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Magnetic Flux Quantization and Josephson Behaviour in Living Systems

E. Del Giudice

INFN Sezione di Milano, Via Celoria 16, 20133 Milano, Italy

S. Doglia and M. Milani

Dipartimento di Fisica dell'Università, Via Celoria 16, 20133 Milano, Italy

C. W. Smith

Dept. Electronic and Electrical Engineering, University of Salford, Salford M5 4WT, U.K.

and

G. Vitiello

Dipartimento di Fisica dell'Università, 84100 Salerno, Italy

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Abstract

The proposal of coherent electromagnetic processes as the engine for biological dynamics suggests that Josephson effects could be present in living cells. Positive experimental evidence is reported and discussed.

1. Introduction

We consider in the present paper the Josephson phenomena as a test for the existence of possible long range correlations and coherent processes in living biological matter.

A coherent dynamics has been proposed in the last decades by many authors as the fundamental driving force of living processes [1–3].

Contrasting reports have been produced concerning the experimental evidence relating to phenomena connected with coherence.

We have proposed to look at the possibility of a Josephson phenomenology in order to have further insight in this matter.

Coherent excitations in biological systems have already been proposed to arise as a consequence of a long range correlation among the phases of the oscillating electric dipoles which are the microscopic components of living systems [4, 5].

In 1962, Josephson suggested that peculiar phenomena might occur when two superconductors are separated by a very small distance giving rise to a so-called "weak-link-junction" [6]. At a theoretical level the basic mechanism of the Josephson effect is the quantum tunnelling across such a junction barrier of the bosons [7] responsible for the correlations existing in two separate superconductors. In this way an interaction between two neighbouring although separated domains is established.

The Josephson effects are seen as an anomalous (non-ohmic) current (I)–voltage (V) relationship. Furthermore, electromagnetic fields appear whose frequencies are precisely proportional to the voltages existing across the Josephson junction.

Since the Josephson phenomenology is both typical and unique, in the sense that it can be easily related to fundamental parameters and constants, it might be used as a tool for testing for the existence of correlated regions within the system. The Josephson mechanism is very general indeed and applies beyond the special case of superconductivity. When we have two neighbouring although separated regions where the same kind of correlation holds among the components, quantum tunnelling of the carriers of the correlation must be expected, and a Josephson-like phenomenology becomes a distinct possibility to be looked for.

From a microscopic point of view, the correlation among electric dipoles, which has been proposed to be at work in living matter, points to the correlation being among electron pairs. An appropriate modelling of the microscopic mechanism of the above correlation has not yet been formulated. In Section 2 we summarize the results of the dynamical approach to living matter as a correlated medium. In Section 3 Josephson effects are discussed and in Section 4 examples of possible Josephson-like behaviour experimentally observed in biological systems are presented. Conclusions are discussed in Section 5.

2. Coherence in biological systems: the role of electromagnetic interaction

A living system can be considered as a set of many microscopic components whose interplay occurs through a network of mutually coupled and sequentially ordered chemical reactions. This macroscopic ordering could be considered as emerging from the collective behaviour of the elementary components. Collective behaviour is possible because the dynamics is able to produce "quasi-particles" playing the role of long-range messengers.

The existence of a correlation implies that the physical states of the system must also be coherent states of these "quasi-particles".

Most biocomponents are polar molecules, some being in excited metastable states.

Following then the proposal of Fröhlich [4], the dynamics of an assembly of electric dipoles might be considered as the fundamental biological dynamical process, and consequently the density of electric polarization P be assumed to be the relevant order parameter in the sense of statistical mechanics.

The theoretical framework of the above assumptions has been discussed elsewhere [5], but let us sketch the main points:

(i) The set of electric dipoles is assumed to represent an early stage of the biological system; it is described by a rotationally invariant Lagrangian.

(ii) A non vanishing electric polarization P is found in the minimum energy state and P may acquire many possible values. Correspondingly, we get a large number of degenerate "minimum energy states". The system evolves in time across all the Hilbert spaces generated by the ground states.

(iii) In each Hilbert space the non invariance of the ground state with respect to the electric dipole rotation implies that the physical states of the system must be coherent states of the electric polarization field (dipole wave quanta).

Macroscopically, coherent modes of electric polarization appear in the system and consequently the phases of the dipole fields get correlated.

(iv) According to the general mechanism at work in superconductors, the propagation of the electromagnetic field into a medium whose components are correlated cannot be Maxwellian. Rather, electromagnetic fields propagate within filamentary domains, where the correlation is removed, according to the non-Maxwellian law.

$$(\Box + M^2)A_\mu = J_\mu; \qquad \Box = \frac{1}{c^2}\frac{\partial^2}{\partial t^2} - \frac{\partial^2}{\delta x^2}\frac{\partial^2}{\partial y^2} - \frac{\partial^2}{\partial z^2} \tag{2.1}$$

$$M^2 = f(P) = \frac{q}{m}\frac{2P}{\delta} \tag{2.2}$$

where q is the charge, m the mass and δ the dipole length; M denotes the mass of the e.m. field quantum, and A_μ denotes the vector potential components.

In the simplified case of a completely aqueous medium where all the dipoles are oriented, $M \sim 13\,\text{eV}$ and the filament radius

$$d = \frac{\hbar}{c}\frac{1}{M} \sim 150\,\text{Å}$$

(v) The above non-Maxwellian mechanism [eq. (2.1)] implies a superconductive-like regime for the medium in respect of electromagnetic interactions.

Magnetic fields are excluded from the correlated region and quantization of the flux ϕ flowing across a surface bounding the region holds according to

$$\phi(H) = \frac{h}{q}n.$$

As a consequence, a pair of nearby correlated domains, namely a pair of living systems or subsystems, may be considered as a Josephson junction.

Equation (2.1) holds only when an electromagnetic field is able to couple with the field of the correlation among dipoles. The oscillations of the electric dipoles are able to produce coherent electromagnetic fields whose phase is locked to the phase of the matter field. An external electromagnetic field can become phase correlated only if its energy does not disrupt the coherence. Very strong electromagnetic fields can actually disrupt the correlation and consequently probe the system in an uncorrelated manner, in which case no non-thermal effects would appear. The coherent structure of the matter field in biological systems may reveal itself only when probed by low intensity electromagnetic fields whose frequency lies in a suitable range to interact with the frequencies of the correlations.

3. Josephson phenomena

Let us derive from eq. (2.1) the quantization of the magnetic flux which is the main ingredient of the Josephson effect. In eq. (2.1) $\boldsymbol{J}$ is expressed as:

$$\boldsymbol{J}(x) = \frac{1}{m}\varrho(x)\,[\hbar\nabla\theta(x) - q\boldsymbol{A}(x)]. \tag{3.1}$$

Here m is the mass of the charge carrier responsible for the current and charge spatial density ϱ and the phase θ appear in the wave function for the charge carrier

$$\psi(x) = \sqrt{\varrho(x)}\,e^{i\theta(x)}. \tag{3.2}$$

The condition that the electromagnetic field does not propagate into the region where the dipoles are still correlated gives

$$\boldsymbol{J} = 0 \tag{3.3}$$

namely

$$\hbar\nabla\theta = q\boldsymbol{A}. \tag{3.4}$$

By integrating over a closed contour enclosing a filament, we get

$$\hbar\oint\nabla\theta\,d\boldsymbol{r} = q\oint\boldsymbol{A}\,d\boldsymbol{s} = q\phi(H). \tag{3.5}$$

Single-valuedness of the wave function ψ implies that:

$$\phi(H) = \frac{h}{q}n = n\phi_0, \tag{3.6}$$

where n is an integer number.

When $q = 2e$, then $\phi_0 = 2 \times 10^{-7}\,\text{Gauss cm}^2$.

Equation (3.6) is the starting point for the derivation of a Josephson effect.

Let us consider the simplest case of a pair of coherence domains namely, in our case, two regions where eq. (2.1) is valid, separated by a thin barrier of material in which eq. (2.1) is not valid (junction). A pair of neighbouring biological cells could represent the above model. As in the standard case of metal superconductors, it could be expected that a tunnel current of charge carriers as described by eq. (3.1) passes across the barrier, due to the energy gap 2Δ in the carrier states density of two such coherence domains. This current varies strongly with the applied bias voltage especially near $V = 2\Delta/e$. Following Josephson we could expect that the barrier could also sustain a "supercurrent" through the tunnelling of charge carrier pairs.

In the standard superconductor case, it was suggested by Josephson [6] and experimentally found by Anderson and Roswell [8] that the tunnel supercurrent determines the phase difference

$$\theta_2 - \theta_1 = \phi$$

between the two superconductor wavefunctions ψ_1, and ψ_2.

For a tunnel junction, the relation between the current density J along the junction axis and the electric and magnetic fields is expressed by the Josephson equations

$$J(x, y, t) = J_j \sin \phi(x, y, t) + \sigma_0 V(t) \tag{3.7}$$

$$\frac{d\phi}{dt} = \frac{2e}{\hbar} V(t) \tag{3.8}$$

$$\nabla\varphi = \frac{2e}{\hbar} DB \times z \tag{3.9}$$

where J_j is the peak pair current density, σ_0 is the quasi particle conductance, $V(t)$ is an externally applied voltage, $\boldsymbol{B}$ is the magnetic field in the barrier, $D = 2\lambda_L + d$ with λ_L the London penetration depth in the superconductor and d is the thickness of the barrier.

The current $J(x, y, t)$ is along the z-direction. It can be seen that the Josephson current depends in a remarkable way on the magnetic field across the barrier.

If we restrict the magnetic field to the x-direction and integrate eq. (3.7) over the junction area, then, when capacitive currents are included, one finds the following current voltage relation for the junction

$$I = \beta I_j \sin \phi + C \frac{dV}{dt} + \frac{V}{R_J}. \tag{3.10}$$

The critical current I_J, the shunt resistance R_J and the capacitance C are the macroscopic parameters for the description of Josephson tunnel junctions.

We recall that coupled electron pairs can go over the barrier when a nonzero voltage is applied across the barrier.

The only requirement is that the excess energy $(2e)V$ gained (or lost) by the electron pairs in crossing the potential difference V be invested into an electromagnetic field of frequency such that

$$h\nu = 2eV \tag{3.11}$$

or more generally

$$nh\nu = 2eV, \quad (n = 1, 2, \ldots)$$

if more than one photon is absorbed or emitted.

Although the above results have been derived for a tunnel junction, they also hold for a more general class of junctions called "weak links" [9]. These links, depending on the geometry (e.g. point contacts and microbridges), can in different ways originate tunnel junctions phenomena.

4. Josephson-like behaviour in biological systems

As a matter of fact the search for a Josephson phenomenology in living systems is a very complicated task and has been possible only after having acquired a big amount of expertise in dealing with the electromagnetic properties of living matter [10–18].

One of the authors (CWS) and his co-workers have, over many years, found evidence that Josephson-like phenomena are occurring in living systems. The first piece of evidence [10] came in 1975, when in the course of measurements on enzymes it was found that magnetic fields of the order of 60 mT could lead to very large changes in the dielectric constants of dilute solutions of enzymes. While the dielectric constant is very sensitive to the displacement of single ions, the magnetic field to be effective must be able to overcome thermal fluctuations in ion motion over a volume of the order of a micron in diameter. This clearly indicates the presence of a cooperative phenomenon capable of increasing the susceptibility in a magnetic field. The effect disappeared above a critical value of the field suggesting a Meissner effect. Also the effects disappeared, if the solution was completely sterile biologically. These results have been discussed on the basis of a model in which there is a small superconductive region with linear dimensions smaller than the London penetration depth. A dispersion of such regions will act like a diamagnetic medium and could give rise to an a.c. Josephson effect. These preliminary results were repeated more extensively and using a different experimental technique for measuring the diamagnetic susceptibility [11].

Access was then obtained to an apparatus specifically designed for the measurement of magnetic susceptibility; it used a micro-balance to measure the mechanical force on the specimen in a magnetic field gradient. On this, the temperature variation measurements of the diamagnetic susceptibility of lysozyme [12] were confirmed and extended; it has been also demonstrated that the anomalous effects were absent for poly-nucleotides. A further interpretation of these experiments was given subsequently [13].

The assumption was made that if room temperature superconductive effects exist in association with living cells, some of the other basic experiments of superconductivity should also work.

The above investigations formed the bases on which we were able to look for direct evidence of Josephson phenomena in yeast cells.

4.1. *Yeast cell preparations*

Saccaromyces cerevisiae (normal diploid strain) from Griffin and George Ltd were grown at 30°C in a sterilized medium of 50 mM potassium hydrogen phtalate containing 10 g/l potassium acetate, 6.7 g/l Difco yeast nitrogen base, 1 g/l Difco yeast extract and 40 mg/l adenine.

After 48 h of incubation at 30°C, the cells were harvested in late log phase by cooling to 24° C and then stored at 4° C. Before the experiments the cells were raised from 4° C to 24° C and their concentration in the suspension was adjusted (by centrifugation and decantation of the supernatant) to an optimum value of 1.9×10^6 cells/ml.

The cells were then washed 3 times in deionized 0.25 M sucrose and resuspended in 0.25 M sucrose at 30°C for 3 h. By this experimental procedure the cells were approximately in the same growth phase with a mean generation time at room temperature of about 4 h.

The measurements were performed on the cells during the interval 3 to 5 h after the start of this incubation.

4.2. *Current–voltage characteristics*

Apparatus which had been set up for dielectrophoresis measurements on yeast cells by the method of rate of collection of "pearl-chains" of cells at point electrodes under the attraction of alternating electric fields [17, 18] was suitable for investigating their current–voltage characteristics to look for Josephson-like steps.

Thus a pair of electrodes (78 RPM steel gramophone needles) were sealed into a well in a perspex (PMMA) block mounted on a standard microscope slide. The well contained the suspensions of yeast cells prepared as described above.

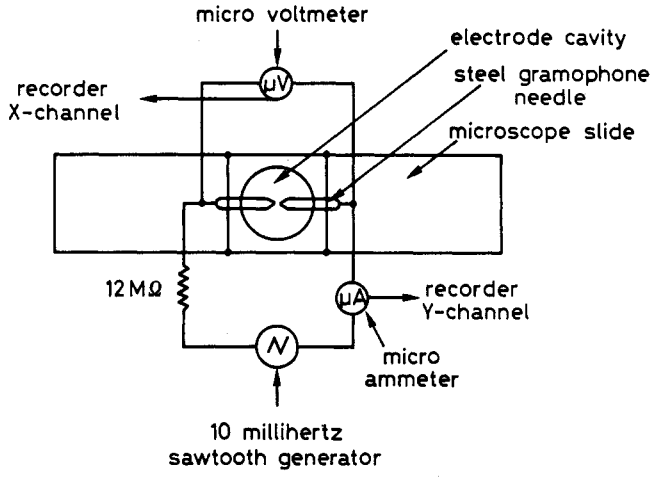

Fig. 1. Experimental configuration for current–voltage measurements on yeast cell clusters.

In order to control the distance between the electrodes from 10 μm down to 1 μm, a sliver of mica was used to separate the electrodes while they were cemented into position with an epoxy resin. Based on the current-voltage measurement, the 1 μm configuration appeared to be the best for finding radiofrequency Josephson emissions. Prolonged illumination at the microscope was avoided in order to reduce undesirable thermal effects on the cells as well as the interfering magnetic fields produced in the base of the microscope by the transformer and by the illumination wire. All the experiments were carried out in an electrically screened laboratory with filtered power supplies.

To form a cluster of yeast cells at the electrode gap an alternating 50 kHz electric field at about 30–40 V was applied to the electrodes by an audio frequency signal generator (Advance, type J1). Within an interval of time of two to five minutes, the space between or around the electrodes as viewed through a microscope (Wild M11) with 200X magnification was seen to be filled with cells. The electrode chamber was then removed from the microscope stage.

At this point the oscillator was replaced by a 10 mHz sawtooth current source connected to the electrodes in series with a resistor of 12 MΩ so as to pass a constant current between the electrodes. A microvoltmeter (Keithely 150B) was connected across the electrode gap, and the current was measured by a microammeter (Level DC type TM10). (see Fig. 1) Their outputs were connected to a X–Y chart plotter and current–voltage tracings were recorded.

Steps in the current–voltage characteristics as shown in Fig. 2 and 3 were observed with these cells for a brief interval of about 2–3 min before cell division. Over this period the amplitudes of the steps were reasonably constant, but over many experiments, the steps had a skew distribution in their amplitudes with the mode at 474 nV in the case of experiments carried out in the geomagnetic field of 50 μT (Fig. 4). Experiments were also performed in presence of external magnetic fields up to 0.4 T. In the case of experiments carried out in a magnetic field of 400 mT the distribution of the steps was more complicated; the main mode was at 1580 nV but there were subsidiary modes at multiples of 790 nV. Thus, increased magnetic field gave larger steps.

Therefore, in order to make it worth looking for an a.c. Josephson-effect corresponding to voltage steps, we needed to ensure that the frequencies would come within the range of

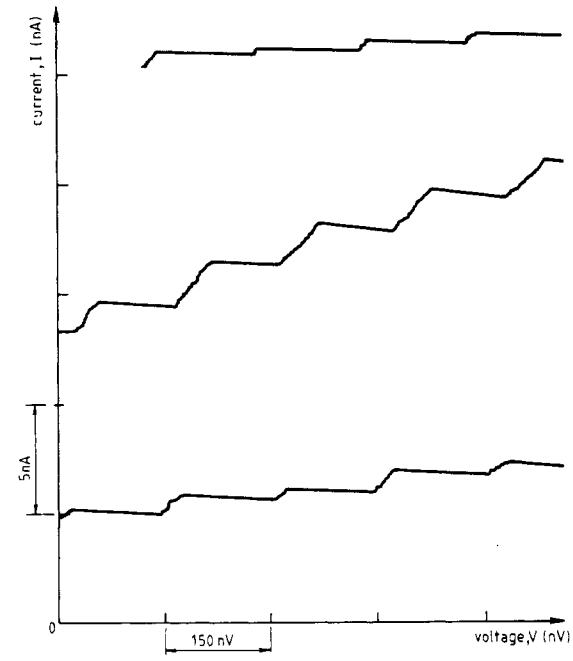

Fig. 2. Steps in the voltage–current characteristics of live yeast cells (*S. Cerevisiae — normal diploid strain*) observed during the 2–3 min before the occurrence of cell division (i.e., after 4 h of incubation). External magnetic field 0.36 T and temperature 22°C.

the available spectrum analyser, that is we needed to get smaller steps. This was done by reducing the electrode gap to about 1 μm and carrying out the experiment in the darkness of a mumetal screening box. These responses were not observed for killed cells, where a linear ohmic behaviour of I/V was maintained, and are therefore associated with the cell division activity. Examples of these and other control experiments are also given in Fig. 3. These gave current–voltage tracings which were linear down to the noise levels of the amplifiers when: the well was filled with 0.25 M sucrose in deionised water, sterilised yeast cells in 0.25 M sucrose in deionised water, and in presence of applied magnetic fields up to 400 mT and a 100 kΩ resistor applied across the input.

4.3. *Radiofrequency oscillation measurements*

For these measurements the two point electrodes sealed on the slide well were connected respectively to the inner conductor and to the grounded screen of a BCN connector, which

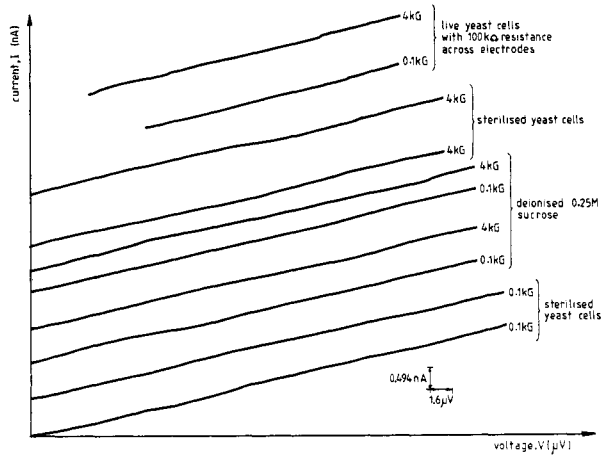

Fig. 3. Voltage–current characteristics from a series of control experiments. No steps were observed with sterilized yeast cells, deionized sucrose, live yeast cells with electrodes loaded with a 100 kΩ resistor.

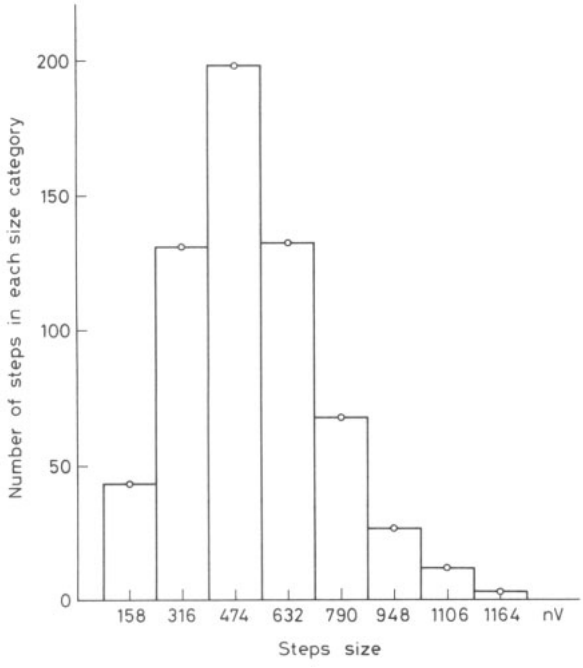

Fig. 4. Amplitude distribution of voltage steps in current–voltage characteristics for *S. Cerevisiae* in different experiments taken during the 2–3 min before the occurrence of cell division (total number of steps = 208). Laboratory ambient magnetic field 50 μT and temperature 22°C.

was also mounted on the slide by an epoxy resin. This was connected to a spectrum analyser H.P. 8553B (see Fig. 5) through a short coaxial cable coming out from the mumetal screening box, all wrapped in a grounded screen for additional electrical and light shielding.

At a time corresponding to cell division a signal was observed, coming out from the 0.1 μV noise level of the spectrum analyser with a band width of about 2 MHz.

Within a short time of approximately 1 min, an oscillation emerged from the spectrum narrowing rapidly down to about 50 Hz band width centered around a 7–8.5 MHz frequency (see Fig. 6 and Ref [17]). Oscillations at 1 MHz and at 60–80 MHz occurred simultaneously.

After a few minutes, the spectrum broadened again and fell back into the noise. To take photographs as shown in Fig. 6, it was necessary to set the spectrum analyser to cover the spectral region 5–10 MHz at maximum gain. As soon as the signal begins to come up from the noise, it must be centered on the screen and the scan reduced to be able to

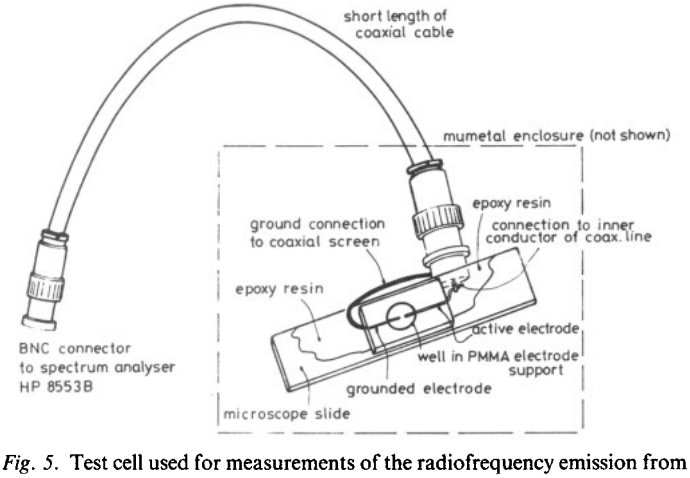

Fig. 5. Test cell used for measurements of the radiofrequency emission from dividing yeast cells (*S. Cerevisiae*).

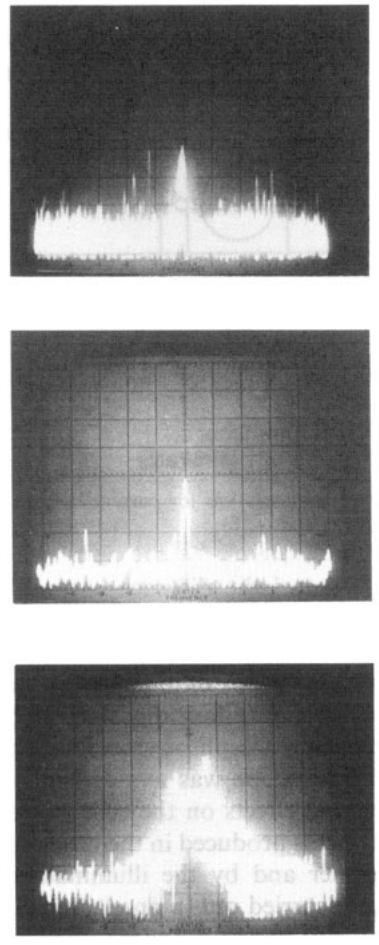

Fig. 6. Radiofrequency emission spectra from yeast cells (*S. Cerevisiae*) taken at 1 min intervals during the 2–3 min before the occurrence of cell division. Centre frequency: 7.0 MHz (horizontal: 2 kHz/div; vertical 0.1 μV/div linear).

resolve the band width of the signal. At a suitable instant, the image is stored and photographed and then the oscillation needs to be found again and the spectrum analyser reset. With practice up to four spectra could be obtained during the 2–3 min that the oscillation lasted. The maximum level of the oscillation observed was about 0.5 μV, the noise level of the spectrum analyser was 0.1 μV.

These oscillations were stopped while being shunted by a capacitance of 100 pF (187 Ω at 8.5 MHz) and separately by a d.c. leakage resistance of 10 MΩ. This showed firstly that the 50 Ω input impedance of the spectrum analyser did not have to be reduced much further in order to stop the oscillations and that the d.c. leakage introduced by 10 MΩ was sufficient to upset the ionic equilibrium established. Thus the use of a short connecting cable and the highly deionized solution employed were justified. The yeast cells withstood this osmotic shock.

From the experimental configurations employed for these two measurements it is clear that it was not possible to measure simultaneously the voltage steps and the radiofrequency emissions. As discussed above it was however possible to calibrate the occurrence of cell division 4 h after the starting of cell cycle. Both voltage steps in the I–V characteristics and radiofrequency oscillations were observed at the same time (cytokinesis) before cell division, and lasted for approximately the same interval of time (of a few minutes).

Although voltage steps and radiofrequency emissions were

not measured simultaneously on the dividing cells, as stated in the previous paragraph, the observed 15 nV steps were compared with the 7–8 MHz frequency emissions observed at the same time of the cell cycle and under the same external condition. A frequency of 7.5 MHz would then correspond to a Josephson-voltage of 15 nV with a conversion factor of 500 MHz/μV. This value appears to be the same as that found in the Josephson effect occurring in superconductors where electron pair carriers are involved.

To actually measure the oscillations and voltage steps simultaneously, the following experimental conditions would seem to be necessary:

(1) The cell and spectrum analyser as described above;

(2) A current sawtooth (10 mHz) connected through a resistor $\gg 10\,\text{M}\Omega$ to the active electrode. Also, a voltmeter of input resistance $\gg 10\,\text{M}\Omega$ and capacitance $\ll 100\,\text{pF}$ connected between the electrodes to give the circuit shown in Fig. 1.

5. Conclusions

The appearance of a Josephson phenomenology in yeast cells is a positive test for the general idea that coherence would be the fundamental feature of biological dynamics. This would open the way to understand why and how external electromagnetic fields could interfere with the fundamental processes of cell division and conversely how this cellular process could induce electromagnetic phenomena.

We point out here a possible consequence that arises from the realization that a pair of nearby cells acts as a Josephson junction. In a set of identical cells, which could be considered as an array of junctions, under suitable conditions the phase among the different cells could be locked-in [19].

The intracellular coherence would then through the Josephson effect give rise to an intercellular coherence.

The investigation of this possibility seems to us a promising outlook emerging from the present research.

A preliminary discussion on this topic can be found in Ref. [20] together with discussion of the relevance of the Josephson mechanism to communication between cells. Moreover Josephson junction approach can be useful in clarifying the interaction between biological systems and electromagnetic fields.

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