CELLULAR SPIN RESONANCE BEHAVIORS OF YEAST

By

SOHEILA HADDAD-DERAFSHI

Bachelor of Science

Central State University

Edmond, Oklahoma

1983

Sumitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1986

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atory for the financial support it provided while conducting this study.

My parents, Rashid Haddad-Derafshi and Farah-Alzaman Balazadeh, deserve my most sincere and deepest appreciation for their unending support and love. Last, but not least, special thanks go to my husband, Baha Jassemnejad, for encouraging me while I strove to finish this thesis, and his support when it seemed it would never get done.

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PREFACE

This study will concern cellular spin resonance of Saccharomyces cerevisiae and it's polarizability. Appendix A with additional information will be included in this thesis.

The selection of cellular spin resonance behaviors of yeast as the topic for this thesis came about for several different reasons. First, my having biological background, studying cells seemed to be a good, logical and natural choice. My desire to learn a little applied physics and computer also contributed to my choice. Most important, the chance to work in collabration with Dr. Herbert Pohl who had started me off in research while I was out of school. I wish to express my sincere gratitude to Dr. Herbert Phol, who is one of the best in his trade, for his patience and encouraging me to carry on.

I also wish to thank Dr. Herbert Bruneau who advised me on academic portion of the graduate degree. My thanks also to Dr. Kent Pollock, and Dr. William T. Philips for helping me and answering my many questions as they pertained to physics.

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CHAPTER I

INTRODUCTION

Suspended living or dead cells can be observed to spin when in a rotating electric field, such as that provided by a four-pole electrode system. The phenomenon is observed to be linked to several factors such as: cell species, age, type, health, and culture age. The spinning occurs in a resonant response to the applied frequency. It has been proved that the spinning of live cells differs from that of dead cells. Live cells usually rotate counter-field with except at very high frequency. By using the technique of cellular spin resonance (CSR), each cell can be individually studied during its advance through the life cycle. This will be the thrust of my research, i.e., to study the changes in electrical properties of cells as they advance through the life cycle (1983a).

Seventeen years ago, Pohl and Crane showed that yeast cells can rotate with a frequency a few HZ in an alternating electrical field in the frequency range between 100HZ and 500KHZ (1971). Research is currently very active in this new area. Pohl and Crane (1971) used the descriptive term, "cellular spin resonance" (CSR) for this phenomenon, because the spinning of the individual cells

appears at a sharply resonant frequency of external field, especially when cells are subjected to a rotating electric field. Nevertheless, it is often possible to find spinning cells at certain applied frequencies even in a non-rotating AC field, mainly in direct contact with electrodes. This may be due to the occurence of natural oscillating dipole fields from within the cells themselves that interact in a resonant fashion with the externally applied AC field.

In normal CSR, cells could start rotating when exposed to AC fields. The rotating of cells is dependent on the electric field. Sometimes cells rotate very fast and other times they rotate very slowly. Several different research studies have been done on yeast cells (Saccharomyces cerevisia) such as: (1) How cells rotate and in what directions, (2) Is there any difference between the spinning characteristics of dead or live cells?--At what frequency do they rotate most rapidly? These experiments have been done mostly with two-pole, three-pole and four-pole chamber electrode chambers (1982).

In the two electrode experiment, it is observed that CSR is affected by age, the physiological condition, and the phase of the cells life cycle (Pohl & Crane, 1971; Pohl and Braden, 1982; Zimmermann, Vienken and Pilwat, 1981; Holzapfel, Vienken, and Zimmermann, 1982). In such experiments, two types of CSR have been studied: (1) that in massive clumps of cells; or (2) that of lone cells. It was thought that "lone-cells" spinning is associated with the

natural cellular oscillating dipoles, whereas the spinning of cells while en masse CSR instead reflects that and yet other electrical attributes. Some experiments have been done with tripole and fourpole chambers; and the results were the same.

The principle of operation of CSR is simple, and requires only that one be able to observe cellular rotation while the fields are applied. At the present time, direct human observation through the microscope aided by a video recorder (VCR) system is used. There are three types of CSR. In the first, lone cell spinning, which appears to be little affected by local field pertubation due to delayed polarization, can be seen to spin resonantly and to spin at a frequency of rotation which is much less than frequency of applied field. In the second type, particles spin because of interactions due to delayed polarization acting on each other at angle off that the main field. The first type of spinning was reported by Pohl and Crane, (1971), Michel and Lamprecht (1982). The observation of lone cells spinning out in the medium is usually only very brief. The cells often move quickly to an electrode or form pearl-chains with other cells near by. This is the usual result of dielectophoretic forces or perhaps other rather more mechanical forces such as streaming, thermal upsets, etc. Lone cells could be easily observed to spin when they are against a mirror-smooth noble metal electrode (e.g. Pt) while they are in the presence of no other visible source

of pertubation by polarizable particles.

The third type of CSR, due to current-carried change deposition on particles can be observed for inanimate particles present in static or AC field of very low frequency. The mechanism for this type of CSR depends on the action of ambipolar current in producing surface charge dipoles on bodies having a conductivity or dielectric constant different from the suspending medium (1983c).

In the above discussion of the effects of cell life cycle phase these can be covered very clearly in the experiments with yeast cells, for the yeast cell almost uniquely shows readily observable morphologically its point in its life cycle. However, there were unanswered questions as to the mechanism of CSR which leave an empty hole in our understanding. One is: Are there observable CSR differences attributable to two different media? What is the effect of different pH but the same conductivity? Such experiments will help show up the role of surface charges and their degree of dissociation in cell behavior.

To sum up, we asked the questions: How does the electrical polarizability of living cells change during their life cycle. There have been increasingly frequent studies of CSR on cells from which much can be learned about the electrical polarizability, a unique and hitherto little known attribute of cells. With CSR it is possible to examine individual cells. The yeast, Saccharomyces cerevisiae was selected for this study because of its

readily observable morphological changes during its life cycle. Because of its sturdiness, it offers a unique opportunity to observe changes in the electrical polarizability of cells during their life cycle.

CHAPTER II

EXPERIMENTAL PROCEDURE

Chamber

A circuit capable of producing a rotating electric field (CRS) chamber consists of four platinum electrodes having shiny spherical tips with a spacing of 300 micrometers between tips across from each other. The electric circuit was capable of spacing the useful range of 100HZ to 2MHZ is shown in Fig. 1. The design was suggested by Karan Kaler and R. J. Adamson, University of Calgary, was built with slight modifications by Douglas West of Stillwater, Oklahoma.

Cell Preparation

Cells of Saccharomyces cerevisiae were grown in sterile sabouraud medium (Difco) for one to ten days. 1.5 ml of cellular suspension was centrifuged for five minutes and the supernatant was discarded. Cells then were resuspended in deionized water and again sedimented by centrifugation for five minutes and supernate was discarded again. This purification was repeated three times. The final pellet was resuspended in distilled water for a very low conductivity.

Procedure

Dielectrophoretic rotation was observed under a microscope type ortholux (Leitz/Wetzlar) equipped with television camera (Philips EL 8000), a monitor (SABA240F) and a video recorder (JVCHR 3660EG). The time for ten cycles was measured directly, and cell morphology was measured by monitor during replay from the video recorder at low speed (see Appendix).

Measurement of cellular spin rate was done by placing a drop of very dilute cell suspension on the electrode region, covering with a microscope cover slip, applying the rotating electric field, and determining the spin rate by direct microscopic observation and taping by video cassette camera. Typically a potential of 2 volts was applied. As each of the several frequencies was applied, the spin rate of ten cells of the desired morphological shape was measured. The mean and standard deviation was calculated. The five measurements of cell morphologies are: SC, budless single cell; SB, cell with small bud, i.e, a bud diameter less than 1/5 the mother cell diameter; MB, cell with a bud diameter between 1/5 and 1/3 the mother cell diameter; LB, cell with a bud diameter between 1/3 and 2/3 the mother cell diameter; C.K., cell within or nearly in the cytokinetic stage, having two etities of closely the same diameter.

Actual performance of the experiment can be broken down as follows:

1. Cells or particles are placed in a pre-washed chamber and covered with cover slip.

2. Power is then applied to four pole circuit and 'balancing' of the circuit is done.

3. The observation of the cell rotation rate is made by using a stopwatch to time the rotation of the cell.

4. Following the reading, the stopwatch is reset, the frequency generated is switched to the next desired frequency and the circuit is rebalanced as in step 2.

5. In this way a series of spin rates versus frequency measurements are obtained for the CSR spectrum.

Temperature

Cells of Sacchromyces cerevesiae was grown at room temperature from 1 to 7 days.

CHAPTER III

RESULTS AND DISCUSSION

The results of determining the spin rate of the yeast cells are shown in Figs. 2 to 7. In Figs. 2, 3, 4, 5, and 6 are shown the spectra for each cell stage. The points are averages of ten determinations on ten different cells of the same morphological type. The standard deviations are shown plotted on the graphs and are most often too small to show. In Fig. 7 are shown the variations of the spin rates at selected frequencies as a function of the stages in the cell cycle (see Appendix).

To begin with, we can comment that the effective dielectric constant of bodies in aqueous media often display the responses shown diagramatically in Fig. 8. The several well-known polarization mechanisms (cf. Pohl, 1978, Chapter 14) usually considered as associated with suspended particles in aqueous media and having ionic double layers: the "tight ions" are more stiffly held, the "loose ions" move more in a sidewise electric field, the stiffly-held ions can follow even high frequency E-field changes; plasmoidal-type regions: anions bound to chains (Coo^-) and cations chich are loose (H^+, Na^+, K^+) (see Figure 10); dipoles: negative and positive charges of cells which act

differently in charged field and from unchanged field (see Figure 11); and interfacial bulk-bulk interactions (i.e. Maxwell-Wagner polarizations): polarization of positive charges from aquaous layer toward the waxy layer of cell wall and reducing the applied field (see Figure 12) are pictured as arising in rather localized frequency regions. With these in mind, we can begin an interpretation of the observations.

Throughout the low-frequency range (i.e., less than 100kHz) of observations of these live yeast cells, the cellular spin is opposite to that of the direction of rotation of the applied electric field. This one can understand as meaning that the total effective polarizability of the cells is less than that of the supporting medium, water of low conductivity. In the high-frequency region, the cell spins in the same direction as the applied field moves. However, there are statistically significant variations of the cellular spin rate throughout the entire range of 3×10^2 Hz to 1×10^3 Hz. One can take all this to mean that various polarization mechanisms of the cells vary significantly in this frequency range. If in the lower-frequency range the cells are seen to spin more slowly in the anti-field direction, this then implies that some cellular polarization mechanism is increased. In the high-frequency range, of course, higher spin rates still imply higher cellular polarization responses, since the cells are spinning with the field. The spin rate of the cells, then,

is a measure of the cellular polarization at a particular frequency.

We suggest the following provisional interpretation of the results:

a. the spin at 1-10 KHz as due to loosely bound ions of the outer ionic double layer (Dukhin and Shilov, 1974),

b. the spin at 10 kHz as due mainly to loosely bound ions on the cell surface, and in the cell wall (plasmoid-type polarization response) (Einolf and Carstensen) (1971),

c. the spin at 30 KHz as due to tightly held ions of that outer ionic double layer (Schwarz, 1962), and

d. the spin at 100 KHz as due to the sum of two overlapping mechanisms, that of the tightly held ions of the outer double layer of the cell, and that due to Maxwell-Wagner bulk-bulk polarization. As can be seen the polarizability of the yeast cells at 100 kHz then remains quite constant throughout the life cycle (Schwarz 1962),

e. the spin at 300 kHz as due more or less solely to Maxwell-Wagner polarization. It increases slightly as DNA doubling takes place, and decreases somewhat as the cell forms its large daughter cell (Schwarz 1962),

f. the spin at 1000 kHz as due to the overlapping of Maxwell-Wagner (M-W) and some higher-frequency-associated dipolar polarization, such that the total response remains rather constant throughout the life cycle, except perhaps in the single cell phase where inner dipole structures such as DNA, RNA, and large protein molecules are beginning to

show relaxation polarization (Schwarz 1962).

Then one can observe that:

a. The "loose ion" and wall-type polarizability is maximal in the single cell and large bud stages.

b. The "tight ion" polarizability is minimal in the single cell and cytokinetic stages.

c. The M-W polarization varies slowly throughout the life cycle; it is maximal at or near the small bud (DNA reproductive) stage, and minimal at the large bud stage.

Said another way: in the single cell (SC) stage, the loose ion condition dominates, and the tight ion condition is repressed. In the budding stages, gradual growth of the tight ion condition occurs until at the cytokinetic stage this trend is gradually reversed so that the ratio of loose and the tight ions' contributions approximates that of the single cell stage. Overall, the polarizability changes indicate that the ions associated with the ionic double layers gradually tighten their association with the outer cell wall during the advance through the cell cycle.

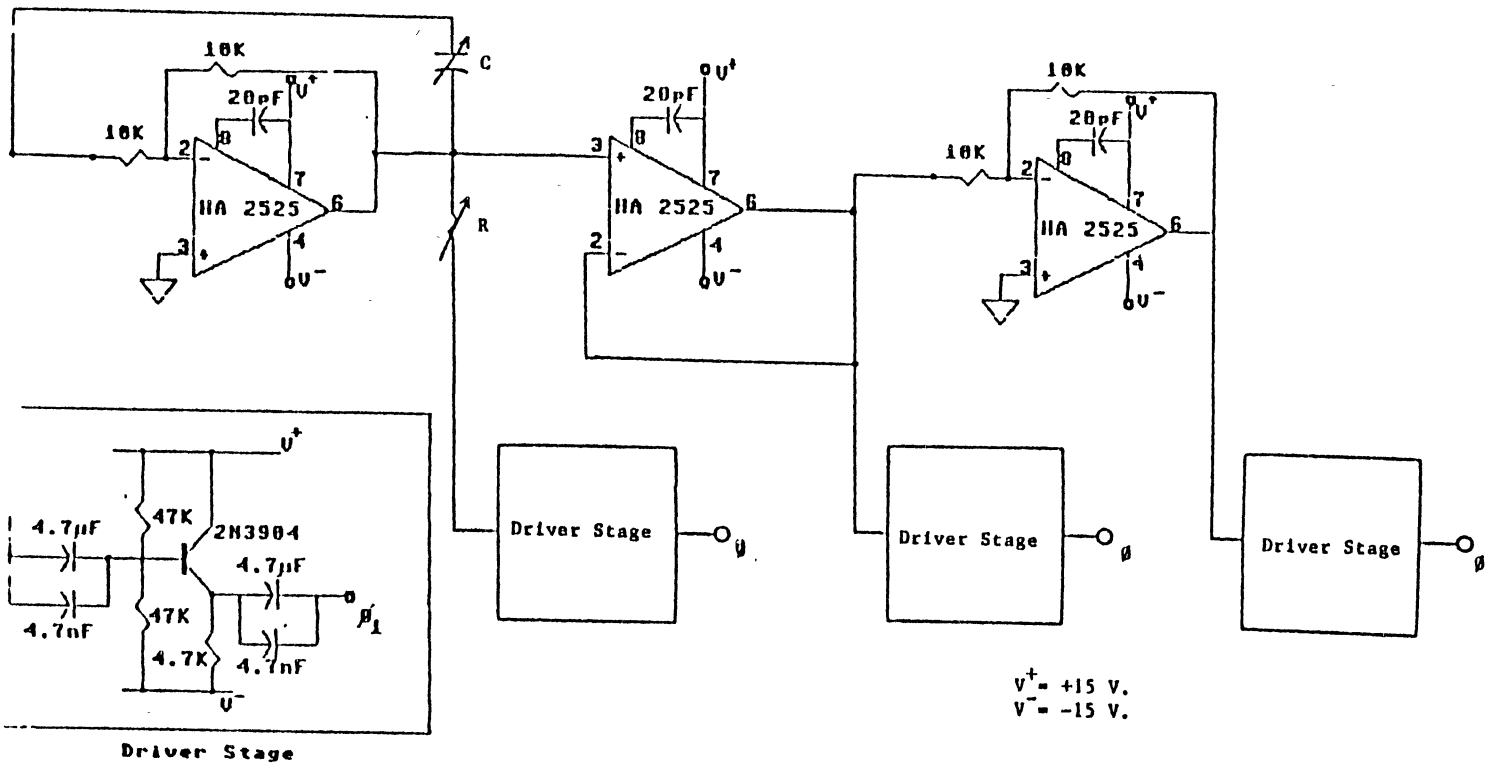

Figure 1. Phase Shift Circuit

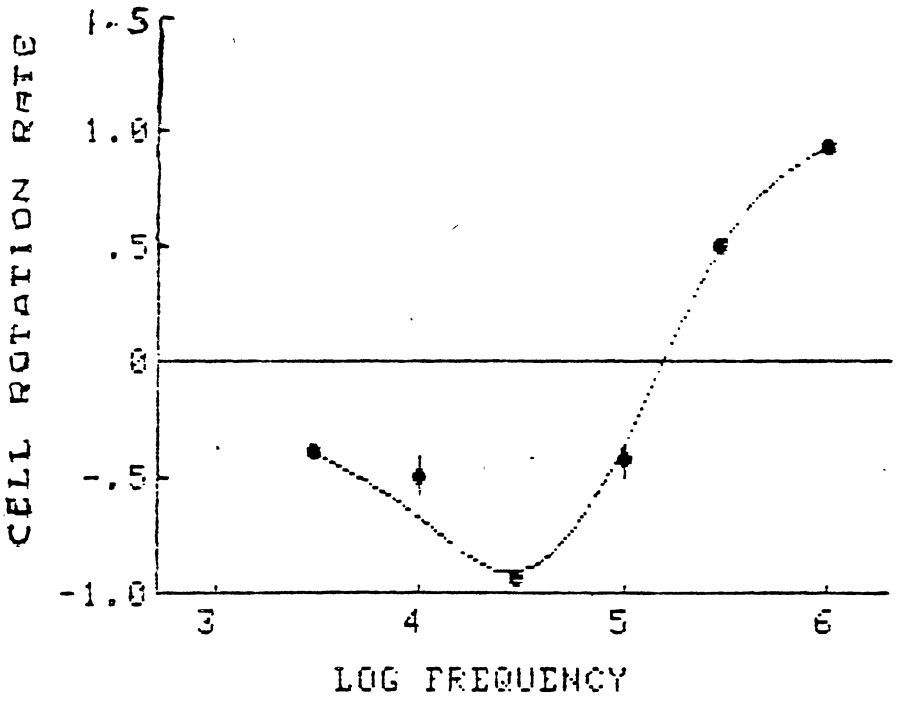

Figure 2. Spin Rate of Single Cell State of Yeast

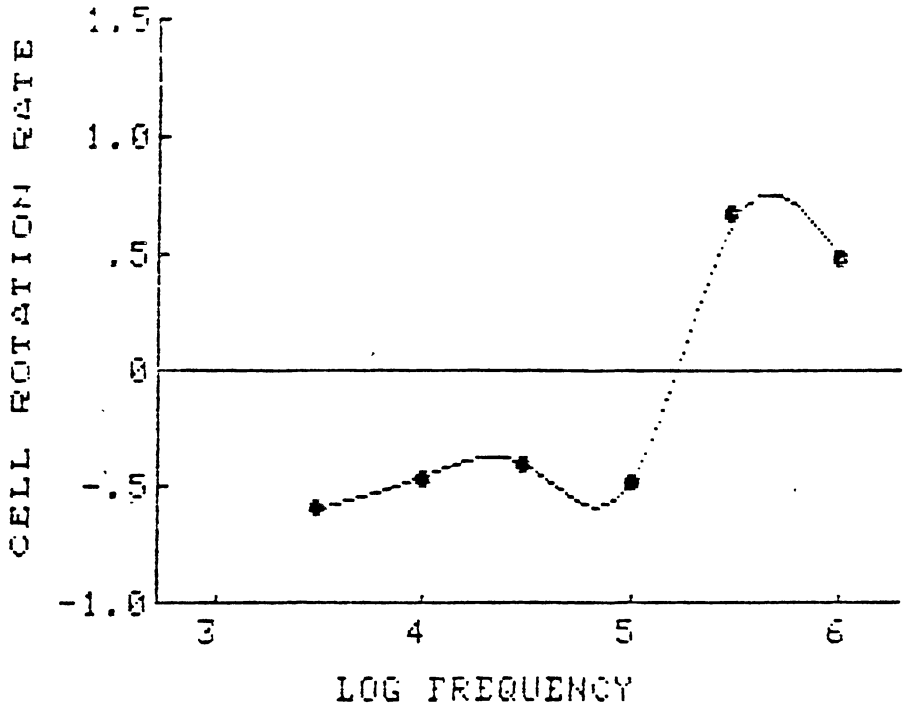

Figure 3. Spin Rate of Small Bud Cell Stage of Yeast

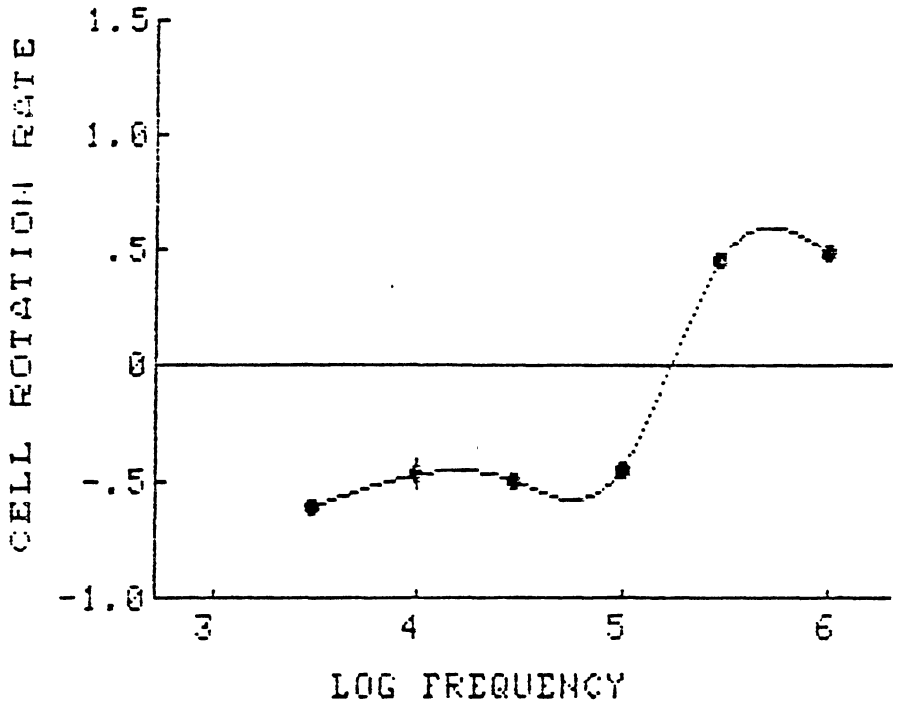

Figure 4. Spin Rate of Medium Bud Cell Stage of Yeast

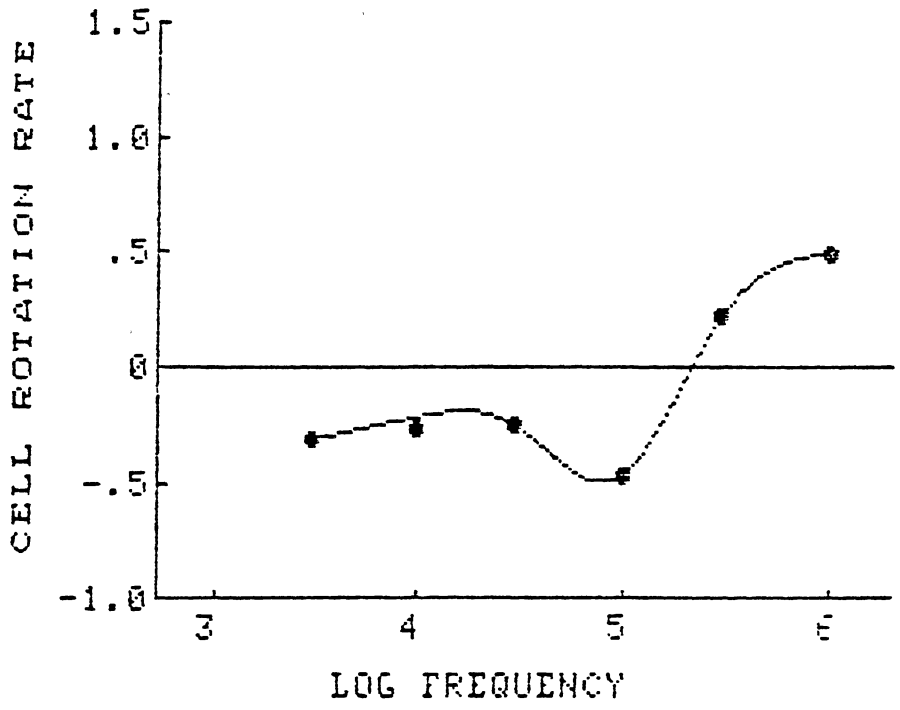

Figure 5. Spin Rate of Large Bud Cell Stage of Yeast

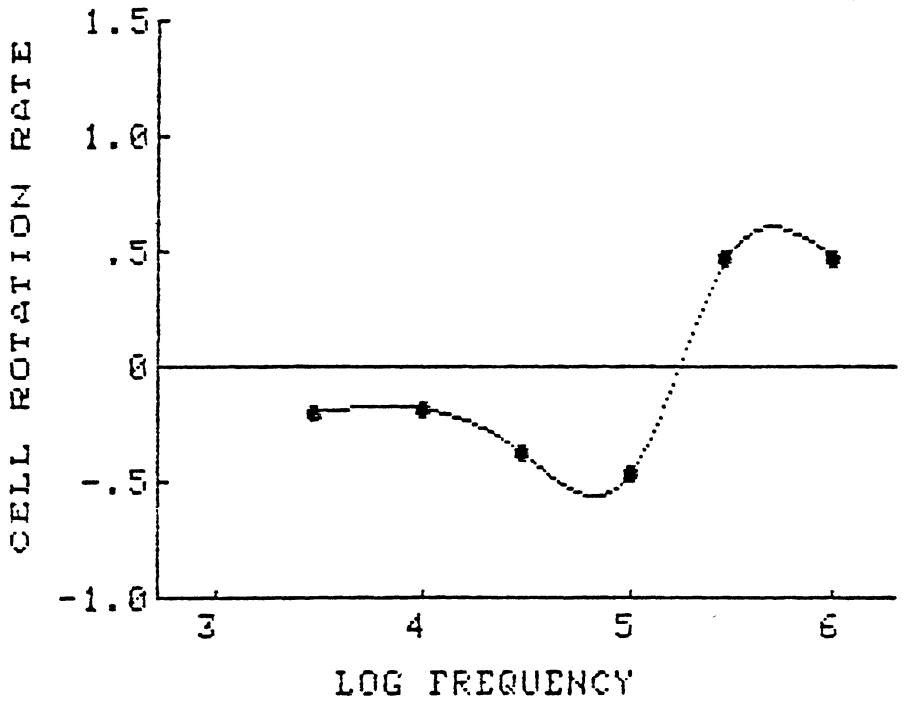

Figure 6. Spin Rate of Cytokinetic Cell Stage of Yeast

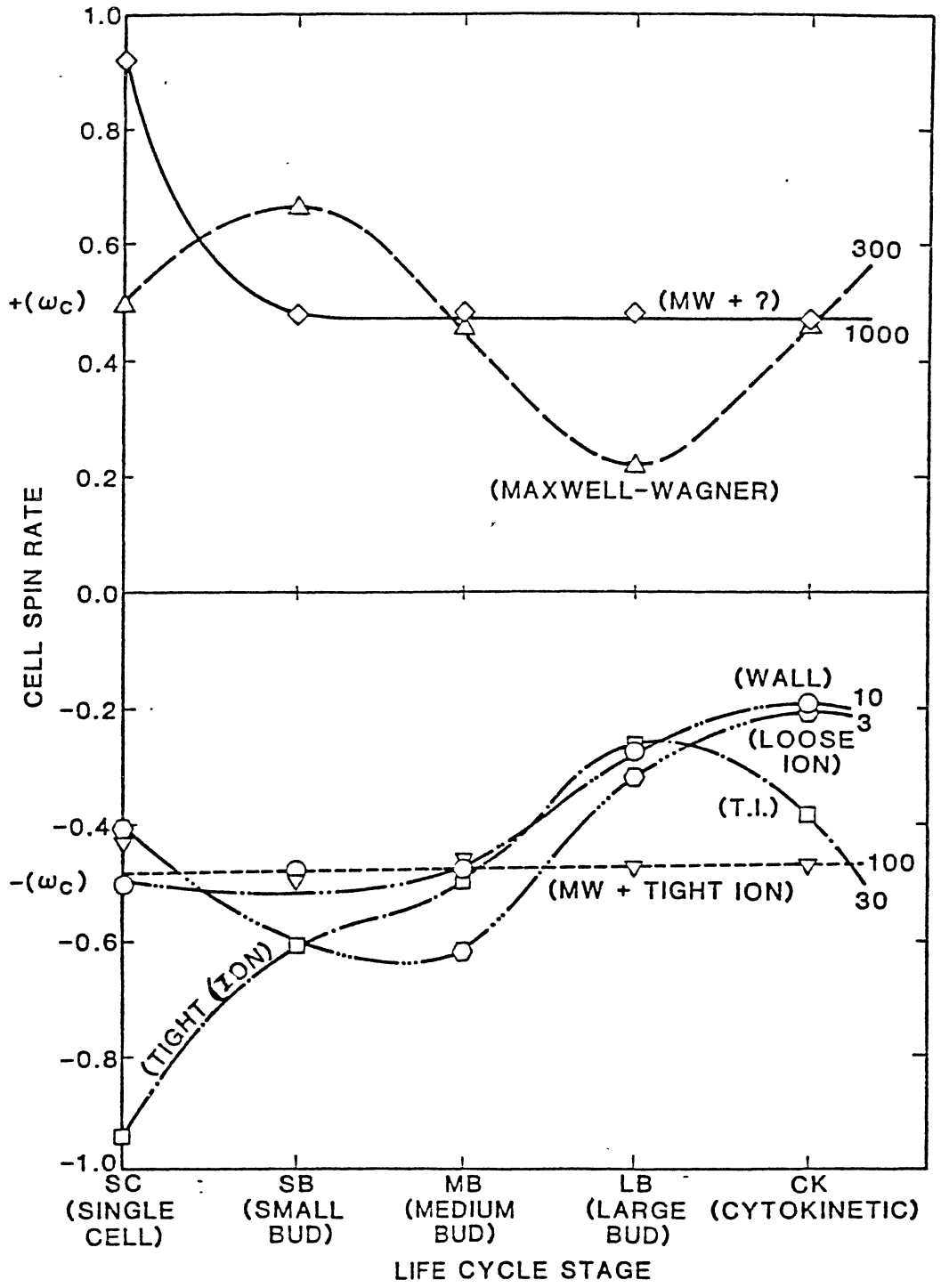

Figure 7. Variation of Spin Rates at Selected Frequencies as a Function of the Stage in the Cell Cycle.

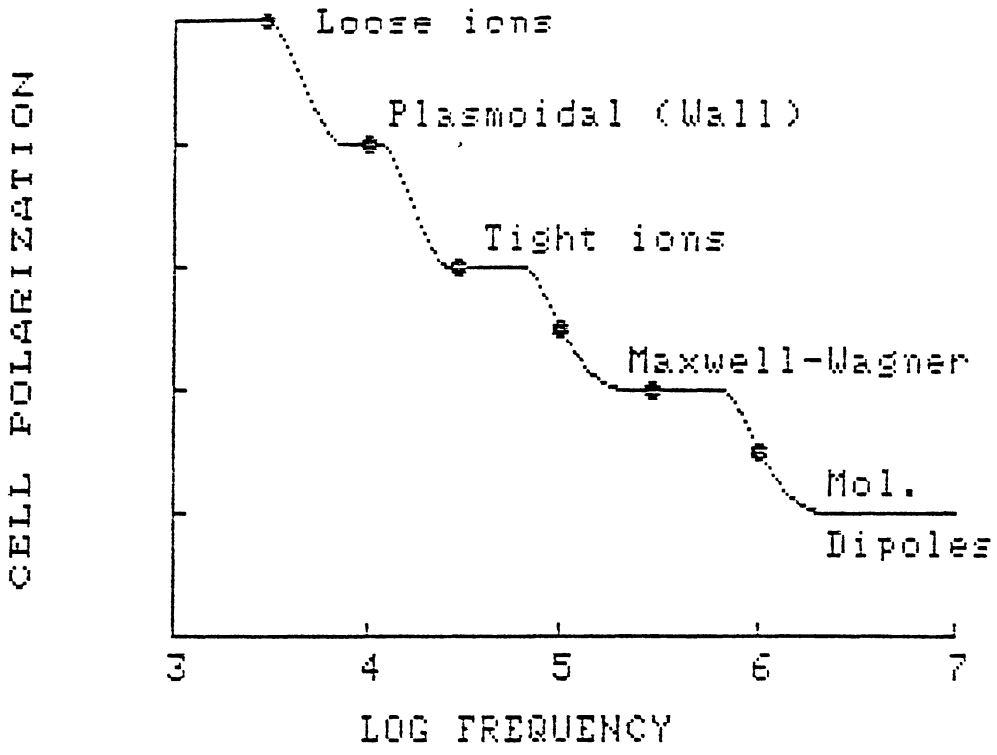

Figure 8. Polarization Mechanisms

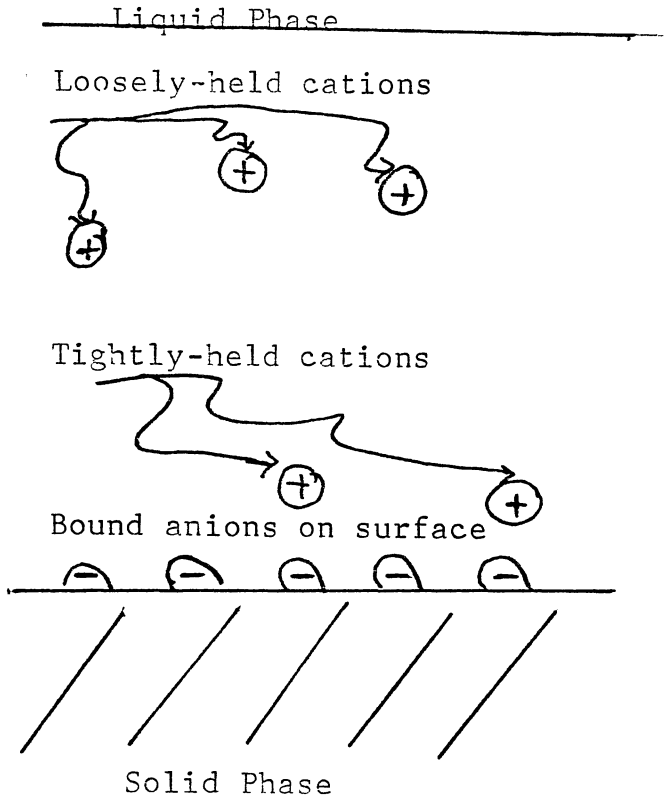

Figure 9. The Ionic Double Layer

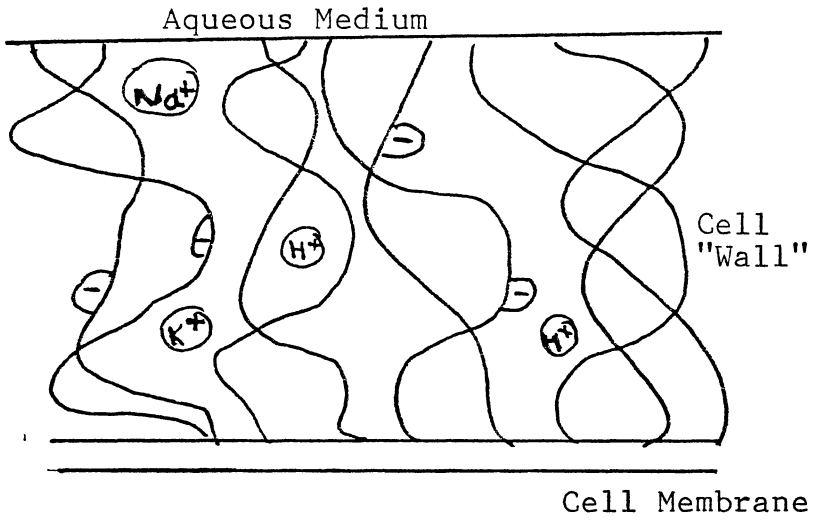

Figure 10. Diagram of the Ion Motion in the Wall of a Cell

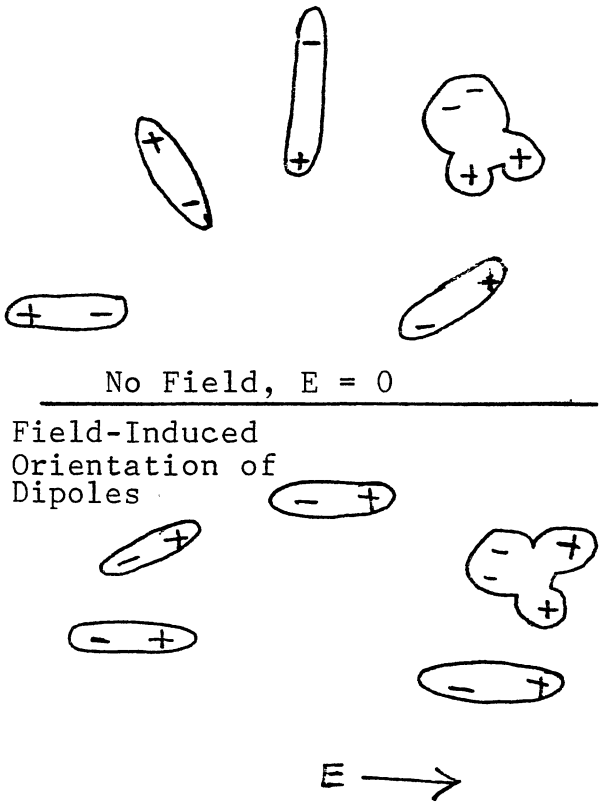

Figure 11. Dipole Orientational Polarization

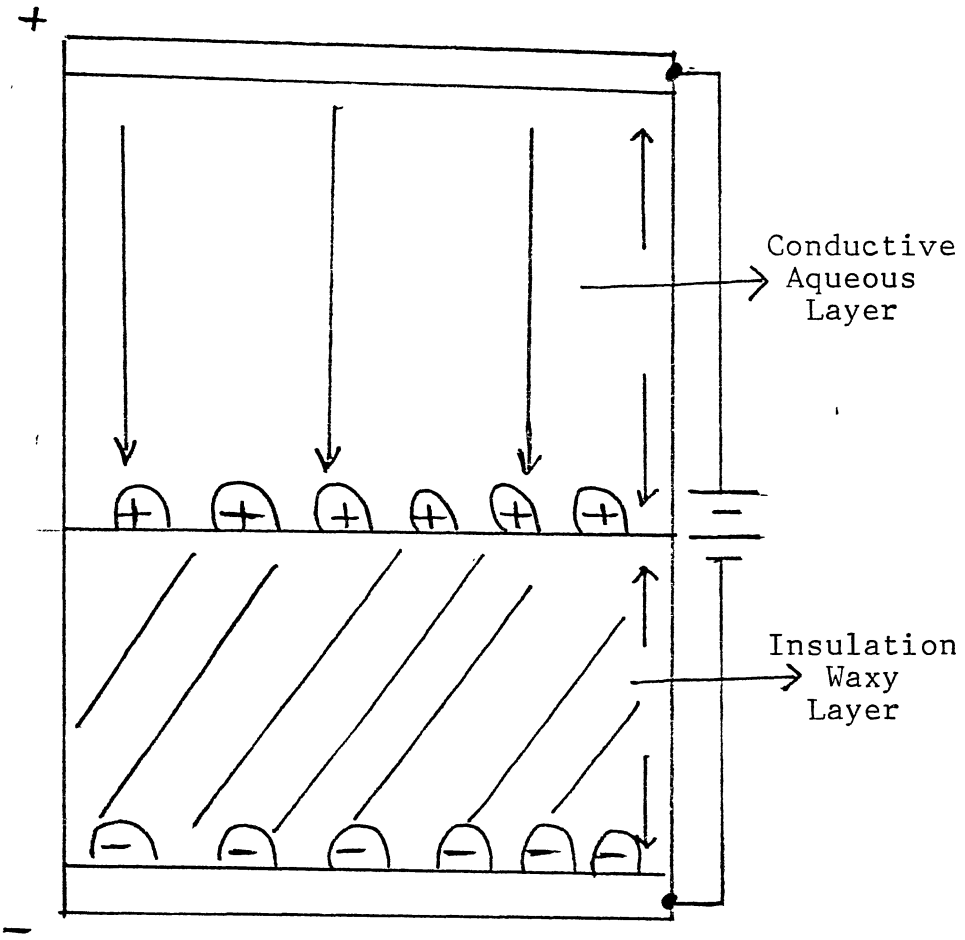

Figure 12. Diagram of Maxwell-Wagner Interfield Polarization

CHAPTER IV

SUMMARY AND CONCLUSIONS

Individual cells may be made to spin by the application of alternating electric fields. The spinning of a given living cell is observed to respond rather sharply and in a resonant manner at several frequencies, ehnce the term "cellular spin resonance" (CSR). The spin rate of cells is linked to several reasons such as type, age and health of cells. All live cells will rotate counterfield with exception of very high frequencies which indicates that polarizability will change because of associated ions with other ionic double layers gradually tighten their association with the outer cell wall during the advance through the cell cycle.

The spin rate at 1-10 kHz is due to loosely bound ions of the otuer ionic double layer. The spin rate at 10 kH is due to loosely bound ions on the cell surface, and in the cell wall. At 30 kH is due to tightly held ions of outer ionic double layer. At 100kH is due to the sum of two overlapping mechanisms, tighly held ions of the outer layer and Maxwell-Wagner bulk-bulk polarization at 300kH is due to Maxwell-Wagner polarization and at 1000 kH is due to the overlapping of Maxwell-Wagner and dipolar polarization.

Looking Ahead

There is still much to be done with CSR such as: the effect of chemicals on cells; continual study between CSR in four pole configuration; difference of cell rotation in several different pH's; effects with the cell age or life cycle of between mamilian cells and their oncogenetic state; effect of different conductants on spin rate of cell.

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APPENDIX

ELECTRICAL ASPECTS OF DIVISION CONTROL IN SACCHAROMYCES

(An Article in Journal of Biological Physics 13, Number 2, 1985)

Electrical Aspects of Division Control in *Saccharomyces*

SOHEILA HADDAD, J. KENT POLLOCK, WILLIAM T. PHILLIPS, and HERBERT A. POHL

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ABSTRACT: Readily discernable changes in electrical character take place during the division cycle of the yeast, Saccharomyces cerevisiae. By using the technique of cellular spin resonance, the electrical polarizability of individual cells can be seen to change with the life cycle, as determined by cell morphology. The polarizability changes indicate that during the advance through the life cycle, the ionic double layers associated with the outer cell wall gradually tighten.

INTRODUCTION

The cell division cycle of yeast, Saccharomyces, has been extensively studied. (Hartwell et al. 1973; 1974; Reed, 1980; Pringle and Hartwell, 1981) The essential physiological and morphogenic events comprising cell division and replication appear to be the result of processes in several interlocking pathways. Their organization appears to converge at the outset of the G1 interval, and diverge at its end. The paradigm in this organism is that cell division is controlled at this period in response to both food limitations and mating phenomenon stimulation. These different signals can initiate operation of a single starting event following which DNA replication and division proceeds in a manner relatively independent of external events. Reed (1984) emphasizes that duplication of the spindle pole body best signals the execution of the "starting event". The spindle pole body is the principal microtubule-organizing organelle of the yeast cell, and is centrally involved in the organization of the mitotic apparatus. The initiation or starting event of such a new cell division cycle can also be monitored morphologically by the emergence of a bud (Hartwell, 1973) In the present study of the Saccharomyces cell cycle we have used the bud emergence and its subsequent growth to monitor the advance through the cycle.

Previous studies of the electrical characteristics of Saccharomyces cerevisiae (Pohl and Crane, 1972; Mischel and Lamprecht, 1983; Mischel and Pohl, 1983) showed the rate of spinning in an applied electrical field to relate to the colony age and the shape of the cell. The natural electrical oscillations of the cell, as exhibited by its own external electric field, was shown to be maximal at or near the "starting event" of division. In the present study we have focussed on making more precise the knowledge of the polarizability changes of the cell during its cycle.

To follow the polarizability of individual cells we use the delicate technique of cellular spin resonance. Here the rate of spinning of individual cells, far removed from others, is measured as a function of the frequency and intensity of an applied rotating electric field. The theory for this phenomenon in rotating sinusoidally varying fields (Mischel and Pohl, 1983; Pohl, 1983a; Block et al., 1984) and in pulsed rotating fields (Pohl, 1983b, 1983c) has been studied. The rate of spinning of a cell (ca. a few hertz), normally is far less than the rate of the field rotation (typically kilo-hertz to mega-hertz). It is readily measured directly or with the aid of a stroboscope. The cellular spin rate, usually measured in an aqueous medium that is isoosmotic with the cell but of low ionic strength, can be related to the effective dielectric constant of the cell. Theory suggests that the spin rate is relatively independent of the cell radius because of the concomitant viscous drag of the medium that is compensatingly radius dependent. Since the polarizability of bodies in aqueous media varies strongly with the applied frequency of the electric field, useful spectra can be derived. The sharpness with which the cellular spinning responds to the frequency has occasioned

the term "cellular spin resonance" (CSR) to describe the technique. The interpretation of the spectral responses can be made in terms of various molecular, interfacial, and macromolecular mechanisms.

EXPERIMENTAL METHODS

Cells of (*Saccharomyces cerevisiae*) in media of low conductivity were prepared by centrifuging freshly harvested cells from growth medium (Difco Sabouraud Liquid Medium), rejecting the supernate, and diluting the cells with deionized water or selected medium for the CSR study. The conductivity of the final suspension was between 5 and 9 microMho/cm. The development through the cell cycle was followed by selecting the following five cell morphological types for measurement: SC, budless single cell; SB, cell with a small bud, i. e., with a bud diameter less than 1/5 the mother cell diameter; MB, cell with a bud diameter between 1/5 and 1/3 the mother cell diameter; LB, cell with a bud diameter between 1/3 and 2/3 the mother cell diameter; CK, cell in or nearly in the cytokinetic stage, having two entities of closely the same diameter.

The CSR chamber was closely similar to that described earlier (Mische et al., 1982) and included four platinum electrodes having shiny spherical tips with a spacing of 300 μm between tips across from each other. The electrical circuit, capable of spanning the useful range of 100 Hz to about 2 MHz, is shown in Fig. 1.

Measurement of the cellular spin rate was made by placing a drop of the very dilute cell suspension onto the electrode region, covering the whole with a microscope cover slip, applying the rotating electric field, and determining the spin rate by direct microscopic observation. Typically a potential of 2 V p-p was applied. At each of the several frequencies applied, the spin rates of at least ten cells of the desired morphological shape were measured and averaged, and the standard deviation was determined.

RESULTS AND DISCUSSION

The results of determining the spin rate of the yeast cells are shown in Figs. 2 and 3. In Fig. 2a-e are shown the spectra for each cell stage. The points are averages of ten determinations on ten different cells of the

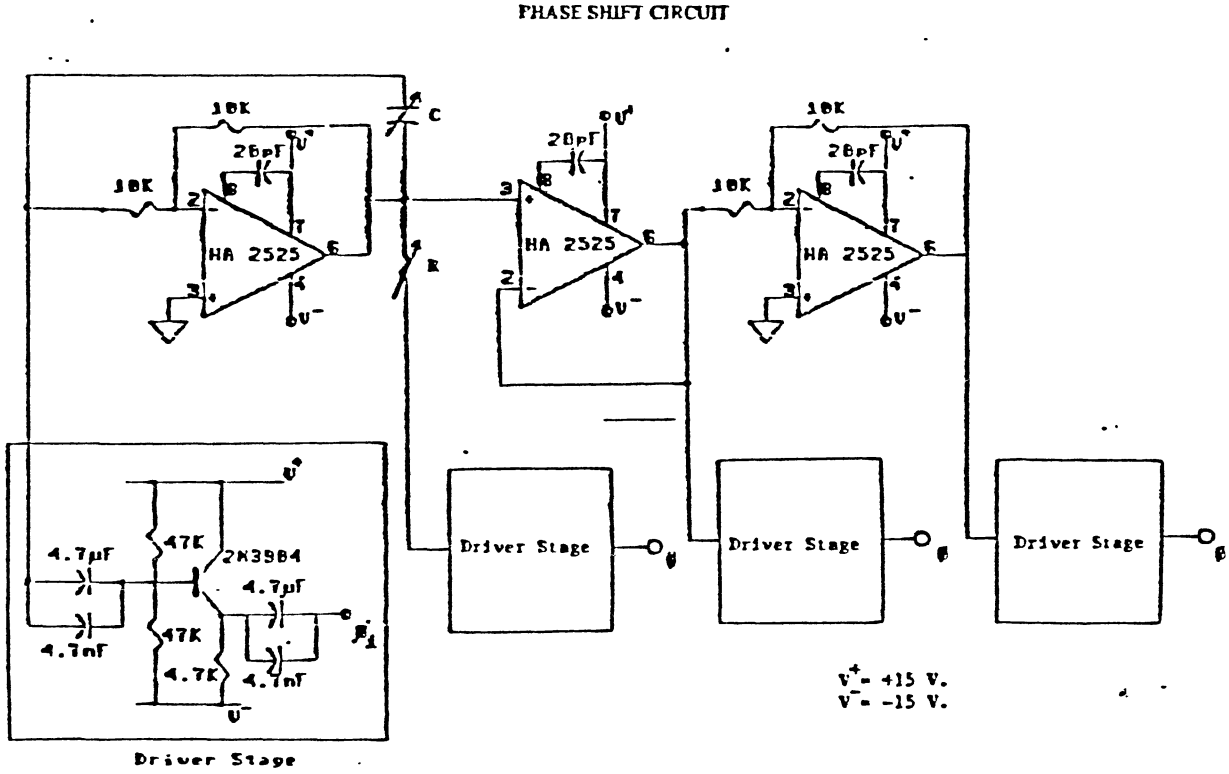

Figure 1.

rare morphological type. The standard deviations are shown plotted on the graphs and are most often too small to show. In Fig. 3 are shown the variations of the spin rates at selected frequencies as a function of the stage in the cell cycle. Looked at in this form, the data are most easily interpretable.

To begin with, we can current that the effective dielectric constant of bodies in aqueous media often display the responses shown diagramatically in Fig. 4. The several well-known polarization mechanisms (cf. Pohl, 1978, Chap. 14) usually considered as associated with suspended particles in aqueous media and having ionic double layers, plasmoidal-type regions, dipoles, and interfacial bulk-bulk interactions (i.e. Maxwell-Wagner polarizations) are pictured as arising in rather localized frequency regions. With these in mind we can begin an interpretation of the observations.

Throughout the low-frequency range (i.e., less than 100 kHz) of observations of these live yeast cells, the cellular spin is opposite to that of the direction of rotation of the applied electric field. This one can understand

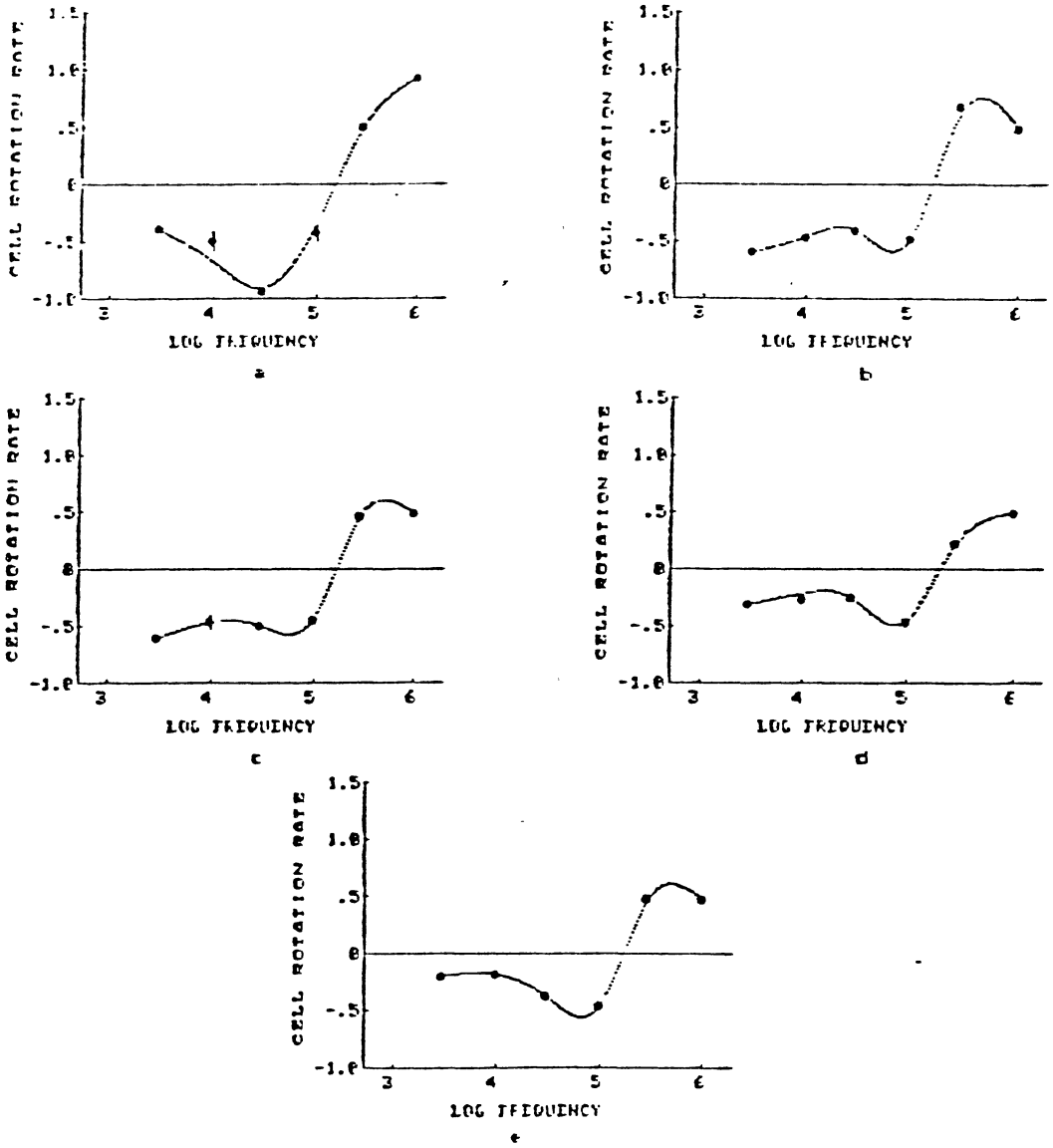

Figure 2.

as rearing that the total effective polarizability of the cells is less than that of the supporting medium, water of low conductivity. In the high-frequency region, the cell spins in the same direction as the applied field moves. However, there are statistically significant variations of the cellular spin rate throughout the entire range of 30 x 10^3 Hz to 1 x 10^6 Hz. One can take all this to mean that various polarization mechanisms of the cells vary significantly in this frequency range. If in the lower-frequency range the cells are seen to spin more slowly in the anti-field direction, this then implies that some cellular polarization mechanism is increased. In the high-frequency range, of course, higher spin rates still imply higher cellular polarization responses, since the cells are spinning with the field. The spin rate of the cells, then, is a measure of the cellular polarization at a particular frequency.

We suggest the following provisional interpretation of the results:

We will assume:

(a) the spin at 1-10 KHz as due to loosely bound ions of the outer ionic double layer (Dukhin and Shilov, 1974),

(b) the spin at 10 kHz as due mainly to loosely bound ions on the cell surface, and in the cell wall (plasmoid-type polarization response) (Pinolf and Carstensen,

(c) the spin at 30 KHz as due to tightly held ions of that outer ionic double layer (Schwarz, 1962), and

(d) the spin at 100 KHz as due to the sum of two overlapping mechanisms, that of the tightly held ions of the outer double layer of the cell, and that due to Maxwell-Wagner bulk-bulk polarization. As can be seen, the polarizability of the yeast cells at 100 kHz then remains quite constant throughout the life cycle.

(e) the spin at 300 KHz as due more or less solely to Maxwell-Wagner polarization. It increases slightly as DNA doubling takes place, and decreases somewhat as the cell forms its large daughter cell.

(f) the spin at 1000 kHz as due to the overlapping of Maxwell-Wagner (M-W) and some higher-frequency-associated dipolar polarization, such that the total response remains rather constant throughout the life cycle, except perhaps in the single cell phase where inner dipole structures such as DNA, RNA, and large protein molecules are beginning to show relaxation polarization.

Then one can observe that:

(a) The "loose ion" and wall-type

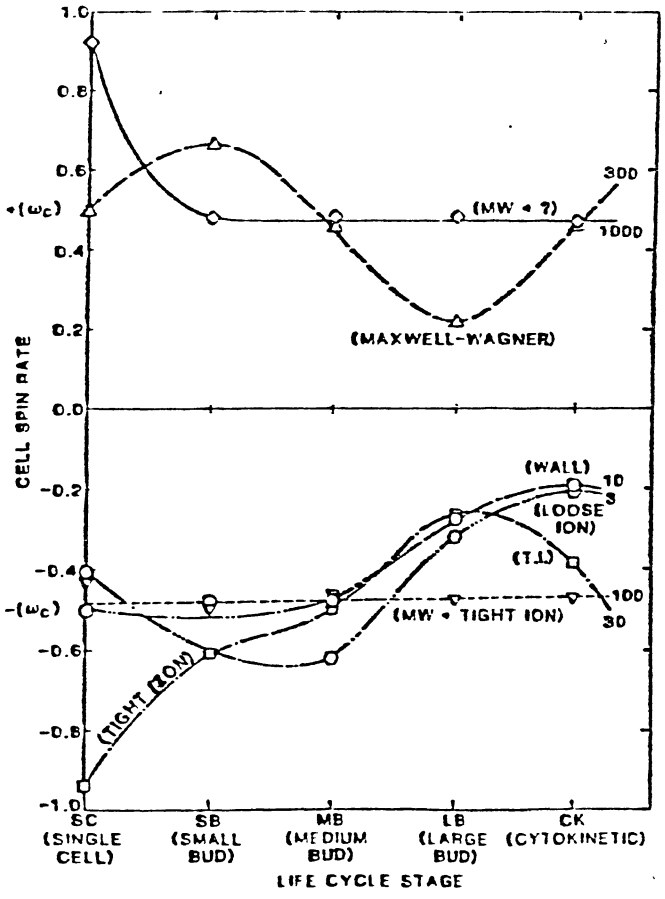

Figure 3.

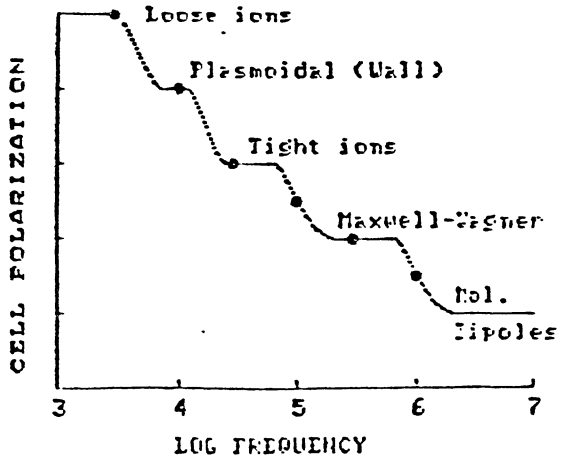

Figure 4.

polarizability is maximal in the single cell and large bud stages.

(b) The "tight ion" polarizability is minimal in the single cell and cytokinetic stages.

(c) The M-W polarization varies slowly throughout the life cycle; it is maximal at or near the small bud (DNA reproductive) stage, and minimal at the large bud stage.

Said another way: In the single cell (SC) stage, the loose ion condition dominates, and the tight ion condition is repressed. In the budding stages, gradual growth of the tight ion condition occurs until at the cytokinetic stage this trend is gradually reversed so that the ratio of loose and the tight ions' contributions approximates that of the single cell stage. Overall, the polarizability changes indicate that the ions associated with the ionic double layers gradually tighten their association with the outer cell wall during the advance through the cell cycle.

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