Evaluation of Pulsed Microwave Influence on Isolated Hearts

Mara Abbate, Giovanni Tiné, and Luigi Zanforlin

***Abstract*— Long-term effects of 2.45 GHz pulsed microwaves on the electrical activity of chick embryo isolated hearts were analyzed. A new analysis procedure, derived in part from deterministic chaos studies, was carried out in order to point out dragging and regularization phenomena of the cardiac frequency, induced by pulsed microwaves. When dragging phenomena occur, results show that cardiac frequency shift on the average towards pulse's repetition rate; this means that the heartbeat maintains its statistical characteristic of frequency distribution and thus, it keeps its natural beating behavior typical of a healthy heart. Moreover in case of arrhythmia, when the regularization is reached, the heart recovers its statistical characteristic of frequency distribution that last even after the end of the irradiation. Dragging effects were confirmed by means of a mathematical model simulating the electrical activity of the sinus-atrial cardiac cells that allowed to suggest an interaction mechanism.**

I. Introduction

FROM the beginning of this century, with the introduction of the radiocommunication, the environmental background of electromagnetic energy grew so rapidly that it reached, in the last years, a value of six order of magnitude greater than in the past.

The first studies carried out about interactions between electromagnetic fields and biological systems shown only an heating effect due to the deposition of thermal energy inside tissues. Since then, a large variety of effects, not linked to a thermal one, have been observed in laboratory both *in vitro* than *in vivo*.

In particular, with regard to cardiac effects, Paff *et al.* observed alteration of ECG of isolated chick embryo hearts after irradiation with 24 MHz [1]; Frey *et al.* reported effects of tachycardia and arrythmia with 1425 MHz pulsed microwaves [2]. Lords *et al.* and Tinney *et al.* found chronotropic alterations in isolated rat hearts irradiated with decimetric continuous waves [3], [4]. Recently, Seaman *et al.* studied chick embryo cardiac cells aggregates exposed to 2.45 GHz square modulated or CW microwaves. They observed a cardiac frequency reduction with CW and an increase with modulated microwaves [5].

Experiments previously conducted in our laboratory, on chick embryo isolated hearts exposed to low intensity pulse modulated microwaves (2.45 GHz), shown that, in case of regular beating, if the modulation frequency was a little higher than the unperturbed cardiac frequency the heartbeat was pushed by modulation pulses until it locked itself to the pulses. Moreover, in case of arrhythmia, a regularization effect of the heartbeat was observed [6]–[8].

In this paper, results obtained following a new acquisition and analysis procedure are reported. Past experiments were performed exposing biological samples for short periods of time (max. 2 min). In the new experiments, instead, samples has been exposed for longer periods (from 10 to 40 min) and a new methodology of analysis has been carried out in order to point out the long-term effects of pulsed microwaves. Heart rate dragging effects were confirmed by means of a numerical simulation of the sinus-atrial cardiac cells electrical activity, some results of which are reported in [9].

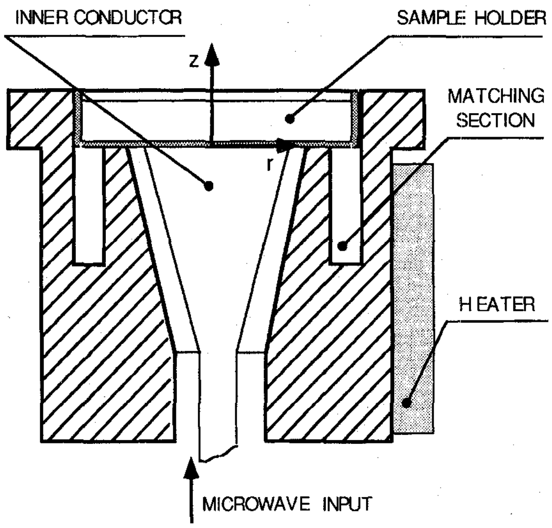

Fig. 1. Coaxial open-ended microwave radiator.

II. Experimental Setup

Experiments were carried out on 9–12 days old isolated chick embryo hearts according to the following procedure:

The heart was isolated and then placed in a Petri dish sample holder filled with avian Ringer's solution, continuously oxygenated and perfused in the holder in order to assure its survival. Solution temperature was kept constant at 37°C, with a precision of 0.1°C to avoid heartbeat frequency shifts depending on temperature variations [6].

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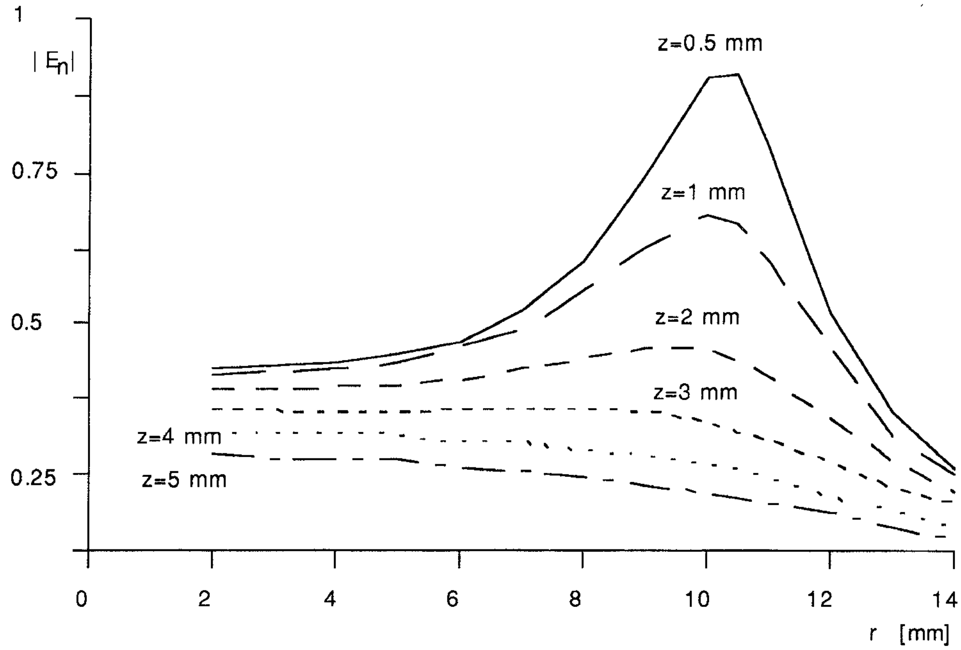


Fig. 2. Electric field amplitude in the same holder for different distancies z from the holder bottom. $|E_n|$ is the normalized field amplitude; r is the distance from the holder axis.

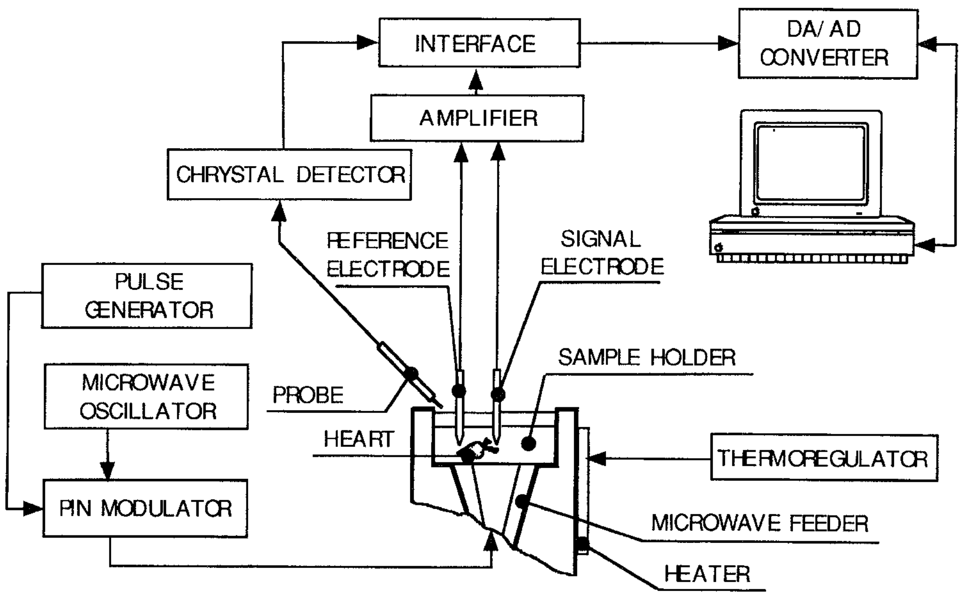


Fig. 3. Block diagram of irradiation and measurement system.

The heart was left in the holder for about half an hour, before starting experiments, so that it could recover and start to beat regularly on a normal rhythm. Then, by means of a micromanipulator, a platinum-iridium electrode (0.5 MΩ of resistence), was placed close to the sinus atrial node to pick up the cardiac signal. Electrodes were placed so as not to touch the heart, this prevents damage to the heart and, moreover, reduces the possibility of artifacts. The reference electrode, put in the holder, was also made in platinum-iridium to minimize the offset voltage and its fluctuations.

Biological samples were irradiated from the bottom of the holder by means of a tapered open coaxial section improved with a matching set-up as shown in Fig. 1. The gap between the inner and the outer conductors was approximately the same as the largest dimension of the heart. The normalized electric field amplitude behavior is shown in Fig. 2. About 3 mW/cm^2 of incident peak power density was evaluated in the sample area (10 mm from the antenna axis). Microwave frequency was 2.45 GHz, pulse modulated with a duty-cycle of 20%; modulation frequency is variable since it depends on the cardiac frequency as explained in the next section. Cardiac signal, after a proper amplification, was digitalized, sampled, and acquired by a desktop computer. The same was done for the microwave modulation signal, detected by means of a probe, placed above the sample holder, followed by a crystal detector. The waveforms were visualized in real time on the calculator screen and, at the same time, they were saved for the successive analysis. The schematic diagram of the measurement set-up is shown in Fig. 3.

III. Experimental Procedure

When hearts beat regularly, usually four data acquisitions of 20 min each are carried out. The first is done without microwaves and, at the end, the mean heart rate is measured. Then the heart is exposed to pulsed 2.45 GHz microwaves, the modulation frequency is set at a value a little higher (typically 10%) than the natural heartbeat, so as to give rise to the dragging effect, and the next data acquisition is done. Afterwards the pulse rate is still increased and a new data acquisition is performed. The last data acquisition is done again without microwaves, in order to observe the heart frequency evolution.

Sometimes the hearts can show an arrhythmic behavior, in this case the aim of the experiment is to try to regularize the beat. So it is necessary to settle the modulation pulse frequency value. If the heart shows the trend to beat at a particular frequency, this value is chosen as microwaves pulse repetition rate, otherwise a typical cardiac frequency, among those observed in previous experiments, is chosen. At last, the heart is exposed to microwaves and the pulses frequency is kept constant, until the regularization is reached (normally within 10–40 min). If it occurs, the last acquisition is done switching off microwaves in order to verify if the regularization keeps itself or not.

IV. Data Analysis

In cardiology, the normal electrical cardiac activity was ever described as a regular sinus rhythm [10]. Lately, a more careful observation shows that cardiac interbeat intervals fluctuate in a complex, apparently erratic manner. It was pointed out that these fluctuations, on living organisms, depend, at macroscopic level, on the complexity of the heart control systems and on the interaction with other systems as, for example, the breathing. At microscopic level, *in vivo* or *in vitro*, they depend on different causes, from the generation of the electrical pulse on the sinus-atrial node, to their propagation on the His–Purkinje system, whose complex branch show a fractal morphology [11], [12]. In both cases these fluctuations are the expression of the normal behavior of the heart and their alteration is index of a physiological disease [13]. In case of electrical activity of isolated hearts, of course, all the macroscopic causes do not occur anymore, and we can observe only the fluctuations depending on microscopic interactions.

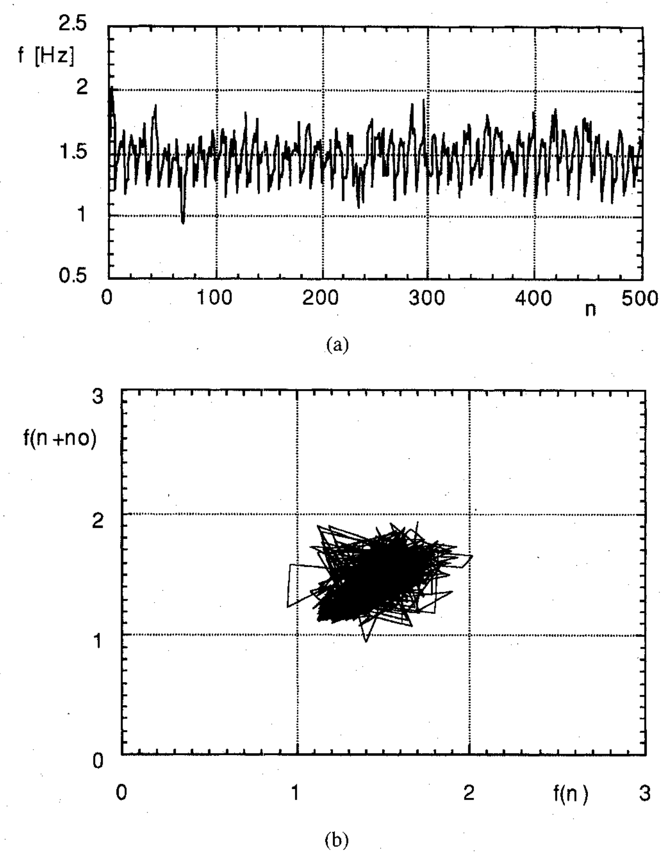


Fig. 4. (a) Instantaneous heart frequency versus number of intervals R–R. The tacogram shows the heartbeat fluctuation behavior of an unperturbed heart. (b) Return map carried out, with no $= 10$, from the same data of the tacogram (a).

This new kind of approach allows to consider the heart as a nonlinear, deterministic system, with a noninteger dimension attractor, so that the heartbeat behavior can be analyzed also by means of techniques coming from nonlinear dynamic and deterministic chaos [14].

Data analysis was carried out analyzing the instantaneous heart frequency values obtained from R-R intervals, namely the time intervals between two consecutive heartbeat peaks. Dragging effects were pointed out plotting histograms (cumulative count of R-R intervals versus cardiac instantaneous frequency) and tacograms (instantaneous frequency values versus number of R-R intervals).

Moreover, microwave regularization effects were studied by means of bi-dimensional views of state space paths called "return maps," obtained plotting the cardiac instantaneous frequency values $f(n+n_o)$ versus $f(n)$, where n is the number of R-R intervals and n_o is a delay, the value of which is chosen so as to emphasize the paths in the best way.

We verified that these maps, utilized by other authors to compare heart behavior of healthy and sick subjects [15], provide also a valid means to point out microwave effects on regularization processes.

At last, interbeat fluctuations were analyzed in order to estimate if microwaves can alter the natural heartbeat behavior. This behavior is shown in Fig. 4 by means of a tacogram (a)

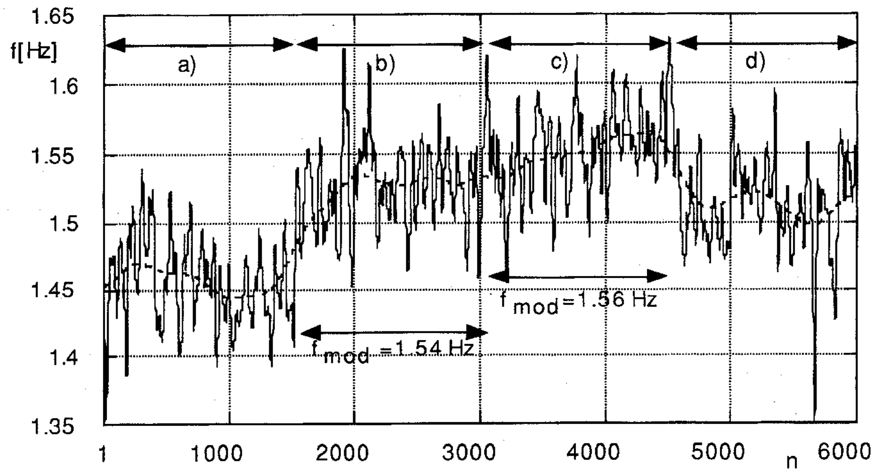
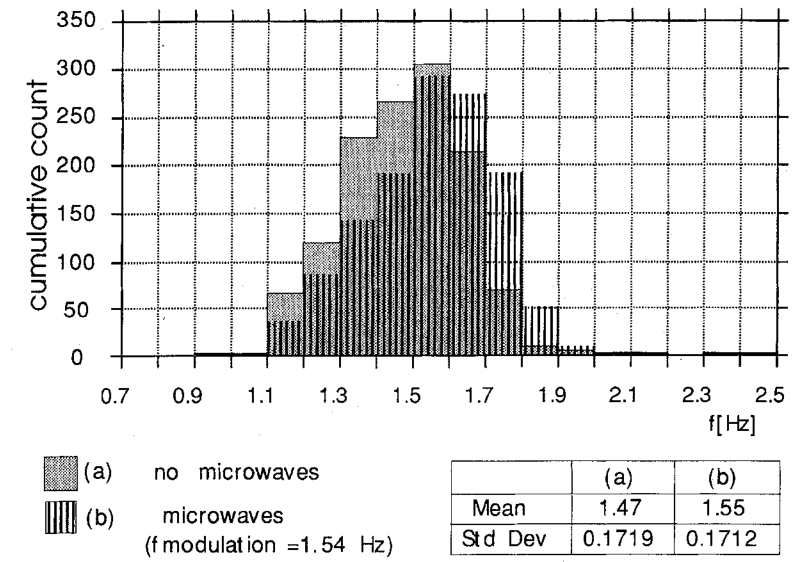


Fig. 5. The tacogram shows an example of microwaves dragging effect on the heartbeat carried out from four acquisitions of 20 min each. a) No microwaves, b) pulsed microwaves: fmod = 1.54 Hz, c) pulsed microwaves: fmod = 1.56 Hz, and d) no microwaves.



	(a)	(b)
Mean	1.47	1.55
Std Dev	0.1719	0.1712

Fig. 6. Cumulative count of R–R intervals versus instantaneous heart frequency. Histograms are related to the period (a) and (b) of Fig. 5. It is possible to see the shift of the heart mean frequency due to microwaves.

and a return map (b), displaying the natural fluctuations of a unperturbed heart.

V. Results

Isolated chick embryo hearts, irradiated with pulsed 2.45 GHz microwaves, show the tendency to follow pulse repetition rate, while they do not show any significant heartbeat modification if exposed to CW microwaves. An example of the dragging effect induced by pulse-modulated microwaves on cardiac repetition rate is shown in Fig. 5. The tacogram has been obtained analyzing four consecutive acquisitions of 20 min each, corresponding to the periods a), b), c), and d). Instantaneous frequency values have been computed and plotted versus the number of intervals R-R. As explained in the past section, the first acquisition a), performed in absence of irradiation, shows the natural behavior of the instantaneous cardiac frequency. The heartbeat is quite regular with a mean value of about 1.47 Hz. Then the heart was exposed to microwaves with a modulation pulse frequency of 1.54 Hz for period b) and 1.56 Hz for period c). The last acquisition d) was done again without microwaves. The figure shows clearly the microwave dragging effect: the heart mean frequency grows, until the electromagnetic field is present, following the modulation pulse frequency, and decreases as soon as the irradiation is interrupted.

It is possible to find the same effect on the histograms of Fig. 6 relating, for shortness, only to the periods a) and b) of Fig. 5. In the period a), the instantaneous frequency values are assembled around their mean value (1.47 Hz). When the field is present (case b) it is possible to observe a shift of the mean frequency that move to 1.55 Hz, very close to the modulation pulses frequency, set at 1.56 Hz. It should be noted that both frequency distribution shapes and standard deviation values, reported in the histogram of Fig. 6, are very similar in the two cases. Moreover, the frequency behavior of Fig. 5 never shows a constant course. This means that synchronization phenomena, when occur, do not last in time even if, as already observed, during the dragging phenomenon it is possible to observe short periods of perfect syncronization between the heartbeat and the modulation pulses [9].

Using long irradiation periods, it is also possible to observe very interesting regularization effects. An example of such effect is shown in the sequence of Figs. 7–9 related to the case of a heart beating with an irregular double frequency as shown in Fig. 7(a). The histogram of Fig. 7(b) is shown clearly that frequencies are placed around two main values, about 1.5 Hz and 2.8 Hz. After a control acquisition of 10 min, microwaves were turned on with a modulation pulse frequency of 2.8 Hz. The heart was irradiated, until regularization was reached, for 40 min obtaining other four registrations of 10 min each, for a total of five. In Fig. 8, only three maps obtained, respectively, from the control case 8(a), the third 8(b) and the fifth 8(c) registrations. The first map 8(a) shows the unperturbed heartbeat paths: frequency values are rather scattered and cover a large area from 1 to about 3 Hz. In the next map 8(b) it is pointed out that heartbeat frequency begin to move towards the higher value, forced by modulated microwaves. In the last map 8(c) the regularization is achieved. So, in Fig. 9(a), related to this last map, we can see a regular heartbeat and the histogram [Fig. 9(b)] shows that frequency values now are all placed around the value of 2.8 Hz with the variability typical of a regular heart.

One of the more interesting phenomena observed during this research is that microwaves do not alter the natural behavior of heartbeat fluctuations even if they are able to induce dragging and regularization effects. This result is confirmed, for example in the case of a dragging phenomenon, analyzing the fluctuations behavior of Fig. 6, showing the same heartbeat variations when the heart is not irradiated (part a and d) and when the cardiac frequency is forced by microwaves to follow modulation pulses (part b and c).

In case of arrhythmia, the same result, can be carried out from the return maps of Figs. 5(b) and 8(c). As we can see in the maps, the shape of the plots show approximately the same area confirming that, when regularization occurs, the heartbeat has the same variability of a regular nonirradiated heart.

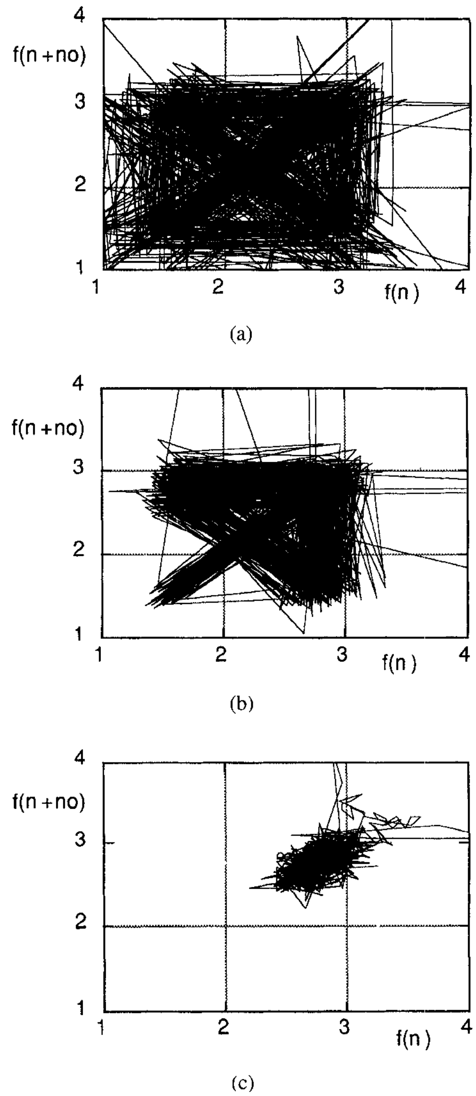


Fig. 7. The regularization process, seen by means of the return maps ($n_o = 10$). (a) Unperturbed heart behavior: the relative histogram is reported in Fig. 8. (b) After a 20 minute exposition (fmod = 2.8 Hz) the heart frequency begins to move to the higher value. (c) After 40 min, the heart beats regularly at about 2.8 Hz. The relative histogram is shown in Fig. 9.

VI. Numerical Simulation

Results reported in literature demonstrate that low-level electromagnetic fields can influence intracellular and extracellular calcium ions exchange, affecting directly the transmembrane protein channel function [16], [17].

In order to suggest a possible interaction mechanism, we verified the hypothesis that pulse modulated microwaves can modify the transmembrane calcium ionic currents, performing a numerical simulation by means of the Noble & Noble (N–N) model [18]. This model refers to the cardiac sinus-atrial cells activity, that carry on the heart beating function. It includes a careful description of the membrane currents and in particular of the calcium ionic current. Moreover, it fully incorporates the currents generated by Na-K and Na-Ca exchange processes and reconstructs the variations in intracellular and extracellular ionic concentrations.

The kinetic of the ionic channels is described by a first order nonlinear differential equations system and the solution of this system displays the transmembrane voltage of the sinus-atrial cardiac cell.

Using a computer program based on N–N model, we simulated the action of the microwave modulating signal on the calcium background current $i_{b,Ca}$ and on the calcium component of the fast calcium current $i_{Si,Ca}$. This was accomplished perturbing these currents at the same rate of the modulation pulses [19]. Fig. 10 shows an example of dragging frequency effect simulation: the repetition rate of

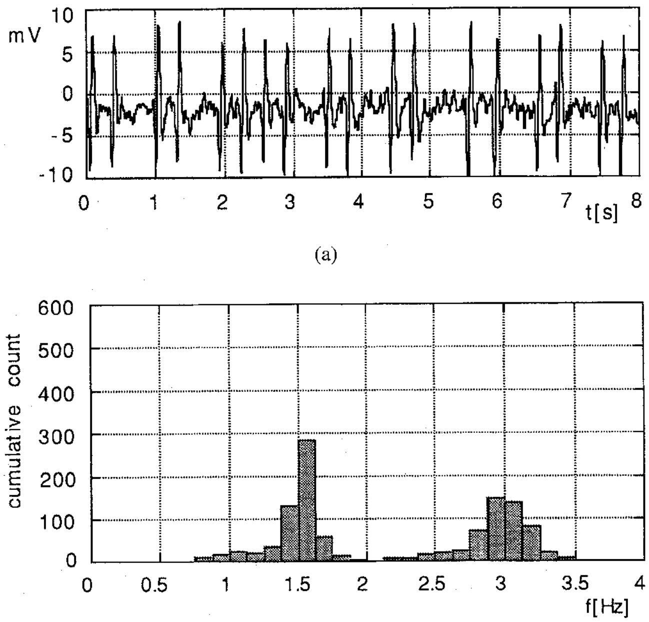


Fig. 8. (a) Example of an irregular beating: the heart shows a double frequency beating. (b) Relative histogram carried out in absence of microwaves field.

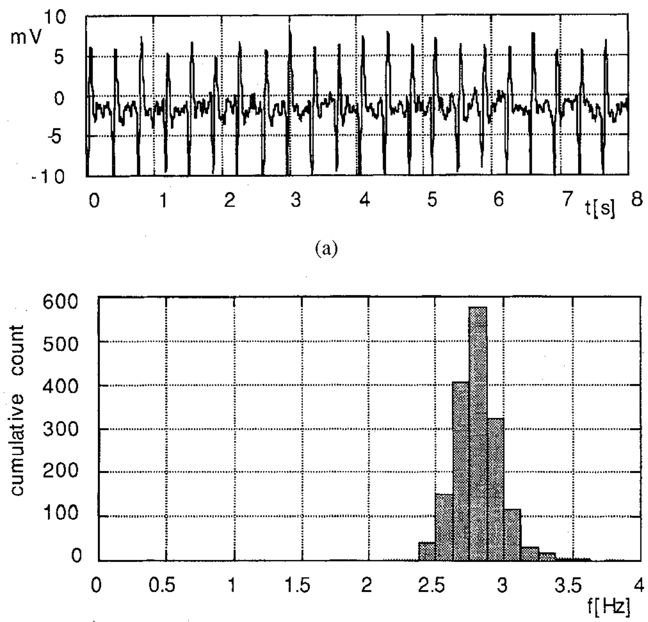


Fig. 9. (a) The same heart of Fig. 8 after 40 min of exposition: the regularization is reached. (b) The double heartbeat disappeared and the frequency, forced by microwaves, stabilized itself around the value of 2.8 Hz.

the perturbing pulses (dashed line) has been grown above the natural unperturbed cardiac frequency (2.35 Hz) from 2.4 Hz to 2.6 Hz in 16 s and with 20% of duty-cycle; the heartbeat (no dashed line) follows the perturbing signal. This result is in agreement with the dragging phenomena observed experimentally and seems to confirm the hypothesis above suggested.

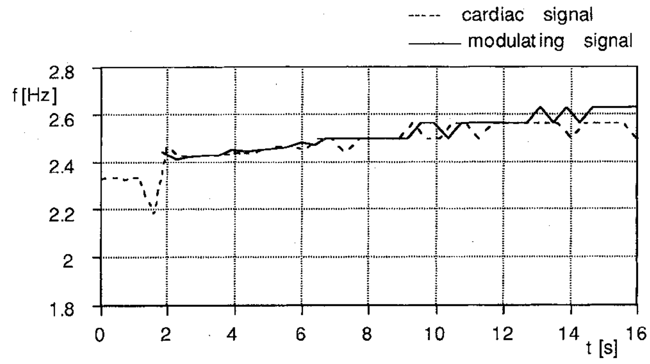


Fig. 10. Numerical simulation of a dragging phenomena; the cardiac frequency follows almost immediately the frequency that simulates microwave modulating signal action on calcium currents.

VII. Conclusion

Microwaves CW irradiation, performed at the same peak power of the experiments with modulation, do not shown any significant modification of the heartbeat.

This suggest a nonthermal effect induced by pulsed microwaves because they originate in the sample a temperature increase lower than that due to CW irradiation; therefore, none heartbeat increase is related to temperature variations.

Experimental results point out that dragging and regularization effects, observed when the samples were irradiated for short periods, appear also during longer irradiation periods and they last all through the exposing time. Moreover, when the regularization is achieved, the heart go on beating regularly also after the end of the irradiation.

Instead, synchronization phenomena of the heartbeat with microwaves modulation pulses do not last in time. Cardiac frequency shift on the average towards pulses repetition rate but the heartbeat maintains its statistical characteristic of frequency distribution.

At the power level adopted during experiments, it was observed that the heart maintains its typical variability when forced to beat at a frequency induced by microwaves. This lets to foresee the possibility to use microwaves in clinical applications to control the cardiac frequency without alter its natural behavior.

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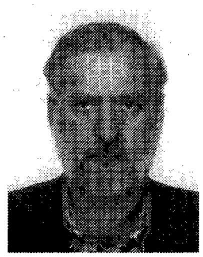

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