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NMR Conditions and Biological Systems

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INTRODUCTION

Nuclear magnetic resonance (NMR) is a phenomenon associated with atomic nuclei having an odd number of either protons or neutrons. The first demonstration of NMR was made by Rabi in 1938 using molecular beams. The first observations in condensed matter were made independently and almost simultaneously by Purcell, Torry and Pound in Cambridge, and by Bloch and Hansen at Stanford. The Nobel Prize was awarded jointly to Purcell and Bloch in 1952.

The physical fundamentals and applications of NMR have been the subject of many texts (1-8). The two major NMR applications involve chemistry and medical imaging. The chemical applications arise because the magnetic field at a nucleus is never exactly equal to the externally applied magnetic field, but depends in many ways on the magnetic properties of the molecular structures surrounding an atom. These give rise to characteristic shifts in the nuclear magnetic resonance conditions of the order of parts per million.

The possibility of using NMR for *in vivo* medical imaging of body structures arises because the relaxation times for the return to an equilibrium condition following the application of a nuclear magnetic resonance perturbation to a biological system vary not only among different kinds of normal tissues, but more importantly between healthy and diseased tissues. Application of electronics and computing techniques have enabled living systems to be scanned while the relaxation times are being measured. This results in a map or image of their spatial distribution in such detail that it correlates with the anatomical landmarks, and shows the distribution of physiological and biochemical parameters.

BASIC THEORY OF NUCLEAR MAGNETIC RESONANCE

NUCLEAR MAGNETIC MOMENT

The magnetic moment μ of a coil in a magnetic field B is the torque Γ per unit

magnetic field and is equal to the product of the current i and the area of the coil S,

$$\Gamma/B = \mu = 1 \bullet S \quad (1)$$

Scaling things down to nuclear dimensions, a current i can be considered equivalent to a charge e rotating at $\omega/2\pi$ revolutions per second where ω is the angular velocity in radians per second.

$$i = e\omega/2\pi \quad (2)$$

Taking an idealized nucleus as an annulus of mass M, radius r and charge e rotating about the principal axis with angular velocity ω, the magnetic moment μ will be from Equations (1) and (2):

$$\mu = e\omega r^2/2 \quad (3)$$

The ratio of the magnetic moment to the angular momentum P, is the gyromagnetic ratio;

$$\gamma = \mu/P = e\omega r^2/2M\omega r^2 = e/2M \quad (4)$$

A fundamental postulate of theoretical physics is that the total angular momentum of an isolated particle can only take on certain discrete values. It is said to be quantized, and can only have values which are integral multiples of the quantity $h/2\pi$, often written $\hbar$, where h is Planck's constant (6.626176 x 10^{-34} J.sec). The nuclear energy levels are quantized as a direct result of the quantized nature of the nuclear angular momentum. It takes on a series of values corresponding to unity changes in a quantity known as the spin quantum number I, which can range from +I to –I. Since the proton has I = 1/2, only the two values corresponding to I = ±1/2 are allowed. The value of I depends on the particular nucleus. Isotopes with equal numbers of protons and neutrons have I = 0 and isotopes having odd mass numbers tend to have 1/2 integral spin values. The highest value is for the isotope $^{176}_{71}$Lu, for which I = 6.

NUCLEI IN A MAGNETIC FIELD

Remembering that magnetic moments are generated by the equivalent of rotating charges, a non-zero angle between an applied magnetic field and the magnetic moment of a nucleus is the equivalent of the classical Larmor precessional motion of a gyroscope with its axis at an angle to the gravitational field. Force is given by the rate of change of momentum, torque is given by the rate of change of angular momentum. If a system with angular momentum P is precessing at an angular velocity Ω, then the change of angular momentum in time dt which can give rise to the torque is

$$dP=P\,\Omega \sin\theta\, dt \tag{5}$$

The torque is given by

$$\Gamma = \mu B \sin\theta = \gamma P B \sin\theta \tag{6}$$

Comparison of Equations (1), (4), and (6) gives the angular velocity of the precession

$$\Omega = \gamma B \tag{7a}$$

or in terms of the Larmor precessional frequency, ν_o

$$\nu_o = \gamma B/2\pi \tag{7b}$$

POPULATION OF SPIN STATES

In most cases, NMR transitions between the energy levels will be the result of stimulated emission or absorption of radiation. Irradiation of a specimen with an intense radio-frequency field would rapidly equalize the populations of the energy levels setting up a dynamic equilibrium in which no changes in emission or absorption would be detected. In matter in general, the population distribution between two energy states can be described by the Boltzmann factor,

$$N_2/N_1 = \exp(-\Delta E/kT) \tag{8}$$

where N_1 and N_2 are the number of nuclei in energy states 1 and 2 respectively, the energy state 1 is less than that of 2, k is the Boltzmann constant and T is the absolute temperature.

In a magnetic field of strength B, the separation of the energy levels ΔE is

$$\Delta E=2\mu B \tag{9}$$

$$N_2/N_1 = \exp(-2\mu B/kT \approx 1 - (2\mu B/kT) \tag{10}$$

if $2\mu B << kT$.

The observation of NMR through some physical process depends on being able to detect a net absorption of energy by the small excess population defined by Equation (10). Living systems are, by definition, systems far from thermal equilibrium. Their population distributions are determined by stimulated emission and absorption, and will not be Boltzmann if the system is alive and active (9).

SPIN-LATTICE AND SPIN-SPIN RELAXATIONS

1. NMR Relaxation Times

If a system is perturbed from a condition of stable equilibrium and can eventually return to the initial stable state, the return may take the form of an exponentially damped oscillatory response, such as the motion of a pendulum swinging in air, or the exponential return of a pendulum in a viscous oil. The relaxation rate is expressed by the constant of the exponent.

The concept of relaxation times for assemblies of magnetic dipoles is of great importance in the applications of NMR techniques. By arranging detector coils in various directions relative to the applied field, the magnetizations and relaxations in these directions can be determined. When a radiofrequency pulse is applied, the magnetic dipoles begin to precess at the Larmor frequency about a direction defined by the resultant of the steady and oscillatory magnetic fields. The magnetization nutates, or nods, away from the direction defined by the applied steady field. The nutation stops when the radio-frequency magnetic field is switched off. If this occurs when the angle of nutation reaches 90°, the magnetization would be left to relax in a transverse plane. A 180° pulse is one that reverses the direction of the magnetization. Experimentally, the NMR signal is usually detected by a coil placed perpendicular to the steady magnetic field and resonant at the radiofrequency. The decrease in the induced voltage measured after the end of the radiofrequency pulse is a measure of the relaxation of the magnetization as the nuclei return to their equilibrium.

2. Spin-Lattice Relaxations

The interaction between the nuclear spin and the nuclear environment is small but finite, and given enough time and an absence of stimulation, the nuclei will eventually come into thermal equilibrium with the lattice. Absorption of radiofrequency energy reduces the population of the lower energy state, and the flow of thermal energy opposes this process. Since spontaneous emission is insignificant, the NMR relaxation is only by stimulated downward transitions from those lattice magnetic fields that happen to be at the Larmor frequency. For protons in biological tissues, a typical time constant for this process is 0.05–3 sec (10).

3. Spin-Spin Relaxations

Each nuclear magnetic moment experiences not only the applied magnetic field, but the resultant local interaction field arising from the neighboring nuclear magnetic fields. The local field may have both static and oscillatory components, and it can result in a broadening of the energy levels. The lifetime of a nuclear spin state in the absence of an applied radiofrequency field, is an approximate definition of the spin–spin relaxation time. In biological systems, the spin–spin relaxation times are typically 0.04–2 sec (10).

4. Saturation

In the absence of radiation to stimulate transitions between different states, the relaxation processes outlined will occur. When a strong radiofrequency field is applied, the spin system becomes saturated. In the case of a nucleus for which I = 1/2, the populations of the upper and lower states equalize, leaving no magnetization in any direction.

OBSERVATION OF NMR EFFECTS

To observe a nuclear magnetic resonance it is necessary to perturb the system from the condition of steady-state precession. Magnetic resonance in bulk matter can be excited in several ways (11). The present concern is less with the observation of magnetic resonances by physical measurements as such, because the application of magnetic-resonance techniques to biological measurements is well documented (12, 13). Rather, it is to seek ways in which the excitation of NMR can result in the perturbation of a living biological system which can in turn be investigated by physical measurements.

The three methods commonly used for the observation of NMR are described elsewhere (4).

MEASUREMENTS OF BIOLOGICAL SYSTEMS UNDER NMR CONDITIONS

DIELECTROPHORESIS

Dielectrophoresis is the movement of electrically neutral particles in a nonuniform electric field due to their polarization (14-16). The dielectrophoretic yield is the number of particles collected at the electrodes per unit time. The small dielectrophoresis cell used in our earlier studies was constructed on a standard microscope slide and consisted of a pair of spherical platinum electrodes (diameter, 0.8 mm) sealed into a shallow well that contained a suspension of yeast cells. We now use evaporated metal film electrodes to achieve short optical working distance at high magnification (17). The electrodes were connected to an audio-frequency oscillator.

The yeasts used in these experiments (18, 19) were *Saccharomyces cerevisiae* (normal diploid strain). They were grown as pure colonies in a suitable medium, harvested, and suspended in an ion-free isotonic solution. Cell concentration was measured by standard dilution and plating techniques.

The clean, dry electrode chamber was filled with 0.5 ml of the cell suspension, mounted on the microscope stage, and the electrodes were connected to the oscillator. When the selected voltage and frequency were applied, the cells migrated toward the electrodes and attached themselves in pearl-chain formations. After 3 minutes the

oscillator was turned off and the average chain length was measured with the microscope eyepiece graticule.

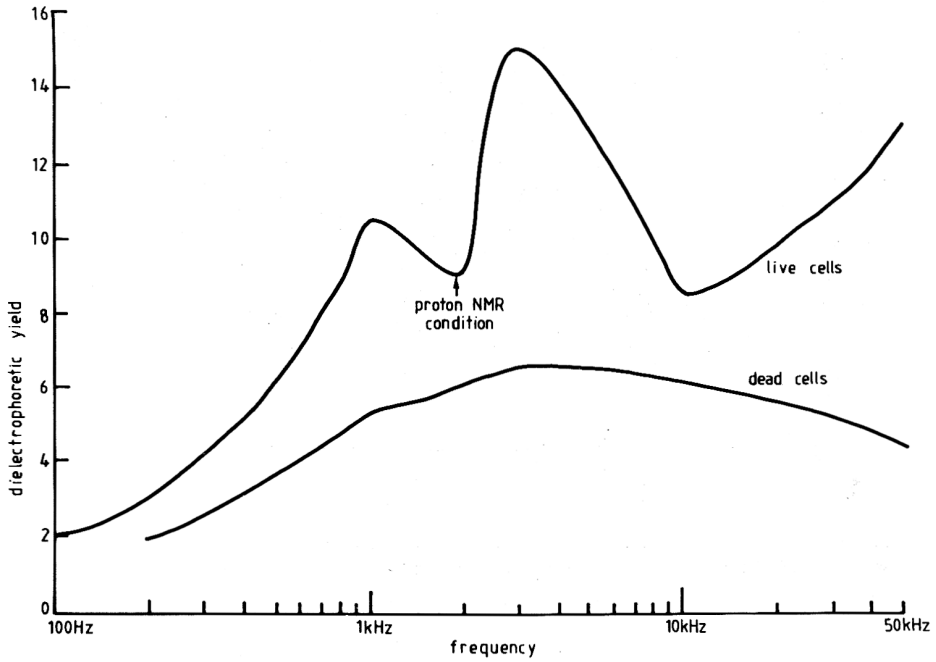

Figure 1. Dielectrophoretic yield spectra of live and dead yeast cells as a function of the frequency of the electric field.

Figure 1 shows the dielectrophoretic yield as a function of frequency for both live yeast cells and cells that had been killed by exposing them to ultraviolet light (254 nm). The anomaly in the region of 2 KHz is clearly seen in the curve for the live cells.

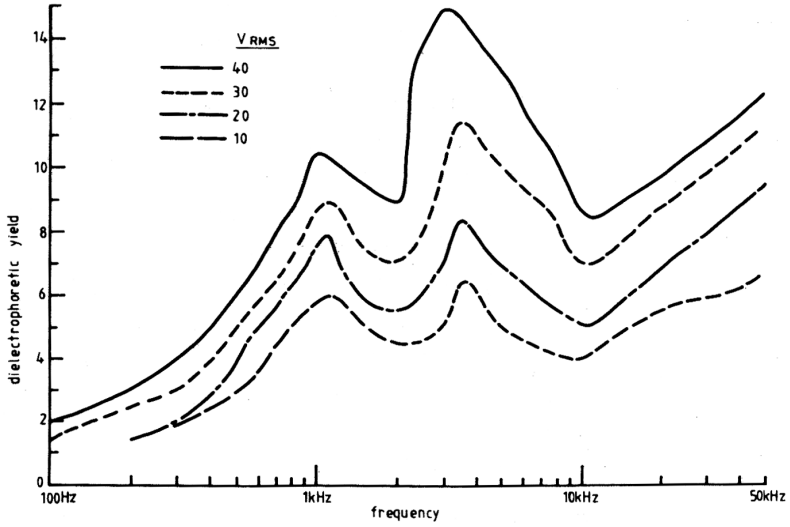

Figure 2a. Dielectrophoretic yield spectra of live yeast cells as a function of the frequency of the electric field.

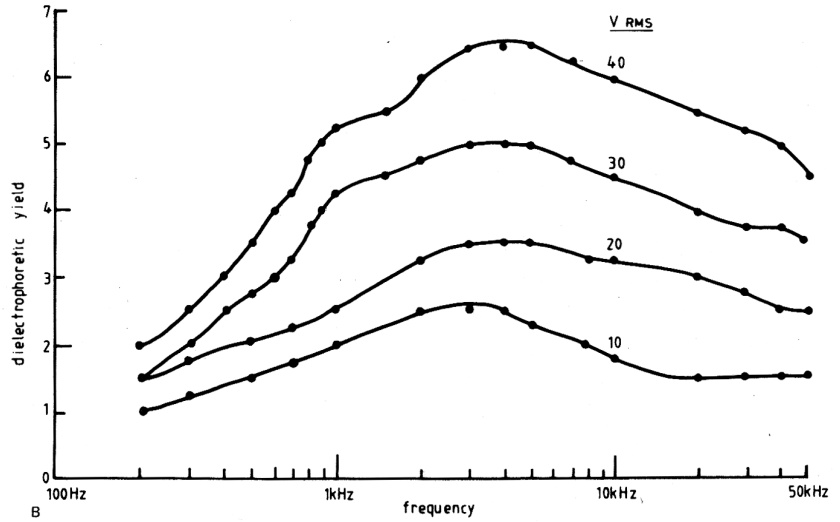

Figure 2b. Dielectrophoretic yield spectra of dead yeast cells as a function of the frequency of the electric field. Yeast cells irradiated with UV light (254 nm).

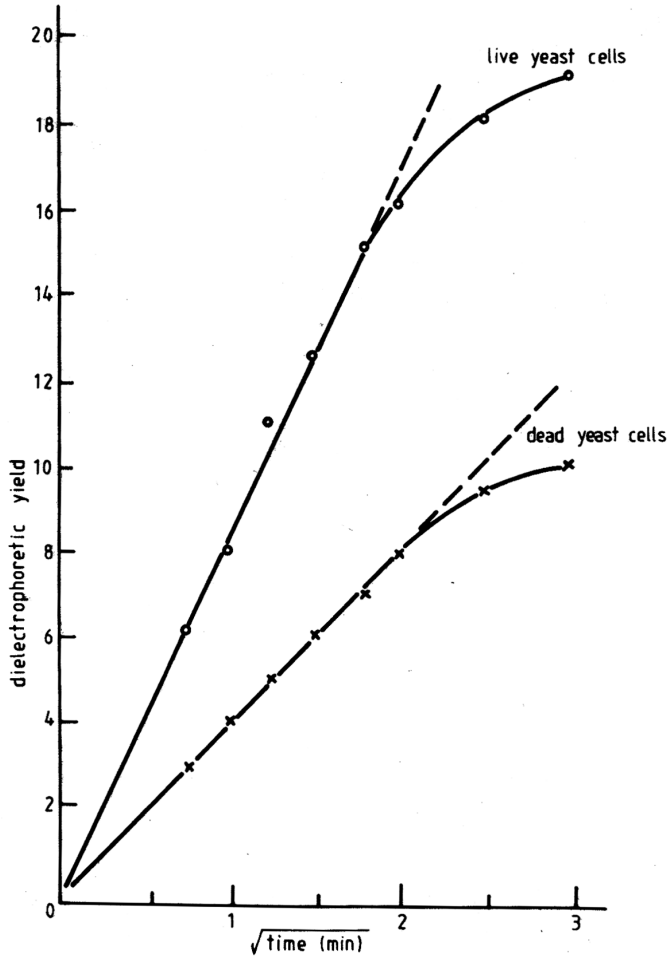

Figure 3. Variation of dielectrophoretic yield with the square root of the length of time the field is applied. V = 40 V rms, frequency = 3 kHz.

Figures 2a and 2b show that the dielectrophoretic yields were proportional to the applied voltage, and that they remained at the same frequency. Figure 3 shows that the yield of live and dead cells differed.

Figure 4 shows the results of measurements on live cells from 0.5 to 5 G (50 μT to 500 μT) normalized by plotting KHz/G as the abscissa. The anomaly then shows up clearly as a sharp resonance at exactly the proton magnetic resonance condition of 4.26 KHz/G from results taken over a 10:1 range of magnetic field strengths. Figure 5 shows the difficulty in repeating these measurements under nominally the same conditions. The variation in the positions of the curves is due to the variation of the laboratory magnetic field at the microscope specimen stage. These experiments took place on three successive days using yeast cells from the same culture.

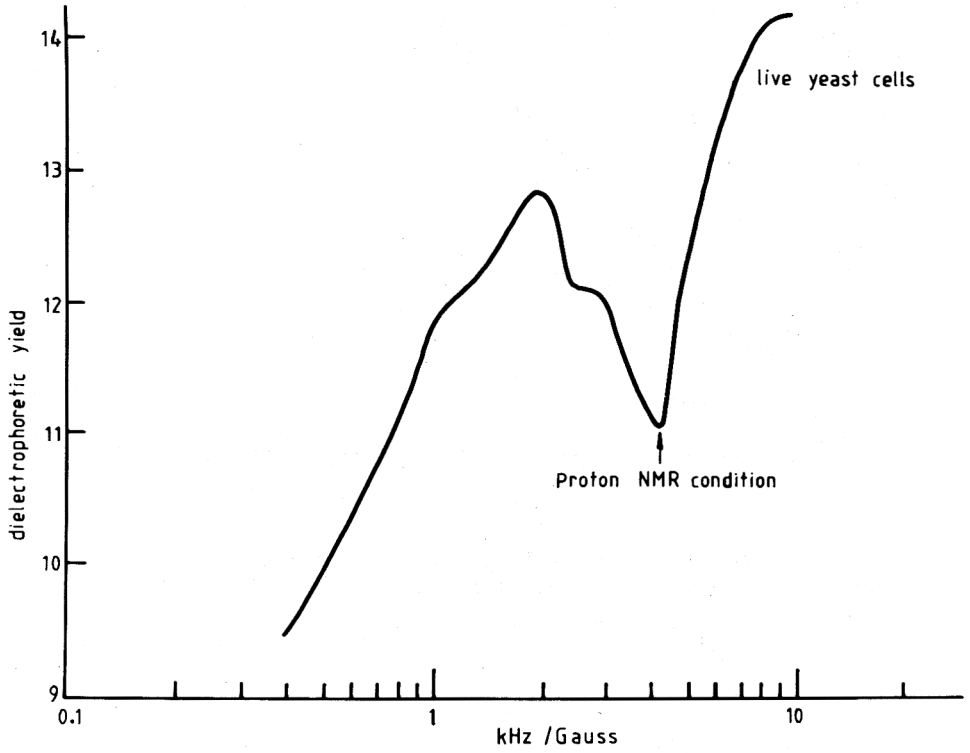

Figure 4. Measurements on live cells from 0.5 to 5 G normalized by plotting the abscissa as KHz per gauss.

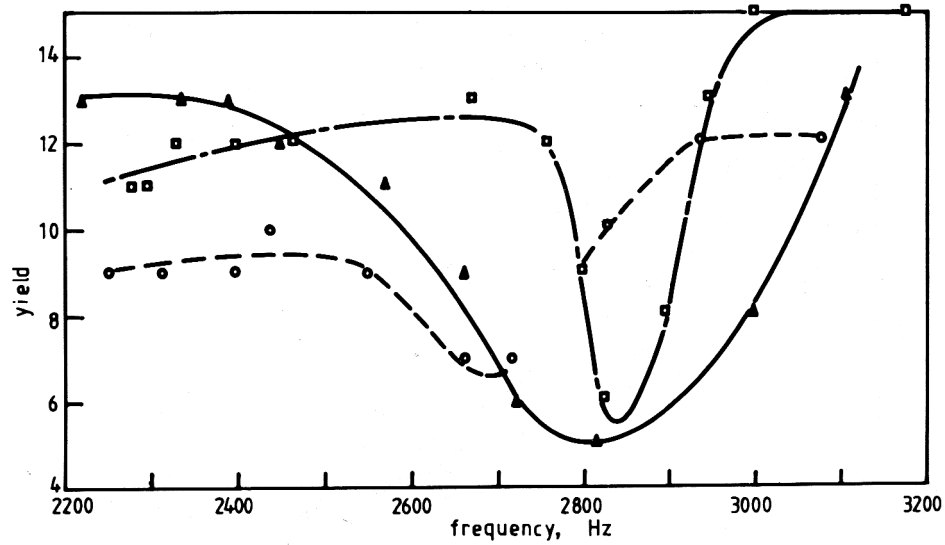

Figure 5. The difficulty in repeating measurements under nominally identical conditions is shown. The variation in the positions of the curves is due to the variation of the laboratory magnetic field at the microscope specimen stage. These experiments took place on 3 successive days using yeast cells from the same culture.

DIELECTRIC CONSTANT AND LOSS

The permittivity and dielectric loss of live yeast cells were measured for a suspension containing 1.9 x 10^6 cells/ml at room temperature and in magnetic fields of 0.5–5 Gauss (18, 19). Figure 6 shows the very sharp resonances that were observed in the dielectric loss. Figure 7 shows values of the frequency and magnetic field for the dielectric loss peaks; the line represents the proton magnetic resonance condition.

More detailed measurements showed peaks in the dielectric loss corresponding to the NMR conditions of ^{1}H, ^{31}P, ^{23}Na, ^{35}Cl, and ^{39}K (Figure 8a). Figure 8b shows these peaks to be absent in the case of dead yeast cells. The magnitudes of these resonances were temperature dependent, as shown in Figures 9–11, where the points were taken at 0.5-Hz intervals and represent the limit of accuracy of the apparatus rather than the shapes of the resonances.

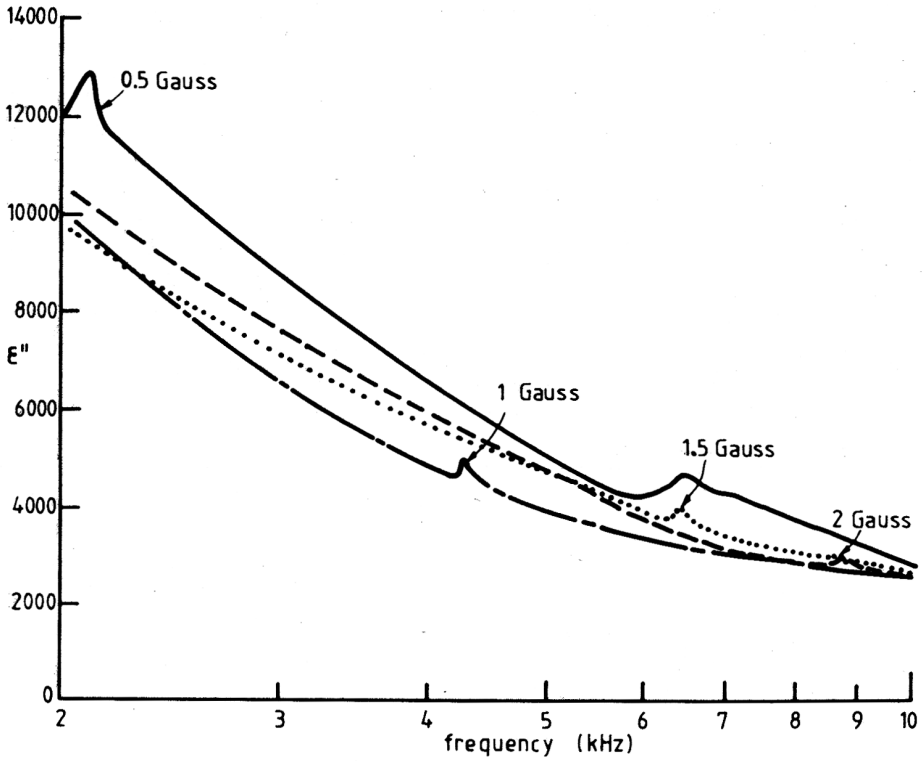

Figure 6. Dielectric loss peaks at NMR field conditions of a 1% by weight bakers' yeast in deionized water at 24°C.

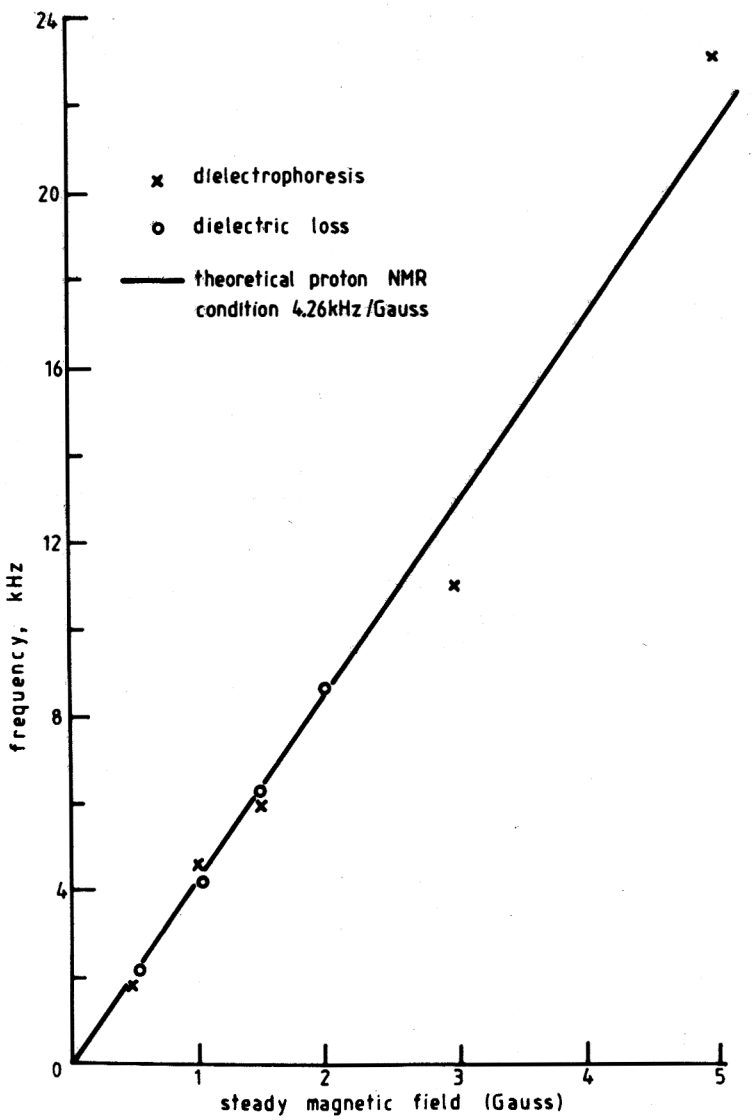

Figure 7. Variation of the proton NMR and dielectric loss peaks with frequency with applied magnetic field.

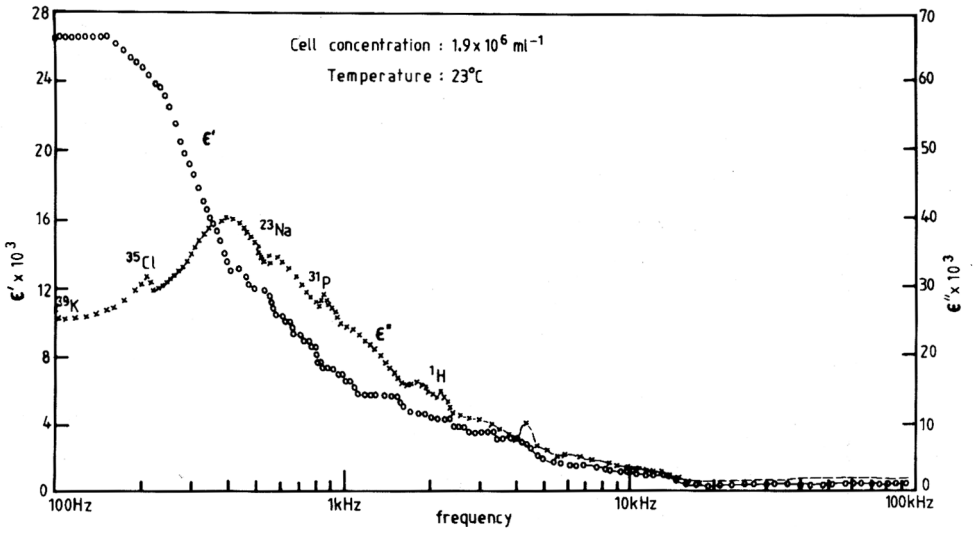

Figure 8a. The real and imaginary parts of the complex permittivity of the live yeast (*S. cerevisiae*) as a function of frequencies. The laboratory ambient-magnetic field was 0.5 G.

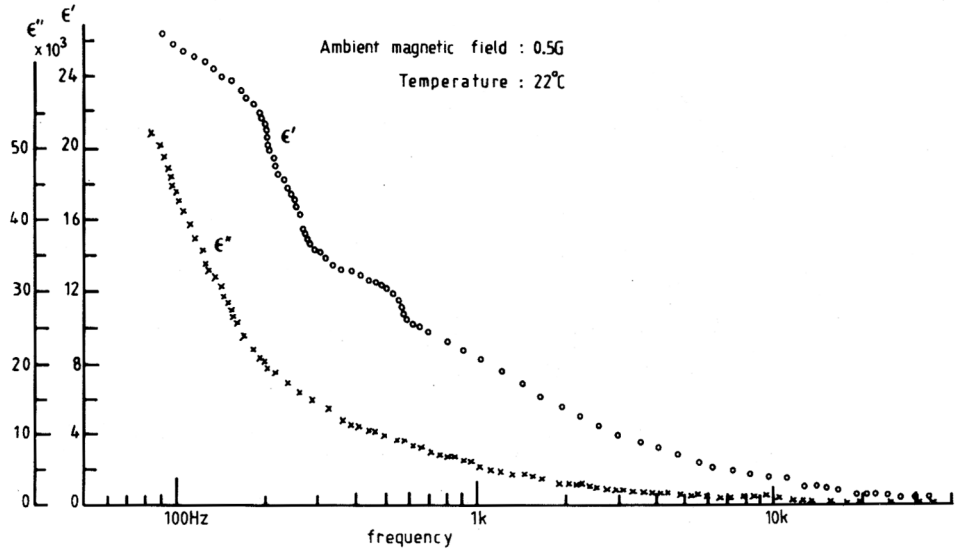

Figure 8b. The real and imaginary parts of the complex permittivity of the dead yeast (*S. cerevisiae*) as a function of frequencies. The laboratory ambient-magnetic field was 0.5 G.

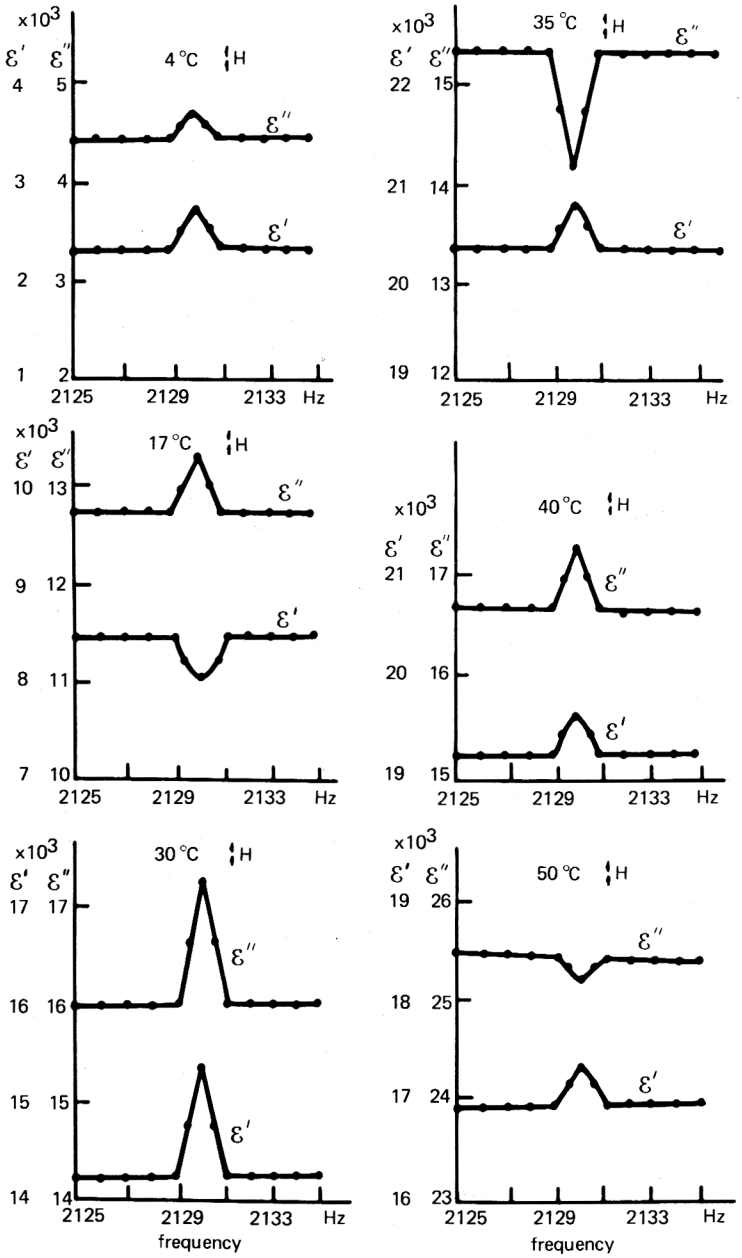

Figure 9. Dielectric constant and dielectric loss of live yeast cell suspensions (1.9×10^6 cells/ml) in a laboratory ambient field of 0.5 G (electric field strength of the order of 20 kV/m). Proton NMR conditions.

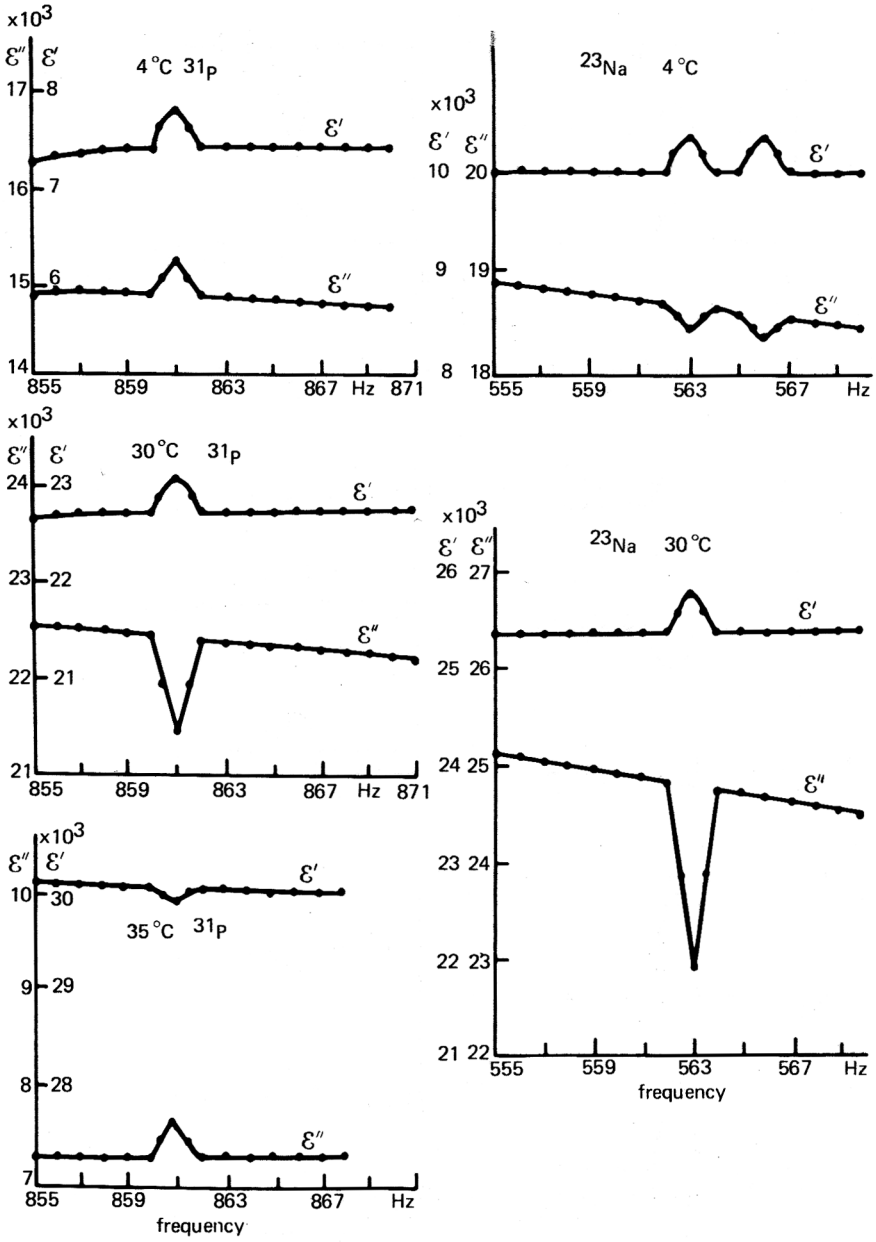

Figure 10. Dielectric constant and dielectric loss of live yeast cell suspensions (1.9×10^6 cells/ml) in a laboratory ambient field of 0.5 G (electric field strength of the order of 20 kV/m). Phosphorus and sodium NMR conditions.

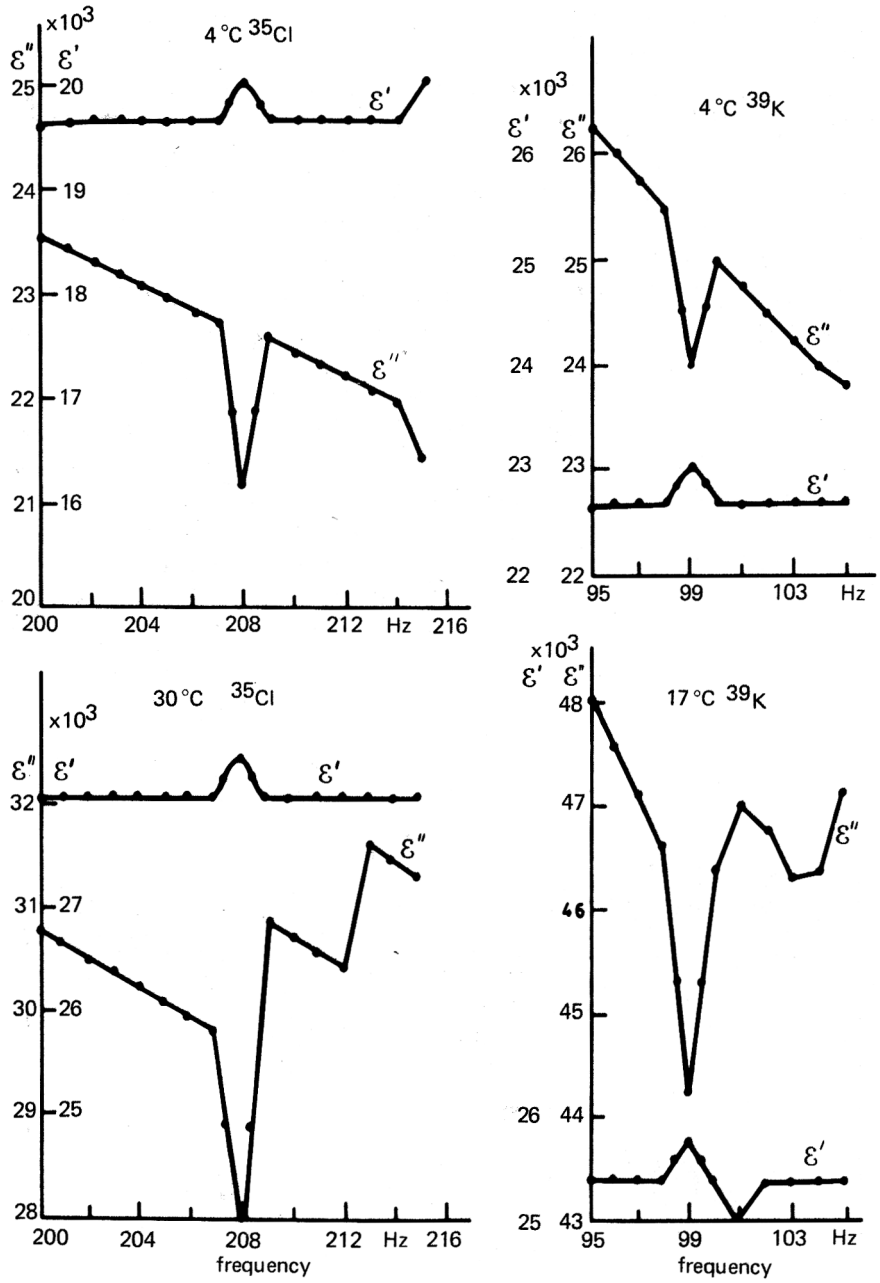

Figure 11. Dielectric constant and dielectric loss of live yeast cell suspensions (1.9×10^6 cells/ml) in a laboratory ambient field of 0.5 G (electric field strength of the order of 20 kV/m). Chlorine and potassium NMR conditions.

There is not enough evidence to determine the specific process responsible for the observed effects on the dielectric constant and loss at NMR conditions when the applied Larmor frequency arises from an oscillating electric field. There is an interaction at proton NMR conditions with the dielectric properties of proton conducting pH meter glass (17). The basis of interactions between electric and magnetic susceptibilities has been given by Van Vleck (20).

GROWTH OF *E. COLI* IN NMR CONDITIONS

E. coli cultures were grown at 39.0°C (21), which is 1.5°C above the value at which the multiplication rate is a maximum. Any additional heating of the test cultures due to the current flowing in the energizing coil of the electromagnet would therefore be expected to decrease the growth rate.

Each culture was grown in a plastic spectrophotometry cuvette. Each experiment involved thirty cuvettes: eighteen cultures were grown in the field, nine were grown inside a closed mu-metal box (giving effectively zero-field conditions), and three were left uninoculated as a check on the sterility of the culture medium.

Approximately 10^4 bacterial cells were used for each inoculation. Each culture was rapidly transferred to the incubator and grown in darkness without aeration for 10–12 hours. This period represents approximately 14 cell divisions at a mean generation time (MGT) of 0.85 hours per division. An electromagnet, the test cultures, the control cultures inside their mu-metal enclosure, and the uninoculated sterility control cuvettes, were all situated inside the incubator. The standard deviation of the MGT between cultures of the same batch was approximately 0.5%.

After incubation, density was determined turbidimetrically at 650 nm using a spectrophotometer (22). A square-wave magnetic field was used at 10, 16.66, 50 and 100 Hz, with a perpendicular sinusoidal magnetic field at 42.6 KHz. The results are presented in Figure 12, where the relative difference in MGT between test and control cultures is plotted as a function of magnetic-field strength (23).

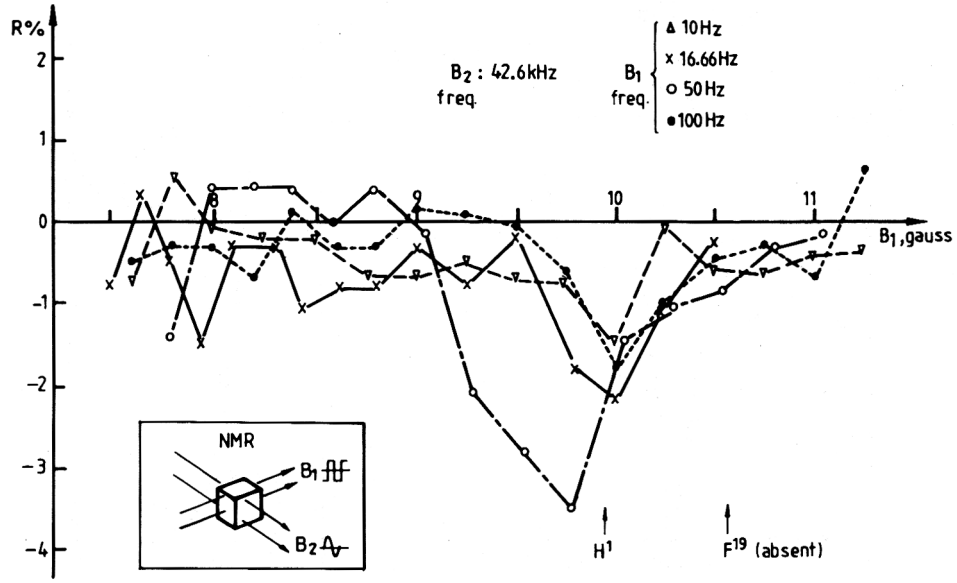

Figure 12. Percentage difference in the MGT of the test and control cultures as a function of the magnetic field strength.

There was a marked drop in MGT when the magnetic field strength satisfied the

proton resonance condition. The effect was most marked for 50-Hz magnetic fields, giving a decrease in the MGT of about 3.5% compared with the zero-field controls. The effect also occurred at 16.66 Hz (about 2.25%), and 10 Hz and 100 Hz (1.5%); the standard deviation of the control cultures was 0.7%. No resonance for F^{19} was observed.

In these experiments it was demonstrated that when satisfying the NMR conditions, which involves the injection of small amounts of energy into protons within the *E. coli*, the bacteria responded by increasing the rate of division by only a few percent. Effects on enzyme activity were also investigated (19, 24).

BOVINE EYE LENSES

Considering all the known biological effects of microwave and radiofrequency radiation, cataract induction appeared until recently to be he only irreversible effect of such an exposure with the notable exception of thermally lethal doses (9, 25-41). We investigated the possible effects of low power density (lower than l0 mW/cm^2) microwaves on the bovine eye lens *in vitro*. The overall aim was to determine the effects of electromagnetic irradiation and NMR conditions on possible cataractogenesis.

A total of 720 bovine eye lenses were studied. An incision was made approximately 1 cm below the cornea, and the eye lens was removed via the vitreous humor and placed in modified Krebs Phosphate Ringer media. The lens could be maintained for up to 240 hours at 35°C using this medium without deterioration.

The microwave radiation studies were performed with the lens on a glass ring in the incubation vessel which was placed within a wire coupling loop. In most cases, the dimensions of the loop were small compared with the wavelengths used.

No significant changes in the sodium and potassium concentrations of the eye lenses occurred following microwave radiation by the time a cataract was visible. It was considered that the microwave radiation had produced an effect on the bovine eye lens if the formation of a cataract in the posterior cortex of the lens could be observed within 24 hours of the commencement of irradiation.

Experiments using the NMR conditions for sodium, chlorine and phosphorus did not result in the production of microwave cataracts in the bovine eye lens. However, the proton NMR conditions at 2000 MHz and modulation frequency of 2.13 KHz in a magnetic field of 0.5 Gauss gave highly developed microwave cataracts in the posterior cortex of the bovine eye lens. The maximum power density of the radiation was 1–2 $\mu W/cm^2$, although there were uncertainties due to absorptions in the nutrient medium. The length of irradiation was 18–20 hr, and all cataracts obtained were located in the posterior cortex of the eye lens, and all were subcapsular.

Experiments were performed in which the various parameters such as power density, time of irradiation and physiological conditions of incubation remained constant, while

the frequency of microwave radiation was the only variable. Microwave cataracts in the bovine eye lenses were then produced at 55–64 MHz; above and below this immediate range no cataracts were obtained. The spectral line-width was about 50 Hz. When the bandwidth was increased to 150–200 Hz under otherwise identical conditions, the cataractogenic effect disappeared. All these microwave cataracts were subcapsular and located in the posterior cortex of the lens. by maintaining the frequency at 55 MHz and starting at a power level that was known from the literature to cause cataracts, the power relationship to microwave cataractogenesis was studied using fixed calibrated attenuators to reduce the microwave power output by known ratios. These experiments determined that microwave cataracts at 55 MHz could be produced over the range of 0.54 mW down to 0.0075 μW (a ratio of 70,000:1 in power).

Microwave cataracts were also produced over the range 900–2000 MHz. The time for development of these microwave cataracts was 1–20 hours depending upon the power density. At low power densities, the length of time of irradiation required to produce a cataract increased to 20 hours. The spectral line width of the 900 MHz radiation was 3 KHz.

The frequency range of 70 MHz–800 MHz was investigated, but microwave cataracts were not obtained in any of the exposed bovine eye lenses. The spectral bandwidths of the oscillators used in this range were up to 1 MHz, and this could account for the observed lack of cataract formation. The above results are summarized in Table 1.

That highly coherent radiation can produce biological effects at very low incident power densities is in agreement with the theoretical predictions of Fröhlich concerning the effects of coherent excitations in biological systems (42). Weak microwave radiation seems able to exert a cataractogenic effect on *in vitro* bovine eye lenses only under two conditions. Proton NMR conditions must be satisfied by a combination of modulated microwaves and ambient magnetic fields such that the microwaves act as a carrier for the modulation frequency which is induced throughout the eye lens on absorption of the microwaves. Also, specific conditions must be satisfied involving frequency, power density, duration of microwave exposure, and coherence of microwave radiation. All these conditions can be met in the context of ambient geomagnetic and other environmental magnetic fields, taking due account of magnetic flux quantization conditions (19, 43, 44). The properties of the water involved must also be taken into account (45).

TABLE 1. Bovine Eye Lenses Exposed to Microwave Radiation

FREQUENCY OF IRRADIATION (MHz)	NO. OF CONTROLS	NUMBER OF EXPOSED EYES	
		Cataracts	No. Cataracts
50–110	35	7	28
110–800	21	0	21
810–890	10	0	21
900	10	3	7
910–950	4	2	2
960	59	9	50
1000–1900	15	2	13
2000	72	8	64
2000	7	7	0
2100–4000	10	0	4
3145	4	0	4
3580	27	8	19
9373	12	0	12
10116	8	1	7

DISCUSSION

Although it happened that the first indications that a biological system might be sensitive to NMR conditions were seen in the results of dielectrophoresis measurements, this technique is not sufficiently precise to stand on its own. It is difficult to work with a chain longer than 10 cells, which immediately limits the accuracy to 10%. In addition, the reproducibility of the magnetic field strength at the microscope stage was only 5–10%. Observation of the distortion of pearl chains subjected to NMR conditions however, does give a qualitative demonstration of the existence of an NMR effect (Figure 13).

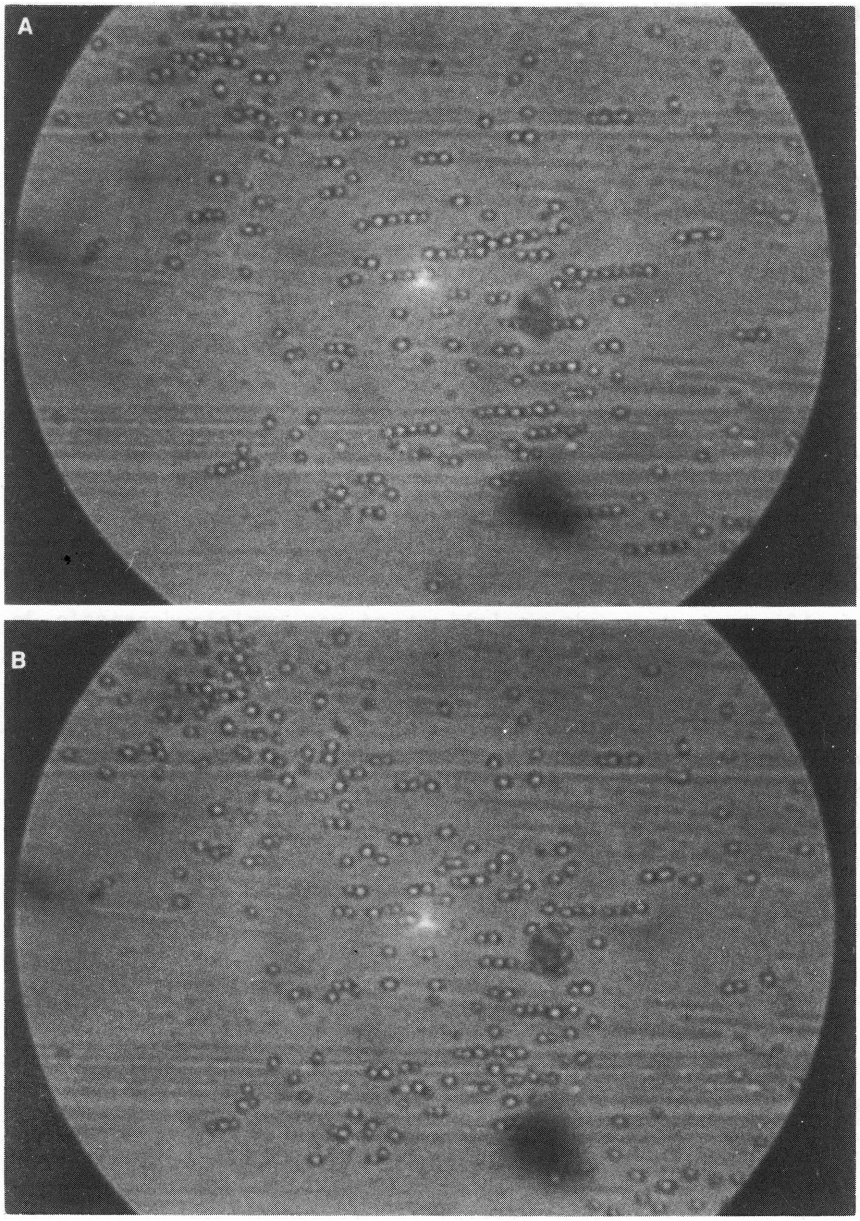

Figure 13. A, dielectrophoretic pearl chain of yeast cells. B, conditions identical, except for satisfying proton NMR conditions.

With the best dielectric measuring apparatus available to us, it was only just possible to detect the small peaks obtained when NMR conditions were satisfied by the bridge frequency and the ambient magnetic field. Experimental points were taken at 0.5 Hz intervals. If these peaks have widths corresponding to NMR relaxation times in biological materials, then readings should be taken at 0.1 Hz to 0.01 Hz intervals. The majority, and largest, effects were found at the proton NMR condition. Water forms 70% of body weight and unlike most physical systems, the electrical conduction processes are largely protonic rather than electronic (45).

MGT of *E. coli* cultures grown in low-frequency magnetic fields showed a periodicity that had a probability of less than one in two million of having arisen by chance (21, 43). However, the basic process whereby an alternating magnetic field between 10–100 Hz can affect the MGT by a few percent remained unexplained.

The formation of cataracts was observed after irradiating bovine eye lenses with microwaves, modulated so that the proton NMR conditions were satisfied in the ambient magnetic field. The cataracts were posterior, subcapsular, and developed within 24 hours.

The posterior cataract is the most important glare-causing cataract because it scatters light rays already nearly focussed by the rest of the lens. The sequence of events that characterize this type of cataract include the disorganization of cells at the equator, some of which subsequently migrate to the posterior capsule where they increase considerably in size to give enlarged nucleated cells called bladder cells or Wedl cells. It is possible that dielectric changes due to the NMR conditions in these cells speed their migration. Alternatively, osmotic imbalance rather than the posterior migration of equatorial cells may account for some kinds of posterior subcapsular cataract (46).

The major metabolic pathways for the lens are controlled by the enzymes, hexokinase, phosphofructokinase, and pyruvate kinase. Future experiments should test the sensitivity of these enzymes to NMR conditions.

Although the presence of high molecular weight (HMW) proteins in a cataract may not be a sufficient condition for opacity (46), water insoluble aggregates of protein can form and reach 0.5 μm in diameter as determined using polyacrylamide-gel electrophoresis. The bands holding the HMW proteins in the normal aging eye show weak non-covalent linkages, whereas the cataractous protein bands are not dissociated by detergents, but only by reduction. Electrophoresis without and with NMR conditions might be worth trying to see whether bond stabilization occurs. There is an indication that cataracts may also have an abnormally high phosphorous content (46). There are several reductase and hydrogenase enzymes in the major metabolic pathways which could give rise to this if they were inhibited, and which might be tested for sensitivity to proton NMR conditions.

In general, there are many biomedical systems that have exhibited sensitivities to low-level electromagnetic fields (42), and which should be tested for sensitivity to NMR conditions. Many of these are given by Becker and Marino (47) in a well referenced source book. It seems that the larger effects are obtained with biological systems which are under stress (48); this suggests that the various electromagnetic field effects involve but one of many redundant control pathways in a system which is normally under good homeostatic control (45). The various pulsed magnetic field therapies may be using NMR effects to generate highly coherent oscillations through spin echoes (49) to mediate in the healing process.

It is possible that the classical waveforms and experiments of electrophysiology may be to biology the equivalent of telegraph telecommunications in the days of Samuel Morse, and that nature is really in the pulse-modulated microwave communications business. Most experiments may only have looked at the modulation envelope of what happened to get detected and demodulated. The most likely spectral region for the carrier frequencies is 100 GHz to 1 THz (9), although optical frequencies should not be excluded from consideration (50). For the ELF region, recent work indicates that frequencies may need to be specified with a precision up to 1–10 mHz if results are to be meaningful; this implies possible latency periods up to hundreds of seconds.

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