for accomplishing a variety of studies of the skin in vitro and in vivo, in animals and humans as well. It is worthy to mention the following applications; study of the skin hydration with and without moisturizers (Lóden and Lindberg, 1991, Lóden, 1996, Held *et al.*, 1997) or irritants (Serup, 1992, Lee *et al.*, 1997, Seidenari *et al.*, 1998, Agner *et al.*, 2000, Choi *et al.*, 2000) and determination of lipid content of the skin stratum corneum (Nicander *et al.*, 1998). Ollmar *et al.* (1994) verified in recent studies that changes in the

electrical impedance could be correlated with the answers of the skin to control the irritation induced by different concentrations of aggressive agents. Although many applications of the electrical capacitance method are presented in the literature, so far, nothing was found about the use of the electrical capacitance to characterize edemas.

This technique seems to be very promising since measurements can be done *in vivo* and in real time, allowing us to follow the time evolution of an edema, for instance. The equipment needed for this method is inexpensive and very easy to handle.

The aim of this study is to monitor alterations of the liquid accumulation in interstitial tissues, i.e. the edema evolution, through a non invasive method, which monitors the time variation of the electrical capacitance. The inflammatory process was induced by the injection of an aggressive agent: carrageenin. An edema was also simulated by the injection of physiologic serum, for a further validation of our method. Since all rats had to be anesthetized in order to carry out the experiment, it was also studied how the anesthetic affects the electrical capacitance.

2. Materials and Methods

2.1. Animals and Drugs

Twenty Wistar rats weighting between 200-300g were used. All animals were maintained in conditions of natural illumination, at room temperature, with food and water *ad libitum*. Animals went shaving to facilitate measurements. The shaved areas were clean with cotton humidified with ethyl alcohol at 70%, in order to improve the contact between the electrodes and the skin.

Animals were first anesthetized with an intra-muscular injection of 0.1ml of Zoletil 50® (Tiletamine zolazepan) for every 100g. of animal weight. They had to be immobilized to reduce interferences on the electrical capacity measurements, due to animal movements. The anesthetic was also used to reduce animal sensitivity to the inflammatory drug injection, and consequently, a possible alteration of its electrical capacitance. The monitoring of the electrical capacitance for all animals was initiated 15min after the anesthetic injection and finalized when animals started to awake.

The twenty rats were equally divided into four groups. To the first group (hereafter called Group A) was injected only the anesthetic. This group was used as the control one. To the second group (Group B) was injected anesthetic and physiologic serum. The third and fourth groups (Groups C and D), received the anesthetic plus the inflammatory drug: carrageenin, at 1% for Group C and 2% for Group D.

2.2. Electrical Capacitance Measurement System

Measurements of electrical capacitance were accomplished through the use of two electrodes connected to a LCR meter, HP model LCR-815B (L: Inductance, C: Capacitance, R: Series and Parallel Resistance) The electrodes, for electrocardiogram use, were round with 10mm of diameter. Electrodes contained a gel layer to improve the electrical contact and were mounted over an adhesive round rubber plate for fixation onto the skin. Electrodes were positioned on the back of the animals, with approximately 5cm separation between them and equidistant from the point where the physiologic serum or the carrageenin were injected.

The following LCR meter parameters were used: frequency of 1KHz, automatic mode, internal trigger and 1.0V bias voltage. The electrical capacitance was measured each 15min, starting 15min after the anesthetic injection, until the end of the anesthetic effect. Measurements of electrical capacitance were performed with rats inside a grounded Faraday cage to avoid interference caused by ambient electromagnetic radiation. Room temperature and humidity were controlled during the experiment to reduce capacitance fluctuations. The values of the electrical capacitance were very dependent of the animal under study. High capacitance values between 20 and 30nF were observed for some animals.

2.3. Experimental Procedure

Group A received 2 anesthetic injections and the variations of electrical capacitance were monitored during a 6 hours period. The second anesthetic injection with a 0.1ml of drug, independent of the rat weight, was applied in order to continue the experiment for a longer time. The time elapsed between the two anesthetic injections was approximately 2 hours.

The group B was first anesthetized with only 1 anesthetic injection and 90min later the animals received 1ml of physiologic serum (sodium chloride at 0.9%). The effect of the anesthesia on the electrical capacitance can be neglected after 90min.

The group C also received an initial injection of anesthesia, and it was submitted 5min after the anesthesia to a subcutaneous injection containing 1ml of a solution of carrageenin at 1% (inflammatory drug). The edema induced by

the carrageenin reached its maximum size 1 or 2 hours after the edema stimulation (Honmura *et al.*, 1992; Honmura *et al.*, 1993).

Finally, animals from group D were anesthetized and the electrical capacitance measurements were initialized 15min later. Animals received an injection containing 1ml of a carrageenin solution at 2%, after 5min from the first measurement. Approximately 2 hours after the beginning of the experiment, the animals received an extra injection with 0.1ml of anesthetic, in this way was possible to extend the measurements for a longer period of time. It was employed a total period of 5 hours for the capacitance monitoring.

The physiologic serum and the carrageenin were injected in the subcutaneous area on the back of the animals, halfway between the electrodes. A schematic drawing is given in Fig. 1, showing the electrical capacitance measurement system, the Faraday cage and the electrodes localization on the back of the rats.

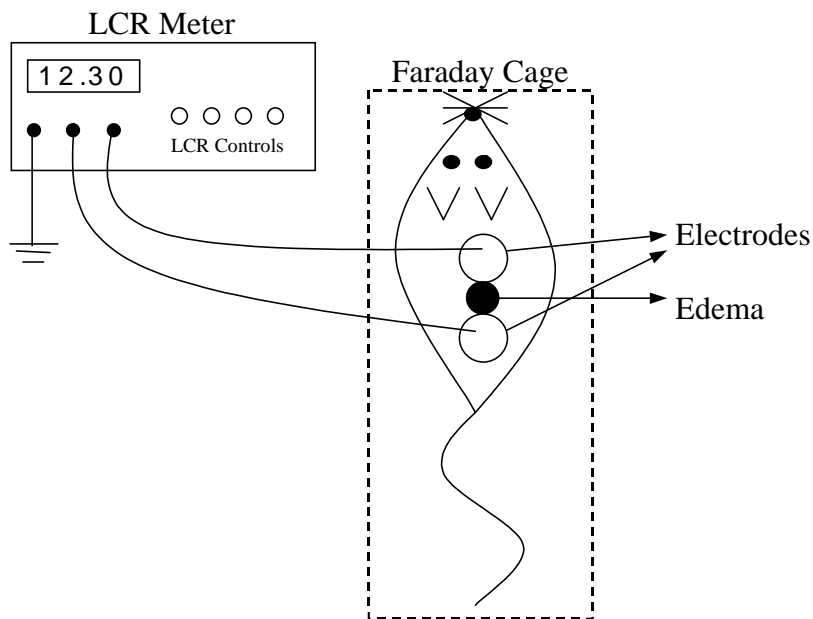


Figure 1: Schematic diagram of the experimental arrangement, showing: the LCR meter, the rat inside the Faraday cage and the electrodes localization on the animal's back.

2.4. Data Analysis

Electrical capacitance data was normalized for analysis purpose, since the initial values varied strongly from one animal to another; for instance from 2 to 22nF. That normalization was accomplished dividing the capacitance values for each animal by its corresponding initial value. Once data was normalized, the effect of each drug on the electrical capacitance (anesthetic, physiologic serum, carrageenin at 1% or carrageenin at 2%) was determined for each animal measuring $\Delta C = C_2 - C_1$, where C_2 and C_1 are the corresponding final (largest) and initial (smallest) values of the electrical capacitance, respectively. It is displayed in Fig. 2 a characteristic curve of the capacitance time evolution, showing how ΔC was calculated for each of the drugs.

A paired T-test was carried out comparing the values of C_1 and C_2 , for each drug. It was accepted a significant level (p) of 5%.

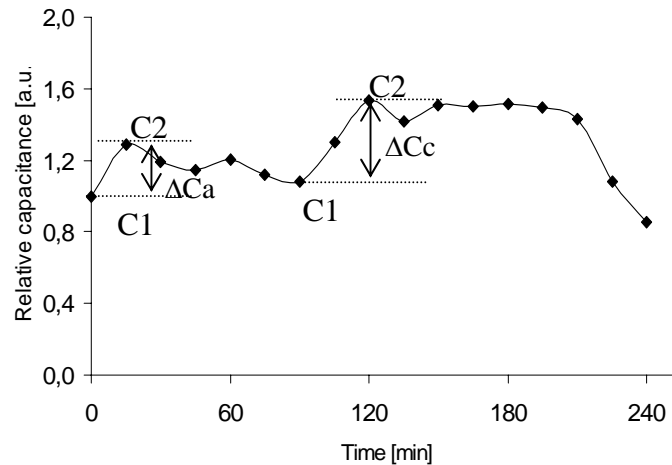


Figure 2: Characteristic curve showing the normalized capacitance versus time and how is calculated the increment on the electrical capacitance ΔC due to each drug. In this graph, ΔC_a corresponds to the action of the anesthetic and ΔC_c stands for the carrageenin.

3. Results

The time evolution of the electrical capacitance for rats belonging to groups A thru D is showed in the Figs. 3A thru 3D, respectively. It was chosen a characteristic curve for each group and the electrical capacitance values were normalized, as it was explained before.

Group A: it is observed an initial increase in the capacitance, reaching its maximum value between 15 and 30min from the beginning of the experiment. After that, the capacitance starts to decrease slowly. The effect of the anesthetic began to dissipated after 90min of the anesthetic injection and rats started to shake. Because of that, it was necessary to inject more anesthetic to each rat (0.1ml of Zoletil 50®) in order to keep them anesthetized during the experiment. It can be seen that there was again an increase in the magnitude of the electrical capacitance up to 150min, followed by a decrease.

Group B: the physiologic serum was used to simulate an inflammatory edema, providing a liquid increase among the electrodes. Since the anesthetic alters the values of the electrical capacitance we chose to inject the physiological serum some time later after the anesthetic injection, when the electrical capacitance reached stability. That occurred at the approximately time of 90min. In such a way, the anesthetic and physiological serum contributions to the capacitance could be separated. The temporal behavior of the electrical capacitance is very similar for all rats of the group. It can be observed from Fig. 3B that after the anesthesia injection there was an initial peak of the capacitance, as it was already found for group A. At 90min, 1ml of physiologic serum was injected into the animals and the electric capacitance increased again, reaching its maximum between 180 and 240min. Contributions to the capacitance from anesthetic and serum were of the same order.

Group C: it is showed in the Fig. 3C a characteristic curve of the temporal evolution of the electrical capacitance for rats of group C. The capacitance presented an initial peak due to anesthetic, as it was already observed for the other two groups. Since the inflammation induced by the carrageenin develops slowly, it is possible to see both contributions to the capacitance well separated: the anesthesia and the aggressive agent. A broad peak due to the carrageenin was observed between 120 and 210min. Then the capacitance started to decline slowly.

Group D: the experimental procedure for animals of this group is the same as for rats of the previous group, except that in this case 1ml of a more concentrated solution of carrageenin at 2% was injected. The trend of the temporal evolution for this group is quite similar to the group C; but, the peak due o the carrageenin seemed to be higher for this group, see Fig. 3D.

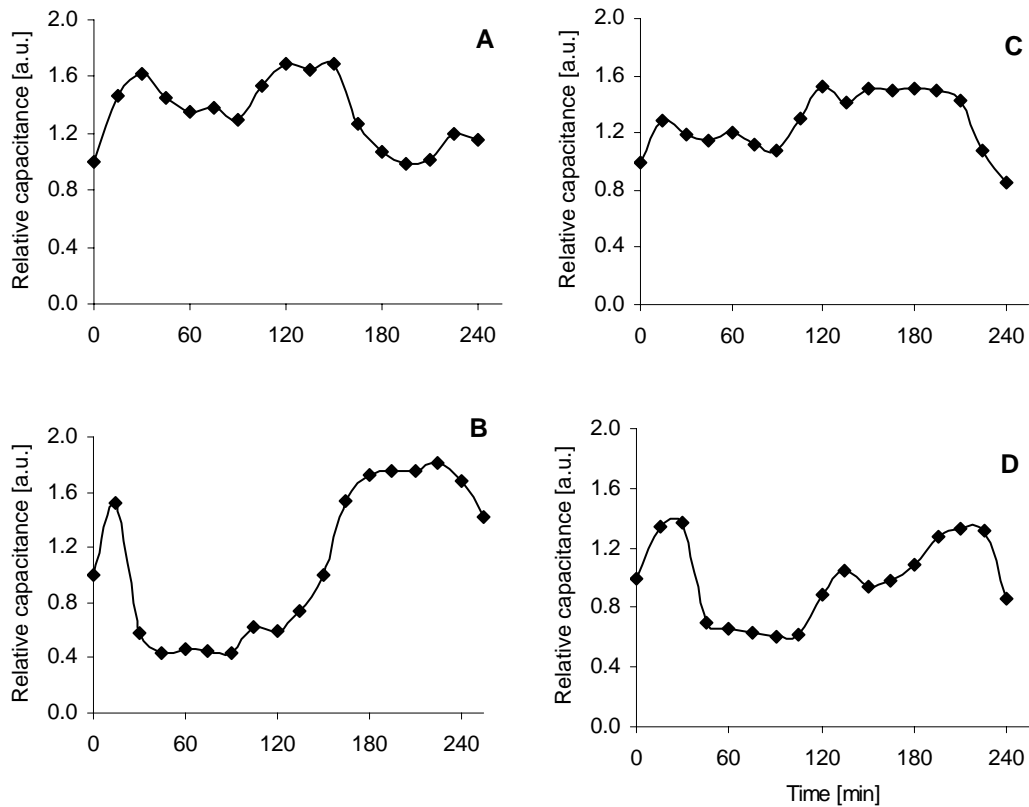


Figure 3: Characteristics curves showing the time evolution of the electrical capacitance. Letters A thru D correspond to groups A to D, respectively. Solid lines are for eyes guiding.

Paired T-test showed significant levels lower than 5% for each of the drugs: anesthetic $p < 0.01$, serum $p < 0.03$, carrageenin at 1% $p < 0.02$ and carrageenin at 2% $p < 0.03$. That means that values of C1 and C2 are statistically different and consequently, that there is an increment of the electric capacitance when an edema is induced by the drug.

It is plotted in Fig. 4 a histogram showing the mean and standard error mean values of ΔC corresponding to the effect of each drug separately. Let us mention that for anesthetic (that corresponds to the first anesthesia injection) the mean value of ΔC was obtaining averaging over all animals, from groups A to D. Increment capacitance ΔC for physiological serum, carrageenin at 1% and 2% were obtained from animals belonging to groups B, C and D, respectively.

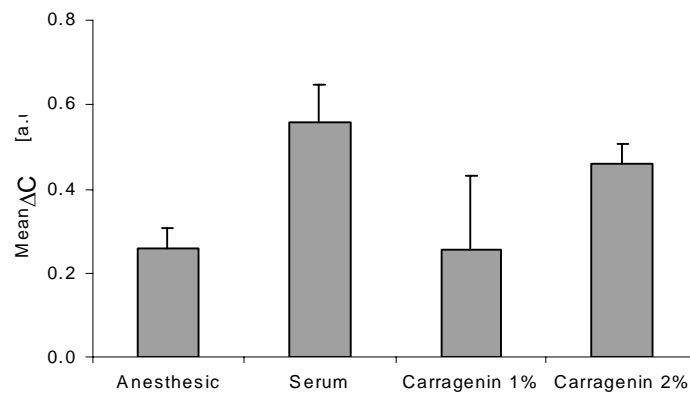


Figure 4: Histogram of the values of the electric capacitance increment ΔC due to the effect of the different drugs: anesthetic (all animals), physiological serum (group B), carrageenin at 1% (group C) and carrageenin at 2% (group D). Note that ΔC corresponds to the action of solely one drug at the time. Bars indicate the error of the measurements.

4. Discussion

It can be seen from Figs. 3A thru 3D that the electric capacitance always increases when the drug is injected into the animals, being the increment dependent on the type of drug. There is an initial peak of the electric capacitance after anesthesia for all groups, presenting a maximum between 15 and 30min and then, the capacitance tends to stabilize at about 120min of experiment. The second injection of anesthetic also induces an increase of the electrical capacitance, smaller than for the first injection, which agrees to the fact that the amount of drug injected in this case is also smaller.

According to Booth (1982), Pauling and Miller formulated in 1961, independently, molecular theories regarding the general anesthesia mechanism, establishing that isolated molecules can be tied to each other by hydrogen bonds forming clusters, due to electric dipole-dipole interaction. The cavities in the clusters can host some other molecules, like the molecules of a gaseous anesthetic, forming micro-crystals of hydrate. That could be the case of some chains of cerebral proteins, that may form stable hydrates at the corporal temperature when in the presence of an anesthetic gas. It is assumed that the micro-crystals formed by such mechanism interrupt the ionic mobility, the electric charge and the chemical and enzymatic activity of the brain, leading to an unconscious state.

On the other hand, a modified Pauling and Miller theory should be considered in order to find a better explanation for the action of some other anesthetics, like the one used in our experiment. One hypothesis is that the biological membranes can expand in the anesthetic's presence, based on the known fact that anesthetic's molecules penetrate and expand films placed in the interface air-water. The proof for that simple mechanism for understanding the expansion of the biological membranes relied on the observation that volume changes; i.e., an increase in the volume of the membrane, takes place when the anesthetic's molecules penetrate into the cellular membrane. The degree of expansion of the membrane volume is of the order of 10 times the volume of anesthetic's molecules. This explanation using the modified Pauling and Miller theory agrees to the results obtained in the present study, regarding the initial peak of the electrical capacitance. The observed increase on the values of the electrical capacitance when animals are anesthetized can be due to the volume expansion of the cellular membranes (Booth, 1982).

It was injected 1ml of physiologic serum to animals of group B, in the subcutaneous area of the animal back, at 90 min of experiment. The corresponding increase of the magnitude of the electrical capacitance can be observed in Fig. 3B. Our results agrees to the observations reported by (Lóden and Lindberg, 1991), that there is an increase of the electrical capacitance when the superficial area of the skin is more moisturized. It occurred in the superficial area of the skin an increase in the amount of water and ions due to the injection of physiologic serum into the region between electrodes; and consequently, of the electrical capacitance too. The dielectric constant K of water is very high ($K=80.0$); so, any increase in the amount of water between electrodes will increase the electrical capacitance as well.

The induction of an acute inflammation using carrageenin is a classic and very well established method for experimental studies of edemas (Di Rosa, 1972). Because of that, we chose the carrageenin to induce the edema used for the validation of our method. The time evolution of the electrical capacitance for animals of groups C and D is shown in Figs. 3C and 3D, respectively. It can be observed that the capacitance always accompany the growing and the subsequent shrinking of the inflammatory edema. These data confirm results obtained by Lee et al., 1997, Agner et al., 2000, Choi et al., 2000, that shown that an acute inflammatory reaction to skin irritants can cause an increase of the electrical capacitance. The injection of a more concentrated solution of carrageenin, 2% instead of 1%, caused a larger increase of the capacitance (Fig. 4), as it was expected.

We found that was possible to separate the effects of the anesthetic and the other drugs, since the edema induced by the physiological serum or the carrageenin took a long time for growing up, in such a way that the effect of the anesthetic on the capacitance can be neglected when the edema reached its maximum. The experimental errors of ΔC range from $\pm 15\%$ to $\pm 30\%$ depending on the drug. These high values are due to the large fluctuations of the skin electrical capacitance during the experiment and to the limited number of animals that we were able to investigate, a problem that is inherent to all animal experimentation.

5. Conclusions

The results seem to indicate that the electrical capacitance technique could be used to detect changes in the skin properties. Such changes may be due to an injection of anesthetic, physiological serum or an inflammation caused by an aggressive external agent, like carrageenin. This technique seems to be very promising, since measurements could be done in vivo and in real time, allowing us to follow the time evolution of an edema, for instance. The equipment needed for this method is inexpensive and very easy to handle.

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