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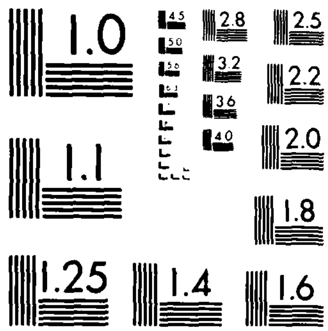

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Electromagnetic Fields and Biological Cells

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The duration of the Erice School was ten days. Therefore, we had sufficient time to discuss the problems of electromagnetic radiations. Vigorous discussions took place during official sessions and during private conversations. The participants of this workshop are mostly those who have been active in the research on bioelectromagnetics, but there are some numbers of speakers who discussed the basic electrical and magnetic properties of polyelectrolytes, biological membranes and tissues. The workshop was unique in that there were participants with a variety of training backgrounds. This enabled us to exchange the information between applied scientists and basic scientists. Also, active exchanges of opinions took place between biological scientists and physical scientists.

Hitherto, the emphasis of the workshop on bioelectromagnetics was mainly on the effects of electrical fields on biological systems and materials. However, during this workshop, it became apparent that the interests on the effects of low level magnetic fields were growing stronger. One of the examples is the discussion presented by Dr. Carl Blackman on the effects of extremely low magnetic fields on the Ca efflux of brain cells. There were a few other presentations with regard to the effects of magnetic fields.

Reflecting the background of the organizers, the main emphasis of this workshop was the discussion of the mechanism of the interaction between electromagnetic fields and biological systems rather than descriptive reports on biological effects.

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INTERNATIONAL SCHOOL OF PURE AND APPLIED BIOSTRUCTURE

DIRECTOR OF THE CENTRE: PROF. A. ZICHICHI
DIRECTOR OF THE SCHOOL: PROF. C. NICOLINI

NATO ADVANCED RESEARCH WORKSHOP

18-22 September 1984

and

5th ISPAB COURSE

24-28 September 1984

INTERACTIONS BETWEEN ELECTROMAGNETIC FIELDS AND CELLS

ABSTRACTS

and notes

1st part

Secretariat of the Centre: Via Guarnotta 26 - 91016 Erice - Italy - Tel. (0923) 29108 - Telex 910366 CCSEM
Secretariat of the School: Temple University, Health Sciences Center, Department of Biophysics and Physiology, 3223 North Broad Street, Philadelphia PA 19140, USA

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BIOLOGICAL AND TOPOGRAPHIC PROPERTIES OF WEAK PULSING ELECTROMAGNETIC FIELDS (PEMFs)

Sylvia Fitton Jackson and C. Andrew L. Bassett

Strangeways Research Laboratories, Cambridge, U.K.
College of Physicians and Surgeons of Columbia University, New York, New York 10032

There is increasing evidence that PEMF can be designed with physical parameters that confer ranges of biological specificity, not unlike pharmacological agents. Both class action and unique effects occur depending upon waveform characteristics. PEMFs have clearly defined topographic specificity in their ability to induce different waveforms in tissues depending upon fabric and orientation within the $\bar{B}$ field. Pulse parameters that affect calcium can be responsible for a variety of biological effects which are dependent on this ubiquitous divalent cation for control; these include messenger/regulators such as cAMP and protein kinase C, transcription/translation and secretion among other cellular functions. In fact, PEMF modulation of Ca^{++} availability may produce cascade effects. Thus, with the broad-band pulses generally in clinical and experimental use, it is likely that a number of cellular events may be triggered concomitantly, such as the deposition of new mineral and the additional accumulation of collagen, events essential for fracture repair. If synthetic and degradative responses, however, are equally triggered within the cell, no net perturbation may be apparent. Furthermore, the direction and rate of cell migration within a given $\bar{B}$ field appears to be determined, in part, by the direction of magnitude of the resultant E field. Several examples of these principles, as they apply in the clinic and laboratory, will be presented.

A CRITIQUE OF MEDICAL USES OF WEAK PULSING ELECTROMAGNETIC FIELDS (PEMFs)

C. Andrew L. Bassett and Sylvia Fitton Jackson

Strangeways Research Laboratories, Cambridge, U.K.
College of Physicians and Surgeons of Columbia
University, New York, New York 10032

PEMFs have been in clinical use for the past 12 years to heal ununited fractures in children and adults. During that interval, nearly 40,000 patients have been treated with a success rate ranging from 70 to 95%, depending upon the severity of the lesions and the management technique. These success rates are comparable to surgical methods which carry greater expense and hazard. In the United States, more than one half of Board certified orthopaedic surgeons have used PEMFs, more than twice in their practice and no untoward effects have been noted. For children with congenital pseudarthrosis of the tibia, the amputation rate has been reversed from nearly 80% before PEMFs to less than 1[illegible]% when the method is used alone or in combination with an appropriate operative procedure. Healing of ununited fractures occurs because pulse parameters trigger calcification of soft tissues (i.e., fibrocartilage) in the old fracture gap. Calcification is a critically important step in the normal repair process which, when arrested, results in a non-union.

By using different waveform characteristics to increase vascular neogenesis and decrease osteoclastic destruction of bone, it has been possible to treat osteonecrosis successfully. This pathologic entity attacks the femoral head in young people for a variety of causes, including steroids, and results in rapid and profound crippling. Surgical approaches have been singularly ineffective, particularly, in late stage disease. PEMFs have arrested and reversed this disabling condition in 90% of patients and spared them total hip replacements for significant intervals in time. General use by the orthopaedic community is expanding rapidly. This same waveform, also, has proved beneficial in treating chronic refractory tendinitis of the shoulder, probably by virtue of its effect on vascular neogenesis and limiting ectopic (dystrophic) calcification of tendon. Each of these clinical uses has been derived from sound laboratory demonstrations of efficacy and safety. Each has been proved in patients by rigorous testing, including double blind and matched case control techniques. Unfortunately, few other clinical uses of pulsed electromagnetic fields are based on scientific foundations. These, generally, uncritical panacea approaches to different illnesses, in the long run, can only cast dispersion on a very valuable therapeutic method.

- **Lecture title:** Phase-contrast, holographic and acoustic cytometries of living cells.

- **Lecturer** Bruno BIANCO

The standard optical microscope in trasmitted light gives an image of the object under observation which depends chiefly on the optical transmittance of the object itseltf. But there are alternative microscopy techniques able to yield very different information, and which can be applied to living, unitained cells. Some are the subject of this lecture. The methods considered have the common characteristic that in each the image formation is well understood only on the basis of the wave behaviour of the excitation, either light and sound. Moreover, they are potentially able to give three-dimensional information on the object.
The phase-contrast microscope (in monochromatic light) gives an image which depends on the refractive properties of the object; in principle, from this image one can recover the phase shift of the light emerging from the object; such an information can be trasformed into an information on the spatial distribution of the refractive index.
The holographic microscope is based on the formation of a hologram in "free space", under the objective; the hologram is magnified by the microscope and reconstructed by a proper processing technique, able to yield views from different points, simulating different position of the observer. The acoustical microscope is sensitive to the spatial distribution of elastic properties of the object.
In summary, these alternative microscopy methods can allow deep insight on the structural/physical properties e.g. of a cell.
The state-of-art in this subject is presented, together with experimental results.

- **Lecture title:**

Interaction of Low-Intensity EM Fields with Cells: Characterization by Ca Fluxes and Possible Mechanisms of Action

- **Lecturer's name:**

C. F. Blackman

- **Extended summary:**

Calcium fluxes have been used as a monitor of electromagnetic field-induced changes in a variety of biological systems. This work was initiated by the finding that current pulses applied directly to the cortical surface of a cat, in vivo, caused calcium release. Follow-up investigations, using radiofrequency (RF) radiation, utilized simpler, in vitro brain tissue preparations from the cat and from the chicken. RF radiation was found to cause changes in the calcium flux only when the radiation was amplitude modulated within a specific frequency range between 6 and 20 Hz. Subsequently, it was shown that the intensity of the radiation was also an important variable; changes in calcium flux only occurred within two narrow, RF intensity ranges. Experiments that used sub-ELF modulated RF radiation are difficult to interpret because the effective sub-ELF frequencies must be demodulated from the RF carrier waves by the sample. Because the efficiency of this demodulation is unknown, the intensity of the effective frequencies in the sample is also unknown. This problem has been circumvented by the finding that sub-ELF signals applied directly to the sample can also cause changes in the calcium fluxes, although at tissue intensities on the order of 10^{-5} V/m, which are approximate four to six orders of magnitude smaller than those produced by the RF radiation exposures. The results, using sub-ELF signals, have been consistent with the modulated RF radiation results demonstrating similar narrow ranges of frequency and intensity that are effective in causing changes in calcium flux from the samples. These responses to intensity and to sub-ELF frequencies have been the source of much speculation regarding the underlying mechanism of action. It is generally accepted that the underlying process must involve a cooperative phenomenon, in which the systems are energetically poised for change, and the externally imposed electromagnetic field serves as a trigger to initiate the process.

More recent experimental work has focused on the electromagnetic parameters that influence calcium fluxes in brain tissue preparation in order to develop a response profile that may reveal clues to the underlying mechanism. Signals of various intensities and frequencies up to 120 Hz have been tested. The results seen in the profile can be interpreted as being caused by two reaction sites, one with a broad frequency response, the other with a narrow frequency response that repeats at odd multiples of 15 Hz. More recent work demonstrates the influence of the magnetic field component in this process. This new data may serve as a basis to develop more specific, detailed mechanisms and to identify the initial reaction sites that eventually give rise to changes in calcium fluxes.

- Lecture title:

Complementary Lectures on Effects Due to Ca Fluxes: diversity and implications of cellular effects associated with field-induced Ca fluxes

- Lecturer's name:

C. F. Blackman

- Extended summary:

Interest in the biological effects of low intensity electromagnetic radiation stems from early reports of changes in human reaction time and in circadian activity caused by exposure to electric and magnetic fields in the sub-ELF range (0 to 30 Hz). Subsequent behavioral studies were designed using sub-ELF modulated radiofrequency (RF) radiation to enhance penetration of the signal into the tissue. These early experiments demonstrated a change in the EEG pattern associated with a conditioned behavioral response. In order to determine whether the behavioral changes were mediated via peripheral receptors or occurred as the result of changes induced directly in the CNS, additional experiments were designed to examine the effects on brain tissue in vitro. Because extracellular calcium and electric currents applied directly to the brain surface had similar effects on the release of GABA and calcium ions, several independent research groups monitored calcium fluxes in brain tissue from young chickens to determine the influence of various electromagnetic and biochemical manipulations on the field-induced changes. These groups demonstrated that the following factors can affect the efflux phenomenon: the frequency of sub-ELF modulation, the intensity and frequency of the RF carrier wave, the spacing between samples, the waveform and type of modulation, and the pH and the presence of other ions in the medium. In addition to calcium fluxes from chick brain tissue, other experimental systems have been tested. The experimental systems include calcium fluxes from rat synaptosomes in vitro, cat cerebral cortex in vivo, and human neuroblastoma cells in culture, and EEG patterns in rabbits. Two research groups have also studied rat pancreatic tissue, which showed changes in calcium fluxes, and mouse T-lymphocytes, which showed reductions in cytotoxic activity immediately following exposure. The unifying feature in these experiments is the critical role of particular frequencies and intensities of the electromagnetic fields.

ELF and sub-ELF fields cause responses similar to those observed with modulated RF radiation. Calcium fluxes from chick brain tissue in vitro were altered in a frequency- and intensity-specific manner, although details of the response may depend upon the particular exposure system used. A variety of biological samples have been exposed to sinusiodally-varying magnetic fields : Physarum - causing longer mitotic cycle and reduced respiration; Drosophila - altered oviposition and viability; human fibroblasts-enhanced DNA synthesis. Pulsed magnetic field studies form an additional dimension to this research area and the results may not be directly related to the calcium flux phenomenon. However, an analysis of the frequency content of the pulses may reveal some common electrical parameters, and ultimately a common reaction site.

- Lecture title:

... ...ECT ... ... AND LIGAND BINDING TO CELL MEMBRANE

- Lecturer's name:

A. ...

- Extended summary:

An em stimulus can trigger biochemical processes by affecting: the rate at which ions bind to the cell membrane or enter membrane channel mouths; the rate at which lectins or hormones bind to the cell membrane.

A general model of such rate processes is presented which is based on the interaction of a particle (ion or ligand) with a generalized binding site (membrane receptor or membrane adsorption site or channel mouth). The solution of two coupled Langevin-type equations allows the computation of the mutual particle-site distance in the mean square sense, in the presence of an exogenous low-frequency electric field. By doing so, the dependence of the macroscopic association and dissociation rate constants on the field can be evaluated.

These results are used to model the effects of low-amplitude em exposure in three different situations: perturbation of the Ca^{++} influx across the membrane of any cell; perturbation of the lectin mitogenic effectiveness on lymphocytes; perturbation of the hormone induced cAMP production in osteoblasts.

It will be shown that, under some different circumstances, the same kind of em exposure can induce opposite types of biological responses.

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ORDERED STRUCTURES AS A RESULT OF THE PROPAGATION OF COHERENT ELECTRIC WAVES IN THE BIOLOGICAL MEDIUM

by

E. Del Giudice, S. Doglia, M. Milani
Dipartimento di Fisica, Via Celoria 16, Milano

ABSTRACT

Experimental findings about biological structures, both intracellular (cytoskeleton) and intercellular (rouleaux formation of erythrocytes) are discussed in the framework of Fröhlich's theory of coherent electric waves in living matter.

It is shown that nonlinear propagation of electric [illegible] waves in an originally homogeneous solution of polar biomolecules produces, through self-focusing and self-trapping of the beam, strong field gradients that eventually induce concentration gradients of selected [illegible] species. The self-association of such molecules [illegible] their assembly along the propagating [illegible]. The formation of filamentous structures can then [illegible], polymerization processes of actin [illegible] will be discussed in terms of nonlinearities [illegible] index of the monomers solutions (Kerr [illegible]).

- Lecture title: Cell membrane structure-function relationship

- Lecturer's name: H.M. Fishman

- Extended summary:

A membrane can be regarded as a thin molecular film (~50Å) which functions both as a diffusion barrier and an interfacial reaction surface. The main functional concepts associated with plasma membranes are the notions of functional compartmentalization (i.e., gates, pores, channels, pumps, etc.) and catalytic optimization (receptors, sites, etc.). The overall objective of this lecture is to outline the physico-chemical concepts of membrane architecture with a view toward the relationship of surface chemistry, model membranes and cell membranes.

CELL POLYELECTROLYTES

Fumio Oosawa, Department of Biophysical Engineering, Osaka University and Institute of Molecular Biology, Nagoya University.

Biopolymers such as proteins and nucleic acids are polyelectrolytes, and biological systems such as muscle, membranes and protoplasm may be regarded as organized polyelectrolytes. Some of the properties of living cells and their constituents can be understood from this standpoint.

The strong interaction between polyvalent macroions (polyions) and counter ions is a source of the characteristic properties of polyelectrolytes. Above a certain critical value of the charge density of polyions, counter ions are condensed on the polyion and a complete selective condensation of counter ions of higher valencies takes place. Small ions from low molecular salts are additively distributed around the polyion and the chemical potential of polyions shows a logarithmic dependence on the salt concentration.

The mobility of condensed counter ions results in a very large dielectric constant of polyelectrolyte solutions. The relaxation time of longitudinal movements of counter ions along the polyion is long, whereas that of transverse movements is short. Therefore, polyions show different local movements depending on the frequency of the applied electric field, although the rotation of the whole polyion is very slow.

Even without the applied field, thermal fluctuation of condensed counter ions produces a fluctuating field. With increasing charge density, the repulsive force between polyions is saturated, while the attractive force due to such fluctuation continues to increase. This force depends on the speed of movements of polyions.

In a muscle fiber, thin and thick filaments composed of actin and myosin respectively are aligned in parallel, interdigitating each other. If an electric field is applied along the fiber, the orientation of actin filaments is made better in one side of the z-line and worse in the other side, suggesting that the filaments have permanent dipoles. Under the field of higher frequency, bending movements of the filaments of higher modes are observable. The relaxation time of the bending movement is much shorter in the resting state of the muscle than in the activated state.

The polar alignment of actin filaments seems to be closely related to the motility and morphogenesis of living cells. It is very likely that the effect of the electric and magnetic fields on the living cells is mediated by the formation of such ordered structures.

PERSPECTIVE ON WINDOWS AND CALCIUM EFFLUX STUDIES

Sol M. Michaelson

Department of Radiation Biology and Biophysics
University of Rochester
School of Medicine and Dentistry
Rochester, NY 14642

Over the last decade, Adey and Bawin and their colleagues have reported extensively on their studies of changes in radioactive-calcium ion ($^{45}Ca^{++}$) efflux from neonate chick brain preparations and isolated samples of cat cortex under very specific frequency and power density regimes of unmodulated sub-ELF fields and of amplitude-modulated VHF and UHF fields. Blackman conducted experiments that verified and extended Adey's findings for chick brain at 147 MHz and later at other modulations and frequencies. Few studies on calcium efflux have sought to determine the source of the calcium involved in the observed efflux, the mechanism by which energy deposition alters calcium efflux, and the functional implications of the induced changes in calcium efflux. It should be recognized that unbinding or release of Ca^{++} may signal either the onset of a temperature change or the occurrence of a thermal challenge in response to which compensatory physiological reaction keeps the animal's temperature stable. Therefore, a thermal effect may indeed cause a change in calcium kinetics without eliciting measurable temperature increase. The concept of "windows" which has evolved from such studies may be reflective of compensatory physiologic responses or rate changes in biochemical reactions. Adey suggests that electromagnetic fields may induce conformational changes of the neuronal membrane resulting in displacement of the surface bound cations. While these studies on calcium efflux are interesting, and potentially important they fail to demonstrate physiologically significant effects of electric, magnetic or electromagnetic fields on calcium function in the brain.

SUBTLE EFFECTS OF RADIOFREQUENCY ENERGY ABSORPTION AND THEIR PHYSIOLOGICAL IMPLICATIONS

Sol M. Michaelson

Department of Radiation Biology and Biophysics
University of Rochester
Rochester, New York 14642

ABSTRACT

We might define subtle effects of RF energy/biologic tissue interactions as those likely to elude perception or understanding. These can be illusory, unsubstantiated, or tenuous, which would be considered of slight importance or significance. On the other hand, several reported subtle effects are unquestionably of considerable interest and, if substantiated, may indicate basic or fundamental biophysical relationships. For pragmatic considerations the question has to be asked and consideration given as to whether such subtle effects have any physiological implications. Subtle effects can be categorized as: 1) influence of field generated forces, 2) membrane interactions--electrosensitive species; nerve cell stimulation; auditory response, 3) biopolymer interactions, 4) changes in calcium kinetics, 5) millimeter wave interactions, 6) orientation, navigation by electrosensing organisms and in ambient electromagnetic fields, 7) modification of circadian rhythms, and 8) behavioral responses to "weak" fields.

Several mechanisms responsible for the interaction of radiofrequency and other electrical fields with biological systems have been described. Foremost is the heat development which results from the absorption of microwaves. The relative contribution to this heat development caused by the various tissue constituents including ions, water, biopolymers, bound water and lipids are noted. Direct field interactions with various biocomponents are also considered. These include membrane interactions, biopolymer interactions, interactions with biological fluids, and field generated forces acting on biological particles and cells. These forces are frequently neglected in discussions and yet pertain to a large number of published observations.

- Lecture title: MM-Wave Spectroscopy on Biological Macromolecules

- Lecturer's name: Dr. Friedrich Kremer

- Extended summary:

Broadband absorption measurements at millimeter-wave frequencies (40 GHz-170 GHz) are reported for several biological macromolecules (lyophylized haemoglobin, lysozyme, silk keratin, poly-L-alanine) The measurements were extended over the temperature range from 4.2 K to 300 K using the novel oversized-cavity-technique (OCT). The millimeter-wave absorption of the dried materials was found to increase nearly exponentially with temperature and approximately as $\nu^{1.5}$ with frequency. This frequency and temperature dependence is quantitatively described as being due to three distinct relaxation processes on a picosecond time scale occuring in asymmetric double well potentials. These processes are most probably assigned to the NH...OC hydrogen bonds of the peptide backbone. For lysozyme the influence of hydration was investigated. The contribution of adsorbed water can be distinguished from the intrinsic processes by its different frequency dependence.

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- **Lecture title:**

Biological Implementation of Electric and Magnetic Orientation Mechanisms

- **Lecturer's name:** Ad. J. Kalmijn

- **Extended summary:**

The ampullae of Lorenzini enable marine sharks, skates and rays to detect dc and low-frequency electric fields up to about 8 Hz at voltage gradients as low as 5 nV/cm.

Well-aimed feeding responses have been observed in response to the bioelectric fields of prey and simulations thereof, both in captive animals and in free-roaming sharks, skates, and rays at sea.

In training experiments, stingrays have shown their ability to detect the presence, direction, and polarity of uniform electric fields, simulating those of ocean currents, and to orient themselves accordingly.

Stingrays have also been successfully trained to orient to the earth's magnetic field, whether the field inclined towards the vertical or was horizontal as at the magnetic equator.

Experiments to prove that the animals indeed use the naturally occurring electric and magnetic fields and to identify positively the electromagnetic principle of orientation are in progress.

The performance of the ampullary system and the actual process of sensory transduction will be discussed for both freshwater and marine electrosensitive fishes and will be related to the conductivity of the medium.

Implementation of a magnetite-based orientation mechanism is known for magnetic bacteria. The use of magnetite for orientation in higher organisms is uncertain however, and will be evaluated critically.

- **Lecture title:**

Physical Principles of Electric and Magnetic Field Detection

- **Lecturer's name:** Ad. J. Kalmijn

- **Extended summary:**

Due to regional differences in skin potentials, aquatic animals produce multipole electric fields in the surrounding water. Electrosensitive fishes use these fields to locate their prey and to direct their feeding attacks.

By flowing through the earth's magnetic field, ocean currents produce vast, virtually uniform electric fields, which provide marine electrosensitive fishes with information about the strength and direction of their drift.

In shallow, protected waters, the fringe fields of nearby ocean currents often yield steady directional cues for the local elasmobranch populations. Tidal flows may modulate those fields to varying extents.

Moving with respect to the water, marine electrosensitive fishes furthermore induce electric fields across their body and in the surrounding water, which could give the animals their magnetic compass headings.

Electrochemical fields of galvanic and electrokinetic origin offer freshwater electrosensitive fishes orientational cues, which they may use when moving about in familiar territory.

Besides by the electromagnetic principle of orientation, biological organisms may also detect the direction of the earth's magnetic field by the use of ordered, single-domain magnetite crystals.

The physics and physical chemistry of the various electric and magnetic fields will be discussed in order to understand how they interact with the recipient organisms and precisely what sensory information they provide.

- Lecture title:

The High-Frequency Interaction: Physicochemical Basis

- Lecturer's name:

K.H. Illinger, Department of Chemistry, Tufts University, Medford, MA 02155, USA

- Extended summary:

The high-frequency interaction [in the microwave, millimeter-wave and far-infrared regions of the electromagnetic spectrum] with cellular systems, and *a fortiori* with biological systems of higher organizational complexity, requires analysis in terms of a hierarchical series of model systems of increasing physicochemical complexity. A salient difference between the spectroscopic and dielectric properties of ordinary fluids and those of biological systems is that the latter contain non-equilibrium subsystems. While the bulk properties are dominated by the equilibrium subsystems, owing to their predominance in concentration, features not predicted by the theory of ordinary dielectrics may arise. We shall attempt to trace the models, definitive or heuristic, which are thought to play a significant role in the high-frequency interaction. The attenuation function and complex permittivity predicted by models of varying sophistication, for equilibrium molecular systems, in the region of 0.1 - 100 cm^{-1} will be discussed and compared to experimental data. The system which dominates such bulk properties of biological fluids and tissue is $H_2O(\ell)$. Systems in the next regime of complexity, apart from the ionic and monomeric molecular components of biological fluids, are biopolymeric systems. The principal coupling mechanism for $H_2O(\ell)$ is the high-frequency tail of its (monomolecular) rotational diffusion and the librational and translational motions of molecular clusters, extending into the region around 1 cm^{-1} from the far-infrared region. Biopolymeric systems may exhibit low-lying vibrational motions in this region as well as in the far-infrared. Recent theoretical models, particularly for DNA and its analogs, have begun to address a realistic description of such motions, including the effect of viscous damping by $H_2O(\ell)$, which plays a significant role in the biological context of such systems. The biophysical entities which may be the irreducible systems that form the loci for non-equilibrium subsystems are *in vivo* cellular systems, including the biological membrane. We shall discuss the spectroscopic properties, in the high-frequency region, of non-equilibrium systems, and their experimental consequences: attenuation function, Raman intensities, the vibrational emissivity, and phonon spectroscopy. Finally, a thermodynamic formulation of the Fröhlich vibrational model will be sketched, which may aid in the application of physicochemical criteria for the experimental study of non-equilibrium biochemical systems.

TOPOGRAPHY OF EM EXPOSURE AND ITS RELATIONSHIP TO BIOLOGICAL EFFECTS ON TISSUES

M. HINSENKAMP.

In previous studies (1982), the topography of the magnetic and electric fields between Helmholtz circular and clinical coils was defined by computation following the Maxwell's equation and by experimental measurements.

We also analyzed the biological effects of time-varying magnetic fields generated by these coils which produced an increase of GAG synthesis on limb buds of mice embryos (1982) and an activation of the skeletal growth of chicken embryos (1983). In this study, it appears that between the coils the position of the stimulated embryos is not meaningless regarding the biological effects.

In the present work, we used quails embryos to have a better definition of the topographical variation of the electrical characteristics.

The eggs were stimulated after four days of incubation and during 96 hours in square shaped coils.

Four groups are studied and characterized by the positions of the eggs between the coils (center, 1 cm inside the edges, 1 cm inside the corner and controls).

This protocol is repeated for two parallel planes between the coils (half way between each coils and 1 cm from one coil).

We analyzed the embryonic weight, the length of each bone segment and the length of the ossification point of each bone.

All these measurements give the same differences between groups.

The group stimulated on the edge of the coils shows the highest growth rate. The group stimulated in the center and the corner shows an intermediate growth between the control and the edges group. The increase of total length of the bone segments is not so pronounced that the increase of the length of the primary ossification point. The statistical analysis of the ratio between these two measurements gives interresting results. Compared to the controls, the mean value of this ratio shows an increase of 10.7 % for the tibia stimulate in the center, 14,2 % in the corners and 25 % on the edges of the coils. After a comparison of the different electric variables and the biological effects we propose a biologically correlated electric characteristic of the electric field.

It appears that the topographical variation of this charasteristic neglected until now in the experimental protocols could be responsible of many inconclusive results due to their apparent unreproducibility.

Another parameter altering the topography of the EMF "in vivo" is the electrical characteristic of the tissues in a limb. The effect on EMF topography in different fresh fractures configurations as well as non-union are studied.

It appears that identical induction pattern or direct current applied to different fresh fractures or nonunions does not produce the same local electrical modification in terms of amplitude and orientation.

This means that before to propose new indications for electrical stimulation, the electrical environment of each pathological situation has to be carrefully defined.

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- Lecture title: Frequency-dependent biological effects of low intensity microwaves

- Lecturer's name: Dr. Werner Grundler, GSF Neuherberg, F.R.G.

- Extended summary:

The existence of biological effects caused by low intensity microwaves and not only due to a tempe:ature increase are of fundamental interest both from the point of view of protection against this radiation and perhaps for an understanding of biological mechanisms in general.

Such a nonthermal microwave sensitivity in biological systems has not been generally accepted. Experimental evidence has been scarce and investigations are needed, where fundamentally it's existence can be proofed. A strong frequency-dependent response of the biological system, especially in a resonant form, points to special mechanisms not compatible with thermal origin. Such results are predicted and supported by theories indicating their acceptance from the point of view of theoretical physics and biophysics. On the other hand, such an experiment allows the critical examination of theoretical models enabling a deeper inside into the possible mechanisms involved.

Due to the scope of this frame, emphasis is taken on frequency specific effects and examples are reported where, at least to some extent, this physical parameter is systematically investigated. These examples are concerned with reactions from molecular to cellular level. Mainly we report evidence of nonthermal resonant action of millimeter microwaves on the growth of yeast cultures. Repetition of earlier photometric studies of aqueous suspension cells and the microscopic observation of irradiated single yeast cells have confirmed that cell growth is affected by weak mm-microwave radiation in a frequency-selective manner.

Additionally, the influence of the same frequencies as used in the yeast experiment will be demonstrated on mammalian cells for testing the question if basic mechanisms are involved common to these different cell systems.

Lecture title: Dielectric properties of biological material: Techniques of measurement

Lecturer's name: E.H. Grant

Exended summary:

Accurate knowledge of the dielectric properties of biological material is required in the areas of dosimetry, NMR imaging, hyperthermia induced by electromagnetic waves and microwave thermography. Complementing these practical situations is the area of academic biophysics where knowledge of the dielectric behaviour of the biological solution or tissue may be interpreted in order to provide information at a molecular or cellular level. Such parameters as molecular dipole moment, cell membrane width and quantity of water of hydration can be obtained by dielectric techniques.

The relative permittivity and conductivity of biological material are determined using frequency domain or time domain methods. In both categories of technique observations may be made on the behaviour of an electromagnetic wave as it passes through or is reflected from a sample contained in a transmission line or waveguide. With measurements in the frequency domain the determinations are carried out directly at individual frequencies; in time domain spectroscopy (TDS) a pulse is sent down the line containing the sample and a Fourier transform performed on the reflected pulse to obtain the appropriate information in the frequency domain. The measured dielectric parameters are fitted to sums of two or more dispersion regions, each of which may be identified by a particular mechanism. To assist in the unambiguous interpretation of each dispersion the dielectric measurements should be performed as a function of concentration, temperature, conductivity and other physical parameters.

In general frequency domain methods provide greater accuracy than time domain techniques, particularly when the measurements can be made by moving a probe through the actual sample as distinct from observing the wave reflected from it or transmitted through it. This is of course only possible in respect of liquids. On the other hand time domain methods are much more rapid and allow the determination of complex permittivity over two or more decades to be made in a matter of minutes. This is especially useful when tissue samples are to be measured and is valuable in any situation where there may be a variation of dielectric properties with time.

In this presentation the various methods of measuring permittivity and conductivity will be discussed, with particular emphasis on recent developments.

Kecture title: Dielectric behaviour of biological material: Molecular properties

Lecturer's name: E.H. Grant

Summary

Accurate knowledge of the dielectr roterties of biological meterial is required in the areas of dosimetry, NMR imaging, hyperthermia induced by electromagnetic waves,and microwave thermography. Complementing these practical situations is the area of academic biophysics where knowledge of the dielectric behaviour of the biological solution or tissue may be interpreted in order to provide information at a molecular or cellular level. Such parameters as molecular dipole moment, cell membrane width and quantity of water of hydration can be obtained by dielectric techniques.

The measured dielectric parameters are fitted to sums of two or more dispersion regions, each of which may be identified by a particular mechanism. To assist in the unambiguous interpretation of each dispersion the dielectric measurements should be performed as a function of concentration, temperature, conductivity and other physical parameters.

In this presentation the dielectric properties of biological material will be surveyed and interpreted in terms of molecular behaviour. New data on DNA, myoglobin, polyvinylpyrrolidone (PVP) and water in the ageing brain will be presented and a novel method of measuring water of hydration in biological tissue by freezing the sample will be described. In this method the unfreezable water is measured and identified with the water of hydration. The advantage of this technique is that by freezing the sample the interfering effects of low frequency dispersions are removed. Consequently by making measurements at microwave frequencies it is possible to study the motion of the water molecules present in tissue without the interfering background of membrane effects and the rotation of polar macromolecules.

- Lecture title: WHAT DATA BASES FOR EXPOSURE STANDARDS?

- Lecturer's name: M. GRANDOLFO

- Extended summary:

A standard is a general term incorporating both regulations and guidelines and can be defined as a set of specifications or rules to promote the safety of an individual or group of people. To protect the general public and persons occupationally exposed to electromagnetic fields, exposure standards are promulgated. Absolute assurances are rarely if ever practicable and specifying maximum permissible exposure limits to different hazards depends on the degree of risk that is acceptable scientifically and socially. With world opinion varying so widely on what exposures to electromagnetic fields are acceptable it is not surprising that very different criteria have been applied in different countries. Among the many factors that go into the development of an exposure standard, the selection of a good scientific biological effects data base plays the most important role: different scientific approaches have produced different philosophies of protection guidelines and thus different exposure limits. In this lecture a comparative analysis of existing or recently proposed standards for extremely low frequency (ELF) electromagnetic fields and for radiofrequency and microwave radiations will be presented, examining the various aspects which are generally taken into account and the rationale on which they are based.

The major problem confronting regulatory agencies evaluating the data on biological effects from exposure to ELF electric and magnetic fields is that they are, in the main, contradictory and controversial. There is not a clearly defined relationship between the ELF field exposure level and patho-physiological effects. This makes the problem of drafting standards much more complex. In most cases it is extremely difficult or impossible to obtain the information needed to draw valid conclusions regarding the dose-effect relationship. Ideally one would like to base standards on firm human data. However, since insufficient data are available, some form of extrapolation from well-designed, adequately-performed and properly analyzed animal experiments is necessary.

Major difficulties exist also in assessing the potential health hazards to man of exposure to microwave and RF radiation, because of the highly complex relationship between the exposure conditions and the energy absorbed. The absorbed dose and rate of energy absorption depend critically on such variables as frequency, power density, field polarization, the size and shape of the exposed subject, and environmental factors. Many of the experiments contain insufficient information on the dosimetry, thus difficulties arise in the exact interpretation of results.

It is important to maintain an adequate research effort so that on the basis of more definite data bases a critical revision of the existing standards can take place on an international basis, possibly reaching unique and certain safety criteria.

- Lecture title: Workshop on measurement techniques.
  Topic: Pseudorandom signals

- Lecturer's name: H.M. Fishman

- Extended summary:

Application of Fourier-synthesized pseudorandom signals is a relatively new means of obtaining rapid linear responses of a system. The discussion will focus on the rationale for use of these functions, how they are generated, and the ways in which they can be used.

- Lecture title: Linear analysis of the responses of axon membrane ion conductances to pseudorandom voltage perturbations

- Lecturer's name: H.M. Fishman

- Extended summary:

The time-varying, voltage-sensitive ionic conductances that underlie excitation phenomena in squid axon and other excitable cell membranes show diverse characteristics when studied in the frequency domain. Rapid steady-state complex admittance measurements by application of synthesized, pseudorandom perturbations of membrane voltage, synchronized with a fast Fourier processor, allow linear analysis of membrane ion conductance responses in the frequency range 1-5000Hz during step voltage clamps. The K conduction system is first order and described by a series g-L branch in parallel with a positive conductance. The admittance of the K system shows resonant behavior with membrane capacitance. The Na conduction system is second order and is described by three parallel branches: g-L, g-C and a negative conductance. The Na system in conjunction with membrane capacitance and residual positive leakage conductance produces an extensive range of complex admittance functions. All of the complex admittances can be fitted with linear models to obtain estimates of conductances and their natural frequencies (or relaxation times) thereby providing a complete linear characterization of conduction kinetics.

CONTROL OF CONFORMATION AND MOVEMENTS OF CELL POLYELECTROLYTES

Fumio Oosawa, Department of Biophysical Engineering, Osaka University and Institute of Molecular Biology, Nagoya University.

Protein polymers in living cells such as F-actin filaments, microtubules and intermediate filaments are all composed of globular protein monomers and have characteris properties as polyelectrolytes. The polymers are formed above a certain critical concentration of monomers, and usually the critical concentration decreases with increasing ionic strength and raising temperature. These polymers show continuous and discontinuous polymorphic transitions depending on the environmental condition.

Recently, a new technique has been developed to make single filaments of protein polymers directly visible under an optical microscope by dark field illumination or by labelling with a fluorescent dye. The filaments are very thin; nevertheless, the overall shape of the filaments is well defined under the microscope and their movements can be recorded in a movie film. (The film is shown at the lecture.) This technique is applicable not only in vitro but also in vivo.

The bending movements of single filaments of F-actin in solution are directly observable. The relaxation time of the bending movement of F-actin of 10 um in length is a few sec. The relaxation time of bending or the flexibility of F-actin is changed by adsorption of small ions and binding of other protein molecules. In the presence of soluble myosin fragments and ATP, F-actin filaments show large and fast bending movements which are not thermal but active movements consuming free energy released by splitting of ATP.

At a high concentration of F-actin filaments, their bending movements are suppressed and only a reptile movement is observed under the microscope. In this condition, myosin filaments and ATP induce a slidinglike movement of F-actin. This movement is fast and unidirectional, while the reptile movement in the absence of ATP is slow and bidirectional.

A typical example of the polymorphic transition of protein polymers is found in bacterial flagella. They assume helical structures having different pitches and different diameters. The transitions among them are caused by changing ionic strength, pH and temperature. The progress of the transition along the polymer can be followed under the microscope. A mechanical force or a flow of the medium can also cause the transition.

The effect of the electric and magnetic fields on these transitions and movements of protein filaments is being examined.

- Lecture title:

Modulation of in vitro and in vivo biological processes by means of low intensity EM exposure

- Lecturer's name:

Arthur A. Pilla, Ph.D.

- Extended summary:

This lecture will present the details of an electrochemical approach to the interactions of EM induced currents and cell function modulation. The concept of electrochemical information transfer at living cell membranes suggests that weak pulsating currents act on the cell via a set of well defined electrochemical pathways. These pathways can consist of ion-functional site interaction, dielectric charging, and passive and active ion fluxes. Any of the above will respond to current flow if potential dependence is exhibited. These non-faradaic electrochemical processes have characteristic kinetics and are therefore optimally stimulated by waveforms containing the appropriate frequency ranges within which sufficient energy is transferred to the relevant electrochemical pathway. A correlation therefore exists between the frequency and power spectra of the induced current and the observed biological effect. Perturbation of any of the possible electrochemical pathways may result in a time dependent change of intracellular ion concentrations (e.g. Ca^{2+} or Na^{+}) which in turn affects the rate of cell differentiation and/or division. There exists both a biological window (cell receptive to intracellular ion changes) and an electrical window (via frequency spectra correlations). Examples of all of the above will be given for Na/K ATPase, complement mediated cell lysis, cell/cell interation, hormone response and fracture repair.

OSCILLATING ELECTROMAGNETIC FIELD EFFECTS OF AND BY CELLS

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Some time ago, Froehlich (1968, 1977, 1980, 1983) suggested that the chemical energies of living cells could couple into their dipolar systems. Much as in a laser, he predicted, if the power density S exceeds a certain minimum level S_o, then a giant dipole mode of oscillation could come about. This cooperative oscillatory state could then be expected to mediate characteristic emissions and absorptions of electromagnetic radiation.

INTRODUCTION

Davydov (1980) presented a theory of solitons applicable to biological systems wherein solitons might be expected to participate in the nearly loss free transmission of cellular energies. Del Guidice et al. (1982) have since amplified these ideas as applied to cells. Pohl et al., Pohl, (1979, 1980a, b, 1982, 1983, Pohl and Braden, 1982, Pohl et al., 1981) proposed that spatially organized periodic chemical reactions involving ionic species were a potential source of such EM transmissions. The limit cycle regulation of chemical processes was discussed by Froehlich (1977, 1983) and Kaiser (1983a, b).

The experimental search for such EM radiation by cells was taken up actively by numerous laboratories. Natural cellular EM radiation and absorption effects were busily sought for. Early claims in sparse Russian reports described observations supporting the Froehlich predictions of EM responses in the difficult experimental range of 20 to 100 GHz. (Devyatkov, Sov. Phys. Usp. 16, 1974). German (cf. Froehlich, 1980 for extensive review of this work) and American studies Webb et al., (1980) carefully citing the extreme experimental difficulties, partially confirmed the Russian studies. Perhaps more importantly, Canadian studies by Rowlands et al., (1981, 1982, 1983, 1984) showed the existence of cell-to-cell forces of extremely long range (up to 4 micrometers) acting in a manner predicted by Froehlich's mechanism via supra-gigaherz types of oscillations. They also observed super-elastic intercellular fibrils with behaviors for which Davydov soliton action could be suggested.

When using experimental techniques well suited for detecting a wide range of frequencies, especially those in the lower frequency range below say, 100 MHz, Pohl and co-workers (Pohl, 1980a, b, c, 1981a, b, c, Pohl and Braden, 1982, Pohl et al, 1981) detected natural rf oscillations from a wide variety of cells, including those of bacteria, fungi, algae, aves, and mammals. The techniques included (1) micro-dielectrophoresis, (2) cellular spin resonance, (3) "hanging drop" figures in which patterns of variously polarizable powders develop about living cells while suspended in a drop of medium, and (4) direct observation as by metallic electrodes coupled to a signal analyzer, Smith (1983), Rivera et al. (1983). There are observations cited by Popp (1983) in which ultraviolet radiation was detected from yeast cells. There are well-studied cases of bacterial and algal cells, Gonyolux, e.g., which emit visible light. Infra red light from the alga Gonyolux was first detected by George Reynolds in 1976. Mormyrid and gymnotoid fish emit EM signals of about 1kHz to 50 kHz, Westby and Kirschbaum (1982).

There are, in addition to the above evidence for the emission of EM radiation by cells, growing evidences for the specific non-thermal effect of radiation on living cells, tissues, etc., Adey (1981, 1983) Blackman et al., (1979, 1980, 1982, Liboff, Pill).

In this brief overview we see that it is well established that living cells generate and emit electromagnetic radiation, and that cells can be affected by EM radiation itself in non-thermal modes. The natural rf oscillations from cells appear to be universal across the species, and to be associated with the mitotic process, at least in the case of yeast, Pohl and Braden, (1982).

These observations taken together suggest that the process of cellular reproduction is correlated with the natural rf oscillations of cells. They suggest that the observed natural electrical ac emissions of cells reflect some critically important chemical processes such as periodic reactions during reproduction. Since human cellular reproduction is critically essential in four phases of life, namely during embryonic, natural regrowth, would-healing, and oncogenic growth, then the study of such growth-related electrical processes is probably worthwhile. Research as to what, when, why, and how of these natural cellular oscillations is sure to prove important.

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BIOLOGICAL DIELECTROPHORESIS: THEORY AND PRACTICE

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ABSTRACT

Dielectrophoresis, the phenomenon whereby neutral matter is attracted into the region of strongest electric field, Pohl (1951, 1958, 1978) is now widely useful in sorting, fusing, analyzing, characterizing, and handling cells. Dielectrophoresis (or DEP for short) depends upon the polarizability of the material being subject to the nonuniform field force. As a consequence it is, unlike the dc phenomenon of electrophoresis, capable of showing a spectrum of responses as the frequency is varied. This DEP spectrum of responses as the frequency is varied. This DEP spectrum turns out to be unique for each cell type Pohl (1978). It sensitively reflects the physiological state, thus making DEP techniques useful.

INTRODUCTION

Biological DEP can be applied in quite a number of ways. The DEP force is used to gather cells or spheroplasts and form "pearl chains" by the sub-phenomenon of mutual dielectrophoresis, Pohl (1978) following which the application of a sharp electric pulse can evoke highly efficient fusion of the cells or protoplasts to form new entities ("electro-sex" or electro-fusion). Following this discovery in the U.S.A. by Pohl and its confirmation by R.E. Buckner and by J.K. Pollock in 1972, (Rivera et al., 1983) and by Mischel and Lamprecht in Germany in 1979, Zimmermann's group in (Zimmermann et al., 1982) Germany has made much progress. The field and potential conditions requisite for fusion control have been computed by Lafon and Pohl (1981).

Cellular spin resonance (CSR) is the sharp rotational response by particles (live or dead) to externally applied rotating fields. Pohl, (1981a, 1983a, b, c; Pohl and Braden, 1982, Mischel and Pohl, 1983, a, b). Here, the DEP force can be used to evoke spinning of cells and other particles. The cell (or inanimate particles, e.g.) may spin at few herz while the field oscillates at much higher frequencies. The rotating electromagnetic field may be one deliberately provided from multiple electrodes, or may arise, as pointed out by H. Schwan, in a two-electrode system by the action of delayed polarization fields coming from other matter nearby the particle.

There is a rarer type of lone cell CSR arising from the direct interaction of natural cellular oscillating dipoles with a simple two-pole sinusoidal field, Pohl (1983). It can be observed when certain lone cells spin out in the pure medium or against a clean smooth Pt electrode, for example. Its presence is an exciting evidence of the presence of ac fields arising from cells.

The DEP force is also useful in sorting cells and in generating DEP-derived spectral responses of the electromagnetic polarizability of cells and their parts, Pohl (1978, 1982, Pohl and Kaler, 1979). Cell sorting with the aid of DEP is done by passing a stream-centered stream of cells between curved electrodes providing a nonuniform electric field at a selected frequency. Alternatively the deflections of a stream of given cell type as the applied frequency is varied gives a polarization spectrum by which the cells can be analyzed and characterized. Such an instrument is currently being commercially developed in the U.S.A.

By means of the vertical action of a nonuniform field, a cell or other particle can be levitated against gravity. In this case the dielectric properties of even single cells or organelles can be sensitively ascertained over a wide frequency range, Chen and Pohl (1974) Crane and Pohl (1977) Kaler and Pohl (1980).

Further applications of DEP to biological systems are being developed, or should be. For example, the electro-filtration of aerosols, virus particles, bacteria, and spores from air environments is quite efficient, Pohl (1978). In Russian hands, DEP orientational effects on bacteria have been used to compare the effects of antibiotics, (Fonchenkov et al., 1979) (V.M. Fonchenkov, V.N. Brezgunov, B.K. Gavrilywk, V.V. Smolyaninov and Z.F. Bunina, 1979). There remains, for example, a major challenge in the construction and operation of the proposed mass-polarizability molecular beam spectrograph based upon DEP and molecular polarizability, (Pohl and Crane, 1983). A similar challenge resides in the model using the ring laser, Pohl (1978).

The theory of DEP has made recent good progress in the hands of F. Sauer, (1983). Previous attacks on the theory of dielectrophoresis (Pohl, 1951, 1958, 1978, Sher, 1968, Denner and Pohl, 1982) acting in real dielectrics, i.e. where there is conduction as well as polarization in the media involved, were imprecise to some extent by neglecting momentum conservation, a necessity when conductive (i.e. frictional) losses occur. The elegant solution to the problems by Sauer is sure to add significantly to our understandings of the various phenomena occuring in real materials in electromagnetic fields.

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- Lecture title: NON LINEAR ION FLUXES IN EM EXPOSED CELLS

- Lecturer's name: SANDRO RIDELLA

- Extended summary:

The goal of this work is two compute the ionic fluxes produced by a low frequency low amplitude electrical field applied to a cell modelled by an Hodgkin and Huxley membrane and by an external and internal medium characterized by a complex dielectric constant.
Strong frequency dependent behaviour has been found in the linear analysis, expecially in the sodium current through the membrane. Since the membrane equations are non linear and the applied signals have a low amplitude, the Volterra approach has been used to compute the rectified fluxes.
The Volterra method consists in evaluating all quantities at all positions in linear situation and in inserting ideal current or voltage generators in points where non linearity are located. Their values are computed approximating the true value of the generators controlling variables with the linear values previously obtained.
Then the values of the variables are computed considering the new boundary conditions.
The rectified K, Na and Cl fluxes have been used to compute the modifications of the ions concentrations inside the cell. These quantities have been suggested to be the cell controlling biological variables. The first result obtained is that rectified ionic fluxes are strongly frequency dependent both for amplitude and for sign.
Considering the frequency where the sodium flux entering into the cell is equal to the potassium flux flowing outside, it is possible to estimate the triggering time based both on the sodium-potassium exchange inside the cell and on the doubling of internal sodium. This time results to be in the order of some weeks for an applied external field about 10 V/m.
The pattern of the ionic fluxes shows similarity with ions current loops found during early development state of egg of the seaweed *Fucus* and that suggests possible effects on early stage of cell development.

- Lecture title: MODELLING OF PROTEIN BOUND WATER

- Lecturer's name: SANDRO RIDELLA

- Extended summary:

The behaviour of the complex dielectric constant of human proteins of normal and pathological individuals seems to differ significantly, as was pointed out by English and Russian authors in recent years.

In order to give a further insight about this question, we measured the dielectric constant of human sera of both normal and sick individuals. The measurements were taken in the frequency band 50-1000MHz.

In order to analyze these measurements, we made a mathematical model of the complex permittivity of a human serum in this frequency range. Being a serum fundamentally a solution of proteins and salts in water, our assumptions were:

- The protein permittivity is constant and equal to 2.
- The water has, whichever salts are diluited in it, an equivalent NaCl normality; its permittivity is given by Stogryn's formula.
- The water bound to the proteins has a Debye-type behaviour.
- The permittivity of the mixture proteins bound-water is given by Fricke's mixture formula.
- The permittivity of the mixture of salt water with proteins and their bound water is given again by Fricke's formula. These two applications of Fricke's formula introduce further relaxations in the complex permittivity.

The volume fraction of proteins, the volume fraction of bound water, its relaxation frequency and the equivalent normality N are calculated by minimizing the sum of the absolute differences between the theoretical and experimental data. The average of this absolute error is always less than 1%.

Examining these results, we evidenced some interesting differences between the groups of sera obtained from normal and sick donors.

- Lecture Title: Mechanisms For Cell Membrane Signal Transduction.

- Lecturer's Name: Dr. Gideon A. Rodan

- Extended Summary: The maintenance of vital cellular processes require effective separation of the intracellular milieu from the extracellular environment and the ability to communicate with the outside world for the recognition of nutrients and poisons and for functional coordination with other cells. The mechanisms for membrane mediated signal transduction include: (i) Regulation of adenylate cyclase activity, (ii) opening of ion channels, primarily calcium channels and (iii) regulation of phospholipid metabolism.

(i) Adenylate Cyclase (AC). Many peptide hormones, catecholamines and neuramines convey their signal via modulation of AC which controls intracellular levels of cAMP. The current model for the modulation of AC includes the following steps: (i) Hormone binds to receptor (ii) Receptor interacts with guanine nucleotide binding subunits causing their dissociation and hydrolysis of GTP. (iii) Activated subunit interacts with the catalytic AC unit and stimulates (or inhibits) it, respectively. The only known mechanism for cAMP mediated regulation is via the cAMP dependent protein kinase, a protein phosphorylating enzyme.

(ii) Ion Channels. Acetylcholine receptors contain calcium channels as one of their subunits and binding of acetylcholine to receptor is associated with opening of the channels. Other hormones may act through similar mechanisms. An elevation in intracellular calcium is bound to activate a large number of processes via interaction of calcium with high affinity calcium binding proteins such as calmodulin, troponin C or a variety of cytoskeletal proteins such as gelsolin. Each cell also contains a number of calcium dependent protein kinases which phosphorylate intracellular proteins.

(iii) Phospholipid Metabolism. Studies conducted primarily in platelets have shown that thrombin activates a phosphotidylinositol (PI) specific phospholipase C which acts primarily on phosphatidyl-4,5,-diphospoho-inositol (PIP2) to yield triphosphoinositide (IP3) and diacylglycerol. The IP3 can act as a calcium mobilizing agent from intracellular calcium stores. The diacylglycerol can stimulate the calcium-phospholipid dependent protein kinase C. Protein phosphorylation thus appears as a central mechanism for the regulation of cell function in response to membrane mediated signal transduction. A diacylglycerol lipase can cleave the arachidonic acid, usually found in position 2, and provide the substrate for a large number of arachidonic acid products, many of which serve as local hormones in intercellular communication (prostaglandins, thromboxanes, prostacyclin and leukotrienes). Some of these agents act via stimulation of adenylate cyclase generating the possibility for multiple feedback loops. In some systems, platelets for example, calcium and cAMP oppose each other's effects whereas in others, such as salivary gland, calcium and cAMP have similar effects. In summary, we have now significant insight into membrane mediated signal transduction mechanisms which seem to be common in principle to most mammalian cells, but vary in the extent to which they are present and the way they interact with each other.

- Lecture Title: Control of Cellular Growth and Differentiation.

- Lecturer's Name: Dr. Gideon A. Rodan

- Extended Summary: The tissues of the mammalian organism can be divided into 3 groups: Non-regenerating (nervous tissue), regenerating under special circumstances (liver), and continuously regenerating (skin, gut, bone and blood). In regenerating tissues, there is a reserve of undifferentiated stem cells which have the capability both to divide and to differentiate. Most of these cells are in a resting state but will enter the proliferative cycle and undergo differentiation upon exposure to appropriate stimuli. Mutation analysis has shown that in yeast 32 genes are activated following initiation of proliferation and the number may be much higher in mammalian cells. Multiple requirements need to be met to start DNA synthesis. These include, cell attachment, for (fibroblast like cells), availability of nutrients, metals, and a combination of growth factors, such as competence and progression factors. The entry of cells into DNA synthesis occurs stochastically at rates proportional to the concentrations of the growth factors. Epidermal growth factor which has both competence and progression effects was shown to promote the phosphorylation of its receptors. Membrane changes occurring after exposure to growth factors include: Increased uptake of nutrients, (amino acids, nucleosides), increased influx of cations such as sodium, calcium, and magnesium. These changes probably trigger additional events needed for proliferation. Intracellular phosphorylations also occur, for example, phosphorylation of the S6 ribosomal protein in response to insulin. Some insights into the mechanism of proliferative control can be derived from the recent findings on oncogenes, which revealed that one of them (sis) codes for platelet derived growth factor, and another one (src) codes for a 60 KD protein $pp60^{src}$, which is a protein kinase specific for tyrosine phosphorylation, the phosphorylation target of EGF.

The division of stem cells is frequently asymetrical yielding two different progeny cells: One which retains the stem cell characteristics and one which can go on to differentiate. DNA replication, therefore, frequently precedes differentiation. On the other hand, the differentiating cells will not proliferate, which is the basis for the reciprocal relationship between differentiation and proliferation. This negative coupling between the two processes is lost in malignant cells. The kinetics of differentiation also exhibit a stochastic pattern. Differentiation factors, analogous to growth factors have been identified. It was believed that differentiation is associated with the coordinate expression of all differentiated properties via the activation of a master gene. More recent data suggest that various properties are probably sequentially expressed. These properties show a normal distribution among the cells expressing the particular phenotype. The stochastic generation of diversity was probably the evolutionary basis for differentiation. This idea implies that such cells could transfer to their progeny the preference for the expression of certain genes and these progeny thus became a tissue. Recent developments in molecular biology and recombinant DNA promise to offer significant insights into the mechanistic basis for the control of growth and differentiation.

MICROWAVES EFFECTS ON CHICK EMBRYO HEART RATE

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Summary

A great deal of theoretical and experimental work has been already done in the field of biological effects of low-level microwaves. However, the interaction mechanism through which such effects are induced is not yet well known [1]. According to some authors, a significant sensitivity of the cardiac activity to low-level microwaves would exist, whereas, according to others,there is no effect on heart rate [2],[3],[4],[5],[6],[7].

In order to investigate this mechanism, we have performed a first set of experiments by exposing chick embryo hearts to 64-74 GHz microwaves. Probably due to the low penetration depth at these frequencies and to the low irradiation power, no effect was observed [8]. Instead, working at 2.45 GHz we observed many effects on heart rhythm.

Experiments were perfomed on isolated hearts of chick embryo of 10-12 days incubation, placed in a small polystirene Petri dish filled with Ringer's solution for bird hearts. The living sample was kept at 38 $^{\circ}$C and continuously oxygenated. The heart was irradiated with an incident power not exceeding 1 mW/cm^2. The extracellular signal was detected through a glass micropipet inserted into the sinus-auricle node and was elaborated by a BERG-FOURIER analyzer in order to obtain the frequency spectrum.

We have observed that a CW irradiation induces only a little bradycardia. With a square wave modulation at a frequency near the natural heart rate, the heart beat was locked to the modulation frequency. This lock was observed for variations of the modulation frequency of 1-4 Hz. In some experiments also a heart rate sensitivity to swept

SUPERDIAMAGNETISM IN BIOLOGICAL SYSTEMS

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We have studied the optical turbidity of multilamellar vesicles formed from bilayers of DMPC, DPPC, DSPC, DOPE, egg lecithin, and several binary mixtures of these lipids. At temperatures above the pre-transition the turbidity decreases significantly in the presence of magnetic fields greater than 0.2 Tesla, and reaches a limiting value at fields greater than about 1.9 Tesla. The diamagnetic anisotropy of individual lipid molecules is too small to overcome thermal effects, however the effect may be attributed to 'superdiamagnetic' molecular clusters formed via van der Waals interactions. Our recent studies have included the microscopic observation of vesicle orientation and angular laser light scattering, in a magnetic field.

pulse, pulse modulated carrier frequency), in order to determine quantitatively the transmembrane voltage produced. Main results of such an analysis and some experimental measurements, made in the giant nerve cell somata from a landsnail, will be shown in detail. We will also discuss these results to give a contribution to the problem of defining the hazard levels of transient electromagnetic fields.

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A COMPARISON BETWEEN THE EFFECTS PRODUCED ON DIFFERENT KINDS OF EXCITABLE MEMBRANES BY TRANSIENT ELECTROMAGNETIC FIELDS.

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SUMMARY

The evaluation of hazard levels of nonionizing radiations from ELF to Microwaves (MW) requires a deep knowledge of the interaction mechanisms between electromagnetic (EM) fields and biological systems. The so-called thermal effect produced by the energy dissipated within the tissue,has been until now the most examined [1],[2]. Since the thermal effect is usually little influenced by the temporal behavior of the absorbed EM field, major literature on the subject is devoted to interactions produced by fields having an harmonic time dependence.

A non-thermal interaction mechanism considered more recently [3]-[5], consists of the displacement of the membrane voltage from its resting value produced by a field acting at cellular membrane level. This mechanism is strongly influenced by:

a) the temporal dependence of the field absorbed within the tissue;

b) the electrical characteristcs of the membrane.

As for a), the electrical behavior of a nerve fibre membrane, excited by e.m.fields having a general temporal behavior, has been analyzed in detail in a previous work [6]. In such an analysis we adopted the Hodgkin and Huxley (H.H.) nonlinear and non-stationary model [7] for studying the influence on the membrane voltage of the various parameters characterizing a transient incident field, such as waveshape, amplitude, and time-width.

The purpose of the present work is to examine the effects produced by a transient field on the membrane of a different cellular structure, namely a nerve cell body [8]. Such a membrane, even if simulated with a model analogous to that of H.H. for the nerve fibre membrane, shows a quite different behaviour, because of the different ionic channels' conductivity and of their different activation and disactivation time-constants. Since this membrane responds to an applied constant current by a train of action potentials, it should be of interest to study how the Connor and Stevens model responds to the current generated into a nerve cell body irradiated by an incident e.m. field.

This study has been carried out assuming incident fields with temporal dependence of various types (gaussian

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INTERACTIONS BETWEEN ELECTROMAGNETIC FIELDS AND CELLS

ABSTRACTS

and notes

2nd part

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Session:

COMPLEMENTARY LECTURES OF DIELECTRO-ELECTROPHORETIC FORCES

Processes involved in electrofusion - U. Zimmermann

Interaction of alternating electric fields and field pulses with cells lead to cell fusion. The steps involved in cell fusion are discussed such as pearl chain formation, membrane deformation and membrane poration. Particular emphasize is given to the field - induced redistribution of membrane components during the application of the alternating electric field.
In this context the interactions of electro-magnetic waves with biological cells leading to cell movement and fusion are also considered.

Session:
DIELECTRO-ELECTROPHORETIC-FORCES

Field-induced cell rotation - U. Zimmermann

Interaction of alternating electric field effects with suspended cells lead to cell rotation at characteristic frequencies.

Rotation of single cells (mesophyll protoplasts of Avena sativa) induced by a planar, homogeneous rotating field has been observed at a frequency of 20-40 kHz (conductivity of the external mannitol solution $6 \times 10^{-5}\ \Omega^{-1}cm^{-1}$). This variation in optimum frequency is largely due to an inverse dependence on cell radius. Rotation direction is opposite to that of the field, and can be reserved at will by reversing the field. The maximum speed of cell spinning was a few cycles per second (and thus always much slower than that of the field) and was proportional to the square of the amplitude of the field.

The rotation of a single cell in a rotating field is expected on the basis of the dipole-dipole theory developed by Holzapfel et al. (J. Membrane Biol. 67, 1-14 (1982)), for multi-cell rotation. Measurements of the dependence of optimum applied field frequency an medium conductivity indicate that the dipole is generated by interfacial (Maxwell-Wagner) polarization. The required frequency is a linear function of the conductivity of the external solution. This relationship is used to derive a value for the specific membrane capacitance. Further applications of this technique for cell and membrane research are discussed.

- Lecture title: The Effects of Pulsed RF Fields on the Shape and Volume of Normal and Sickled Erythrocytes

- Lecturer's name: Shiro Takashima

- Extended summary:

It is well known that electrical fields cause various effects on biological cells and subcellular particles. A few examples are the formation of chains (Pearl chain formation), rotation of cells and cell fusion. These effects are implemented with the field intensities of a few kV/cm. It is also known that the application of pulsed electrical fields causes the increase in the volume of normal erythrocytes.

Sickle cell anemia is a genetic disease which is caused by an abnormal hemoglobin (Hb S). Hb S tends to form filamentous aggregates called tactoids or gels inside the erythrocyte when oxygen is released from them. The intracellular formation of tactoids entails the stretching of erythrocyte membrane and changes the shape from the normal biconcave discoid to a sickle shape. There are many attempts to reverse the sickling process using various chemical agents. However, these attempts had limited successes because of toxicity and other reasons. We found that application of pulsed RF fields causes shape changes of sickled erythrocytes at the level of 3 - 4 kV/cm. We found well defined thresholds for the shape change. Usually, no shape change was observed below E_{th} however, when field intensity reached the threshold, the shape change progress rapidly. The desickling of erythrocytes usually causes extensive swelling of erythrocytes and this entails the hemolysis red blood cells. Hemolysis was prevented only if the field was turned off at the early stage of the shape change. In order to improve the results and minimize the extent of hemolysis, very short pulses of 20 - 50 μsec. with a long interval of 1 second are used. The use of short pulses requires higher field intensities, nevertheless, high intensity short pulses produced less hemolysis than that caused by low intensity long pulses. Moreover, a suspending medium which is very similar to blood serum (Hank's solution) was used in order further to improve the outcome. The use of Hank's solution yielded the results which are clearly better than those in physiological saline solution. The reversal of desickling process apparently takes a very long time and our attempt to implement it was not successful. The mechanism of RF induced shape and volume changes of erythrocytes is believed to be due to the induction of membrane potential across the membrane. The magnitude of the potential can be calculated for sickled erythrocytes using the ellipsoidal coordinates and the results of these calculation are discussed.

- Lecture title: Dielectric Properties of DNA Solution at RF and Microwave Frequencies

- Lecturer's name: Shiro Takashima

- Extended summary:

The dielectric properties of DNA was investigated extensively in ELF region using two electrodes as well as four terminal techniques. It is well known that DNA has a large induced dipole moment due to counterion polarization. However the dielectric properties in RF and/or microwave regions were not well understood. However, the observation that microwaves can affect the growth of micro-organisms, stimulated a series of experimental and theoretical research on the possible absorption of electromagnetic energy by DNA at MHz and GHz regions. The investigation in this frequency range, thus far, is limited to narrow regions and this, in turn, makes the interpretation of experimental results difficult. We intended to carry out the measurements of the permittivity and conductivity of DNA (highly polymerized calf thymus DNA) between 10KHz and 70 GHz using four measuring systems. The results we obtained can be summarized as follows: 1) There is a small dispersion of permittivity and conductivity between 100KHz and 500MHz. The origin of this dispersion is still unknown. However, its characteristic frequency is too low and also the dispersion curve too broad to be due to the resonance absorption predicted by the theory by Prohosfsky et al. Single stranded DNA also exhibited this dispersion, and therefore, double helical structure is not essential for this relaxation. 2) We found that the γ-dispersion (dipolar relaxation of water molecule) of DNA solution is only slightly different from that of pure water, inspite of an enormous viscosity of DNA solution. The permittivity of DNA solution in the frequency range between 1 - 10 GHz is slightly smaller than that of pure water and shows a smooth decrease as frequency increases. The slope of the γ-dispersion of DNA solution and its characteristic frequency were analyzed using several theoretical methods. All these analyses indicate that the presence of highly charged DNA molecules causes slight distribution of the relaxation time (broader dispersion curve) and the shift of characteristic frequency. Although these shifts are small the differences are significant statistically. These observations indicate that the orientational freedom of water molecules is to some extent affected by the charges of DNA because of charge-dipole interaction even outside the first hydration layer. The determination of the amount of irrotationally bound water was not successful.

Physico-chemical models of voltage-dependent gating in membranes

G. Schwarz

Phenomenology. Excitation of a biological membrane (e.g. that of a squid axon) rests on the fact that substantial changes of its ionic conductances are induced by variations of the electric potential drop. A voltage jump of about 100 mV may result in an almost complete transition between two extreme levels which proceeds in times of the order of milliseconds. The underlying process apparently involves an electric field controlled opening and closing of ion-specific channels ("gating"). A detailed molecular mechanism is, however, not known so far.

Structural basis. Possible gating mechanisms naturally must be consistent with the structural features of a biological membrane. According to the "fluid mosaic model" functional entities such as the "gating particles" should be protein structures which are quite tightly incorporated in the lipid bilayer matrix by means of hydrophobic interactions with the hydrocarbon moiety. On the other hand, the channel must also have hydrophilic regions located towards the aequeous phases.

Electro-mechanical effects. It has been suggested that the voltage-dependence of gating may be due to a field induced rotation of a dipole associated with the gating particle. As can be easily shown, this would require dipole moments of the order of some hundred Debye units which have to be turned around over practically 180 degrees. In such a case, however, a considerable activation energy arises from the inherent hydrophobic and hydrophilic interactions which have to be broken. Accordingly the process becomes much too slow in view of the experimentally observed gating kinetics. A similar situation exists with respect to a field induced translational displacement of a charged gating particle.

A basic "chemical" model. It appears reasonable that the gating involves one or more field sensitive chemical transformation steps, e.g. conformational transitions. These would not necessarily be associated with a slow kinetics. The appropriate quantitative aspects of voltage-dependence and relaxation time are rather simply discussed in the light of an elementary two-state mechanism. It permits a satisfying interpretation of early gating current data.

More complex models. Later gating current experiments pointed to the existence of more than two relevant states in the underlying mechanism. By way of example, it can be shown that a three-state conformational transition model permits an adequate interpretation of some squid axon data reflecting two relaxation times.

Simple model systems. A number of chemically well characterized low molecular weight peptides (e.g. alamethicin) form voltage-dependent ion-conducting pores in artificial and natural membranes. For the present, these systems are of some interest because they make feasible a wider range of relevant physico-chemical measurements as they are needed in order to elucidate the actual molecular mechanism.

Electric field effects on rate processes and on cooperative transitions of macromolecular structures

G. Schwarz

Thermodynamic basis. We consider a closed material system which is subject to an externally applied electric field. From the first and second laws it follows that generally for any differential change of state

$$dG \leqslant - SdT + Vdp - MdE$$

(where the modified free enthalpy $G = U + pV - ME - TS$; U: internal energy; p: pressure; V: volume; M: over-all electric dipole moment parallel to the field; E: field strength; T: temperature; S: entropy). The < and = signs refer to spontaneous and equilibrium processes, respectively.

Field effect on chemical equilibrium. It can be shown that the equilibrium constant, K, of a chemical process becomes a function of E as described by the "van't Hoff" relation

$$\partial \ln K/\partial E = \Delta M/RT$$

(R: gas constant) with ΔM, the reaction moment, being the change of M induced by a one molar stoichiometric turn-over. This implies a displacement of equilibrium towards the more polar side of the reaction.

Elementary rate constants. These can be expressed in terms of the transition state theory as

$$k = (k_B T/h)\exp(-\Delta G^{\ddagger}/RT) = k_o \exp(\Delta M^{\ddagger}\cdot E/RT)$$

(because of $\Delta G^{\ddagger} = \Delta G_o^{\ddagger} - \Delta M^{\ddagger}\cdot E$; $\Delta G_o^{\ddagger}$, k_o: standard free energy of activation and rate constant at zero field, respectively). This field effect is, however, negligible under any physically reasonable conditions.

Complex rate processes. Provided a chemical transition involves a great number of elementary steps, its dynamics may definitely show a significant field dependence. Frequently an intermediate rate determining step is encountered which leads to a first or second order kinetics. The corresponding rate constant then turns out to be

$$k = k_o \exp(\Delta M^{*}\cdot E/RT)$$

where ΔM^{*} refers to the formation of the rate determining intermediate state.

Cooperative transition of a membrane protein. This can be dealt with quite readily if an all-or-none mechanism is assumed. Quantitative relations describing the field effects on the degree of transition and on the relaxation time are then derived and discussed. One finds that the decisive factor is Δm, the difference of molecular dipole moment components normal to the membrane taken between the final and initial states of the protein. Once E clearly exceeds $k_B T/\Delta m$ substantial field effects will occur.

A helix-coil transition in an isotropic solvent. Such a case involves not only a large number of different structural states but also on orientational distribution of the individual molecular dipoles. Accordingly the appropriate theoretical approach towards a possible field effect becomes somewhat more sophisticated. This may be briefly discussed with a simple model system.

- Lecture title: Field Force Effects.

- Lecturer's name: H. P. Schwan.

Extended summary: Significant forces can be imparted on particles exposed to DC or AC electrical fields. Some of the effects which have been observed include the following: movement of particles in inhomogeneous electrical fields (dielectrophoresis), alignment of particles (pearl chain formation), orientation of nonsperical particles in the field direction or perpendicular to it, fusion of biological cells, destruction of biological cells, shape changes, microstreaming effects and rotation. Much observational material has been collected, but a full theoretical understanding is incomplete even though there is but little doubt about the basic mechanism involved.

Earliest observations of pearl chain formation go back to Kerr near the turn of the century. In 1926 Muth published a detailed account, and in the 1930's Krasny Ergen published theoretical papers approximating cells by very conducting spheres. Probably the first extensive quantitative studies on the pearl chain formation effect were conducted in our laboratory. The threshold field strength for the effects of pearl chain formation and orientation was studied. Its dependence on a variety of parameters such as pulsing field conditions, particle size, frequency, time needed to cause the effect were investigated theoretically and experimentally. The Langevin criterion, originally developed for permanent dipoles, was suggested by us as an approximate threshold criterion and experimentally verified. It states that $u E_{th} = KT$ with E_{th} threshold field strength u induced particle dipole moment, K Boltzmann constant and T absolute temperature. However, this criterion can only serve as an approximation since each field effect requires its own more detailed analysis. The threshold field strength E_{th} was noted to be inverse to the particle volume and experimentally verified. The time constant for pearl chain formation was predicted to be inverse to E^2 for fields $E > E_{th}$, which also was experimentally confirmed. A precise equation for the force acting between two particles was derived by Sauer and the path chosen before they touch calculated and experimentally verified. Pohl developed similar equations as Schwan, Saito and Sher to predict the behavior of particles in inhomogeneous fields and Sauer carried out an exact calculation of the force acting on a particle in an inhomogeneous field.

The phenomena of rotation of biological cells was studied by Pohl and Zimmermann. Earlier observations go back to the 1920's as pointed out by Glaser. Schwan suggested that rotating fields may be responsible, Zimmermann and Pohl caused rotation with such fields. Glaser demonstrated that even homogeneous, isotropic particles rotate in rotating fields. The effect is optimal at frequencies close if not identical to the characteristic frequency of the β-dispersion investigated by Schwan, Cole and Fricke. The biological significance of these effects remains to be fully investigated.

- Lecture title: Historical Review, State of the Art, Open Problems.

- Lecturer's name: H. P. Schwan.

Extended summary: Interests in the effects of electrical fields on biological materials developed more than one hundred years ago and have ever continued. Activities include:

Electrophysiology: Contributions concerned with the effects of electrical fields on excitable tissues; Electrical properties of cell suspensions and tissues; Macromolecular studies: Electrical properties of macromolecules; Dielectric saturation effects; Pondermotoric forces: Pearl chain formation, orientation effects, other cellular responses to alternating electrical fields.

Parallel to these more basic pursuits various medically or biologically motivated efforts evolved including:

Shortwave and microwave diathermy; The athermal-thermal controversy; Emerging concern about radar and oven induced biohazards; Studies of RF bioeffects, including ELF and microwaves; Concern about the biological effects of ELF fields; Field effects on bone repair and cell growth; Studies of unusual high perception of some life organism.

Most of these efforts developed almost independently with little cross fertilization.

The present situation may be characterized as follows. The more basic pursuits have achieved a high level of sophistication. Membrane physiology and biophysics are highly advanced. Modern instrumentation and techniques have substantially contributed to the channel hypothesis. However, the detailed molecular mechanism for channel transport or transport through biological membranes has not been discovered yet. The macromolecular studies have achieved a degree of saturation even though some details are unresolved as far as the response of highly complex molecules to fields are concerned. The field of pondermotoric forces has experienced a revival with many interesting applications. But a number of basic problems remain to be resolved. So far, the biophysical principles which emerged are not suggestive of very subtle field sensitivities.

During recent years much work has been done which suggests high biological field sensitivities. This includes work on cellular growth effects, bone union repair and modulated RF effects. Extraordinary perception capabilities of certain fish and other organism for ELF fields and very sharp biological resonance effects have been reported in the mm-range. Physical mechanism has been suggested which may account for some of these effects, but details have not yet been worked out.

The task remains to sort out to what extent reported reliable effects can be rationally explained using existing basic concepts and to what extent either modifications of these concepts or entirely new principles must be applied.

- Lecture title: Cellular Properties.

- Lecturer's name: H. P. Schwan.

Extended summary: Extensive work has been done on the electrical properties of macromolecules, cell suspensions, single cells, and tissues, extending from a few Hz to many GHz. The data conveniently can be characterized by three major and some minor additional relaxation effects.

The first effect to be analyzed is the β-effect which occurs at RF frequencies. It is caused by the charging of the cell membranes through extra- and intracellular compartments. The γ-effect largely reflects the dielectric properties of water, which relaxes in the GHz frequency range. The α-effect, at ELF frequencies, may have several causes. Counter ion relaxation, charging of subcellular organelles connecting with the outer membrane are both likely to be strong contributors. Other relaxation effects in the α-range may reflect the complex dielectric structure of the membrane and ion selective channel characteristics.

Smaller relaxation effects are due to the macromolecular content of cells. Between β- and γ-dispersion there exists a weak δ-dispersion largely due to the relaxation of protein bound water. Subcellular organelles inside the cell add to the tail of the β-dispersion of the whole cell.

The insight gained has been useful.

1. The properties of tissue water and intracellular water appear to be very similar if not identical to those of normal water, except for a small volume fraction probably identical with the protein bound water. Static dielectric constant and relaxation frequency are identical for intracellular and normal water. But the conductivity of the cytoplasm is about twice lower than estimated from the suspended ionic species.
2. The capacitance of cell membrances appears to be virtually frequency independent through most of the observable RF-range. However, the apparent membrane capacitance at low frequencies changes, probably caused by the various mechanisms listed responsible for the α-dispersion.
3. The insight achieved permits a determination how electrical fields applied to a cell suspension or tissue may subdivide between membrane and extra- and intracellular field strength values. These values turn out to be frequency dependent. Noticeable membrane potentials dominate at the lower frequencies while significant internal field strength levels acting on subcellular particles exist at higher frequencies. Broad resonance effects exist in the RF-range and at ELF frequencies. Clearly, the interaction of electrical fields with cells must be highly frequency sensitive.

microwaves frequencies and to variations of the pulsed modulation duty cicle was evidenced.

A quantitative analysis of these phenomena may be a useful hint to the knowledge of interaction mechanisms at low level interactions.

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A. Scaglione°, M.R. Scarfi°°°

CHROMOSOMAL ABERRATIONS INDUCED BY ELF FIELDS

Bovine peripheral blood, drawn from the jugular vein, was cultured for 72 hours at 37°C in Mc Coy's 5A modified medium supplemented with 20 per cent autologous plasma, antibiotics and mitogen (Pokeweed). 50 Hz ELF electric fields were continuously applied to the culture flasks by means of external electrodes. Colcemid was added to the cultures 4 hours prior to harvesting. In the flasks containing cells for sister chromatid exchange (SCE), bromodeoxyuridine (BrdU) (10 g/ml, final concentration) was added 30 hours before cell collection. Hypotonic and fixation treatments were performed in the usual manner. Conventional Giemsa staining and acridine-orange staining were used.

Two kinds of cytogenetic observations were carried out on treated cells and on reference cultures:

i) chromosomal aberrations (aneuploidy, polyploidy, deletions, fragments) and

ii) sister chromatid exchanges (SCE)

the latter being a sensitive test for assessing chromosome instability (see e.g. the Bloom's syndrome in humans, were the number of SCE per cell considerably grows).

From the data collected until now, an increase in chromosomal aberrations is apparent (from 8% in untreated cultures up to about 21% in cells exposed to electrical fields of about

30 mV/m.

An increase in the number of SCE has not yet evidenced. Other experiments on different animals, at different field levels are under way.

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Dielectric Properties of Biological Systems and Their Relation to Other Transport Processes

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Abstract

Our group at the University of Pennsylvania has for some years been studying the dielectric properties of biological and other complex suspensions at microwave frequencies, which reflect the rotational correlation times of the water dipoles. The first part of the talk will be a brief summary of the quite similar results that have been obtained from various systems, including single muscle fibers from the giant barnacle, various canine normal and tumor tissues, ionic and nonionic oil-in-water microemulsions, and solutions of organic polymers such as poly(ethylene oxide).

These dielectric data have been analyzed using the Maxwell and Hanai mixture theories, to study the large effects of hydration interactions on the bulk dielectric properties. These theories, however, apply equally well to properties such as water self-diffusion, ionic conductivity, thermal diffusivity, and complex dielectric permittivity. The second part of the talk will compare these respective properties in several biological and nonbiological suspensions, and show that corresponding changes are observed. I suggest that this comparison of different transport properties is a useful and informative approach to studying hydration phenomena in complex aqueous systems.

ELECTROMAGNETIC FIELD EFFECTS ON PHYTOHEMAGGLUTININ INDUCED LYMPHOCYTE REACTIVATION

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ABSTRACT

In this paper ,we describe the effects of weak electromagnetic fields on the process of reactivation of human lymphocytes induced in vitro by the lectin phytohemagglutinin (PHA).

By means of flow microfluorometry and absorption image cytometry,we show that a 72 h electromagnetic exposure reversibly slows down the rate of PHA-induced lymphocyte stimulation,at low PHA doses.

The antagonistic effect of electromagnetic exposure on lectin-induced reactivation is in agreement with previous theoretical findings.

Influence of the Space Charge Distribution on the Maxwell-Wagner Relaxation.

A. García, P. Brito and C. Grosse.

The electric response of an electrolyte with dielectric spheres imbedded in it, is analysed. The continuous medium is characterized by the mobility of its ions, which leads to the appearence of space charge distributions near the interfaces.

This fact diminishes both the dielectric increment and the relaxation time with respect to the values predicted by the Maxwell-Wagner treatment. The results obtained do not agree with those deduced by Bonincontro et al.

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SCIENCE

Time-Varying Magnetic Fields: Effect on DNA Synthesis

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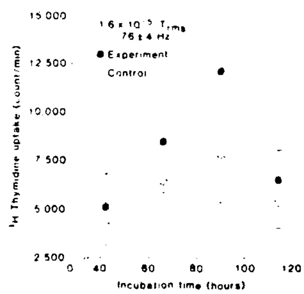
Time-Varying Magnetic Fields: Effect on DNA Synthesis

Abstract. *Human fibroblasts have exhibited enhanced DNA synthesis when exposed to sinusoidally varying magnetic fields for a wide range of frequencies (15 hertz to 4 kilohertz) and amplitudes (2.3×10^{-6} to 5.6×10^{-4} tesla). This effect, which is at maximum during the middle of the S phase of the cell cycle, appears to be independent of the time derivative of the magnetic field, suggesting an underlying mechanism other than Faraday's law. The threshold is estimated to be between 0.5×10^{-5} and 2.5×10^{-5} tesla per second. These results bring into question the allegedly specific magnetic wave shapes now used in therapeutic devices for bone nonunion. The range of magnetic field amplitudes tested encompass the geomagnetic field, suggesting the possibility of mutagenic interactions directly arising from short-term changes in the earth's field.*

Most studies of the effect of static magnetic fields on living systems have yielded negative or inconsistent results (*1*); the exception is studies of species that incorporate ferromagnetic materials as geomagnetic sensory elements (*2*). Experiments on time-varying magnetic fields have been fewer and more difficult to interpret (*3*). A therapeutic technique for accelerating repair in bone nonunions that subjects the site to a time-varying magnetic field (*4*) has achieved a measure of acceptance in the clinical community (*5*). However, the mechanism underlying this procudure is still largely unexplained, in part because the narrow and repetitive pulse shape of the wave form, allegedly specific to the therapy, makes it difficult to perform and interpret in vitro cellular experiments (*6*).

We sought to determine the effect of sinusoidally varying magnetic fields on DNA synthesis in cell culture, particularly the frequency and intensity response, if any. A pair of matched incubators were fitted with modified Helmholtz coils (0.5 m, inside diameter; 0.25 m long), providing a horizontal magnetic field with an active field uniformity no less than 3 parts out of 17. Either could be an experimental or control (no field) unit. Periodically the roles were switched and, at other times, both were used simultaneously as controls. An au-



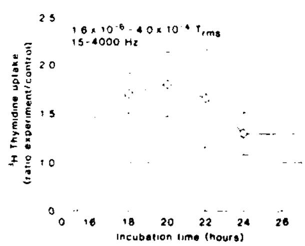


Fig. 1 (left). Tritiated thymidine uptake (mean counts per minute ± standard error) in human foreskin fibroblastic cells exposed to a magnetic field of amplitude 2.3×10^{-6} T oscillating at 76 ± 4 Hz in the horizontal plane (experiment) and uptake in unexposed cells (control). Mean levels differ at $P = 0.0001$, as determined from the pooled variances. The ambient 60-Hz magnetic field in the area of the incubators was < 1 × 10 [illegible] T_{rms}. Note that the approximate intensity of the geomagnetic field is 5×10^{-5} T. Fig. 2 (right). Mean ratios and standard errors of [^{3}H]thymidine uptake in exposed cells to that in control cells for ten different combinations of frequencies and fields. The dotted line indicates the expected response if there is no effect due to a magnetic field. The peak at 20 hours corresponds to the middle of the S phase of the cell cycle. *The dashed lines below 18 and above 24 hours represent the expected, but inexact trends, in these data.*

dio amplifier fed by a function generator supplied 0 to 1.0 root-mean-square ampere to either coil, enabling us to generate magnetic fields up to 1.0×10^{-3} root-mean-square tesla (T_{rms}) at frequencies ranging from 15 Hz to 20 kHz.

Two independent series of experiments were completed, both with human embryonic foreskin fibroblasts (7) as the test cell line.

In the first series, one frequency (76 ± 4 Hz) and one intensity (1.6×10^{-5} T_{rms}) were used for all runs. Cells were seeded into eight 96-well culture plates (8) at densities of approximately 1.0×10^{6} cells per milliliter, and the plates equally, but randomly, distributed between the control and experimental incubators. Following exposures of 24, 48, 72, and 96 hours, [^{3}H]thymidine (9) was added to each well and the plates returned to their original incubators for 18 hours, after which all cells were washed twice in isotonic saline and collected on a filter strip by an automated sample harvester (10). The strips were dried, and the individual filter disks corresponding to each well were counted in a liquid scintillation counter. In 45 out of 48 paired plates, the amount of incorporated thymidine was higher for cells exposed to the magnetic field (Fig. 1).

In the second set of experiments, the cell cultures in the experimental incubator were subjected to various frequencies between 15 Hz and 4 kHz and to intensities between 1.6×10^{-6} and 4×10^{-4} T_{rms}. Tritiated thymidine was added to the cell medium at the time of seeding. The initial inoculation density was limited to 5×10^{4} cells per milliliter. Paired (24-well) plates (11) were removed from both incubators at various times during a 24-hour period. Cell exposure was limited to 24 hours following inoculation to enhance cell synchrony. Selection of plates was not random as it was in the first experiment; seeding and harvesting of these cells were arranged beforehand. Corresponding wells in both plates were sequentially seeded, collected, and compared. In this manner, a fundamental data unit for a given frequency, intensity, and exposure time consisted of the ratio of thymidine uptake in an experimental well to that for a matched control. Averages of these units for all such pairs provided uptake ratios. Preliminary trials indicated that a peak in this ratio occurred after about 20 hours of exposure. Subsequent data collection was then restricted to four exposure times, 18, 20, 22, and 24 hours (Fig. 2).

In separate experiments to determine cell growth as a function of time, the peak in uptake was found to correspond to the middle of the S phase of the cell cycle. Although the enhancement of thymidine uptake was, on average, no greater than 80 percent (Fig. 2), ratios two and three times this occasionally resulted in individual experiments. Figure 3 shows, for two separate exposure times, the relative uptake as a function of the product of frequency ν and root-mean-square field strength B (B_{rms}); this product is directly proportional to the time derivative of the magnetic field. As such, the slopes of the lines plotted will test whether Faraday's law is implicated in the results.

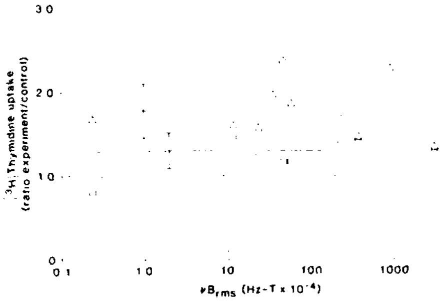


Fig. 3. The mean ratios (from Fig. 2) as a function of the product of frequency and magnetic field intensity. Exposure times of 20 hours (△) and 24 hours (○) are shown. Note the apparent lack of variation in response for four orders of magnitude, indicating that this interaction is independent of the time derivative of magnetic field, $\partial B/\partial t$. The two upper lines represent the mean levels for the 20- and 24-hour exposures, respectively.

These experiments show that there is an interaction, albeit a subtle one (the results from Fig. 2 represent approximately 100 trials), between time-varying magnetic fields and DNA synthesis. Inasmuch as our fields varied sinusoidally, some measure of doubt is introduced as to whether the particular wave shape now used to accelerate bone fusion is indeed as necessary as has been claimed. An alternative, though not a likely one, is that two separate cellular interactions occur at low-frequency magnetic fields.

Although it is reasonable to suggest that Faraday's law is involved in inducing extracellular and intracellular currents according to $\nabla \times \mathbf{E} = -\partial \mathbf{B}/\partial t$ (where $\mathbf{E}$ is the induced electric field and t the time), it should then follow that the observed response will scale as $\partial \mathbf{B}/\partial t \sim 2\pi\nu B$. The lack of such a response (Fig. 3) may mean that induced eddy currents do not play a role in this phenomenon, or it may indicate that the effect is a saturable phenomenon—for example, either a configurational effect or a self-limiting shift in the onset of the S phase.

Our most interesting result relates to the magnitude of the magnetic intensities that were studied. The lowest level of field strength that we used is at or below the intensity of the geomagnetic field (approximately 5×10^{-5} T). In surveying the experimental area, it was found (12) that the 60-Hz ambient field is only two orders of magnitude lower. We estimate the threshold for this effect to be $\nu B_{rms} \approx 5$ to 25 μT sec, close to the value reported (~ 10 μT sec) (13) as interfering with normal chick embryo development. Therefore, care should be taken when studying cell culture that there are no perturbations from local 60-Hz magnetic fields, such as those resulting from fans, motors, and fluorescent lights in close proximity to the culture.

Finally, we can also remark on the potentially mutagenic response that may accompany this phenomenon, if indeed chromosome replication is affected. This would constitute a new type of mutational force and perhaps could be used to explain, in a rather direct fashion, the interrupted speciation accompanying geomagnetic reversals (14).

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4 May 1983; accepted 14 December 1983

- Lecture title:

CELL NUCLEUS AND EM FIELDS

- Lecturer's name:

Claudio Nicolini - Chair of Biophysics, School of Medicine, University of Genoa

- Extended summary:

This opening lecture is aimed to present a broad overview of the interaction between electromagnetic waves and living matter, particularly stressing their role in the acquisition of the most recent understainding of the structure and of the molecolar mechanisms controlling the single most important cell compartment - i.e.,the cell nucleus and its constituents .

Few details will be given on the variuos order of chromatin-DNA organization within the intact nucleus, from the primary (base sequence), secondary (A-, B- and Z- forms) up to the tertiary (nucleosome subunits) and higher order structures, underlying for each level the critical role of the physical state of water, ions and enzymatic protein modifications in determinig the overall DNA structure and the cell function (also through the mediation of interlinked changes in the cell cytoskeleton and of the nuclear membrane).

The complexity of such structural and molecolar alterations - correlated with neoplastic transformation and normal cell proliferation - can be exemplified by the study conducted on isolated nuclei with indipendent non-invasive biophysical probes - all using EM fields (NMR spectroscopy and imaging, complex impedence measurements and differential scattering of circularly polarized light).

Comfortingly the abrupt nature of the experimentally observed nuclear transitions - both in functional and structural (phase-like transitions) terms - and their association with localized or global changes in the electromagnetic environment can be finally explained from first physical principles (within the polyelectrolyte theory and the mean field theory).

Ion interactions with DNA: Results based on polyelectrolyte theory

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Low frequency weak electromagnetic fields (LFWEMF) can affect cell behaviour. When applied to quiescent cells (like frog erythrocytes) the induced cellular modifications result in the decondensation of chromatin and in the resumption of RNA and protein synthesis.
The direct action of LFWEMF on chromatin is extremely unlikely. On the other hand, LFWEMF seem to be able to modify the Ca++ concentration at the cell surface.
We propose, as a working hypothesis, that this modification does result, after unknown intermediate steps, in a variation in the concentration of cations inside the cell nucleus, which causes structural changes into DNA-chromatin.
Inside a nucleus under physiological conditions many cations (K+, Na+, Mg++, Ca++, and even trivalent cations)can influence DNA-chromatin structure. Among the many ways by which this influence does occur, electrical interactions play a fundamental role, and a rigorous description of this kind of interactions for different cations is of fundamental importance in view of a quantitative prediction of the modifications experimentally found in intact cell nuclei exposed to different ionic media.
We tackle this subject by developing a mathematical description of tne space distribution of monovalent and divalent cations near DNA, based on polyelectrolyte theory.

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[illegible] WHICH LOW FREQUENCY [illegible] MAGNETIC FIELDS CAN AFFECT THE DISTRIBUTION OF COUNTER-IONS ON CELL SURFACES.

JUNE 19, 1984

By Charles Polk, Department of Electrical Engineering, University of Rhode Island, Kingston, R.I., U.S.A.

In the recent past several reports have been published indicating effects on cell growth and development of low frequency (<5 kHz) magnetic fields. At least one of these (Liboff et al., Science, 233, 818, 1983) points out that the noted effect is not proportional to the product of frequency and amplitude of the magnetic flux density, as would be expected for any mechanism depending linearly on an induced electric current. It is therefore desirable to investigate the various means by which low frequency and relatively low intensity (<0.01 T) magnetic fields can affect ion motion within the biological milieu. As part of such an investigation the motion and distribution of counter-ions on surfaces of cylindrical cells has been examined. Magnetic fields along the cell axis as well as perpendicular to it have been considered and differential equations taking into account finite ion mobility and ion diffusion have been formulated. Solutions of these equations, employing suitable approximations, have been obtained. It is shown that with an axial magnetic field ions are displaced from their equilibrium position and that the associated currents can be above the thermal noise level at products of radian frequency and flux density as low as ten Tesla second for a cell of 3 μm radius. It is also shown that the ion density as a function of position becomes independent of frequency when $\omega \gg \alpha$ where ω is the radian frequency and α is the reciprocal of the diffusive counter-ion relaxation time. These results, as well as analysis of other field configurations, suggest that some parameter specifications that were usually not given in the past are desirable if experimental data from different investigators involving exposure of tissue or cell cultures to magnetic fields are to be compared.

Evidence of a Chemical Potential in the Optical Range of Biological Systems and Its Probable Significance.

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3) Department of Biology, The University, P.O.B.3049, 6750 Kaiserslautern, FRG

Measurements of photon emission from living tissues show at least in the spectral range from λ = 200 to 800 nm a radiation intensity of a few up to some hundred photons (s.cm^2). The spectral distribution, when projected to the occupation probability of the vacuum phase space, exhibits no distinct wavelength dependence. This fact alone provides at the phase boundary of a thermal equilibrium system (as the idealized external world) and an open system with no wavelength dependence of the occupation probability of phase space (as an idealized biological "core") a thermodynamical probability of a form that indicates Bose-condensation-like phenomena. In fact, experimental investigations (photo count statistics, "delayed luminescence", optical absorption, Ethidium Bromide-induced luminescence etc.) and molecular data point to just those mechanisms, based on the exciton (polariton) structure of the DNA. A variety of biological functions can be understood within this framework.

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Behavior of Heme Like Molecules in Electric Field
by
B. Simic-Glavaski

Abstract

Naturally occuring macrocycle molecules such as porphyrius and synthetically synthetized similar phthalocyanines exhibit a wide range of properties which are involved in catalysis and electro-optic phenomena. Heme-like molecules are also strategically located in a variety of living organisms where they perform fundamental functions.

Discussion will be focused on transition metal phthalocyanines and their unusual electro-optical behavior in electric fields. An emphasis will be placed on phthalocyanines a) catalytic properties in oxygen reduction, b) possible application as an optical read-out system of the nerve polarization state and c) use as generators for fast electro-optic signals.

The electrochemical behavior of the adsorbed phthalocyanine monolayer on an electrode interface has been studied simultanously with the cyclic voltammetry and the surface enhanced Raman spectroscopy.

Phthalocyanines undergo a four step electron process. Optical signals reliably follow electrical changes of the adsorbed macrocycle molecules. Similar effects were also observed for the adsorbed phthalocyanines on nerve membranes during their depolarization.

Electro-optical properties of phthalocyanines can be used for generating fast optical signals with switching times on the order of 10^{-13} seconds. In addition phthalocyanines can be used as multi state memory systems which also provide multioutput and multilevel electrically modulated optical signals.

CLONAL AGE AND CELL SPECIFIC RESPONSE TO LOW FREQUENCY ELECTROMAGNETIC FIELDS: J Smith-Sonneborn, Zoology and Physiology Department, University of Wyoming, Laramie, WY.

When Paramecium were cultivated during their clonal life span exposed to current signals designed to couple to non-faradaic electrochemical processes at the cell surface, age-related increase in cell division rate and survival was detected. Different ages responded with increased survival to specific signals. The induced voltage was supplied by air-gap coils driven by a programmable power supply (Electro-Biology, Inc, Fairfield NJ). The basic pulse waveform, the periodicity (single pulse versus pulse burst and repetition rate) were varied. The pulsating electromagnetic fields (PEMF's) have parameters in common with those used to accelerate bone healing in man, to augment the effects of chemotherapy, and to cause increased transcriptional and translational events in the dipteran Sciara in studies of others.

The effect of the specific signals on cell division rate-acceleration was dependent on the use of wild type or specific mutant type cells. In limiting Ca^{+2} concentration, the rate of cell division for the mutant, pawn was increased more than that of the mutant, paranoia. At least one effect of the fields may be to facilitate the transport of ions (Ca^{+2}), and this in conjunction with other events, may cascade to promote increased survival and/or cell division rate. The swimming behavior is also altered.

Six-Octave Experimental System for In-Vivo and In-Vitro Dielectric Measurements

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Dielectric properties of biological substances including tissues are one of many factors influencing interactions between electromagnetic fields and matter, e.g. cells, tissues. These properties have been investigated extensively since the 1950s with the paramount contribution by H.P. Schwan and his colleagues, as well as important studies by others. Current rapid progress in instrumentation and computers has facilitated development of broadband, accurate, automatic and easy to use systems for dielectric spectroscopy.

During the last five years a computer-controlled system for measurements of tissues and cell suspensions has been developed at the University of Ottawa. The system employs a modified automatic network analyzer and a variety of coaxial-line sensors. The frequency range presently covered extends from 10 kHz to 10 GHz. A single sensor is used to measure the dielectric properties over three-octaves in frequency with uncertainties below 3%. Various sensors have been developed for measurements of cell suspensions at controlled temperature and tissues in vivo. A relatively high accuracy of measurements has been achieved through optimization of the sensors design, improvements in the hardware and effective system calibration procedures.

In this presentation the whole system will be described with an emphasis on the lower range of frequencies, from 10 kHz to 100 MHz. These data have not been previously published. As an illustration of the system capabilities recent dielectric data for animal tissues in-vivo, and changes of the properties vs time after death will be presented.

IN VITRO EFFECTS OF ELEVATED TEMPERATURE ON THE CORNEA

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Rabbit corneas were suspended either in phosphate buffered saline or in medium 199 with 10% fetal calf serum and exposed for a period of 30 minutes to elevated temperatures (35.5 as control, 37, 39, 40, 42, 45 and 50°C). Corneas were removed following incubation and fixed in Karnovsky's fixative at 4°C for 24 hr, then transferred to cacodylate buffer prior to critical point drying and examination by scanning electron microscopy. Approximately one hour elapsed between the time the eyes were removed and corneal incubation began. At all temperatures epithelial cell changes appeared to be more pronounced than endothelial cell changes. Epithelial cells changed from flat cells with elevated intercellular junctions and short cylindrical microvilli at 37°C, apparently swelling and losing their microvilli progressively at 39°C, until at 42°C some cells were missing leaving denuded areas and no microvilli were visible; at 45 and 50°C increased damage was seen, including cell curling to look like potato chips, increased stromal denudation and fibrous cell surfaces. Both these changes and endothelial changes (below) were accentuated in medium containing vitamin E. For endothelial cells also, progressive increases in damage were seen as temperature increased: "potato chip" cells, occasional large processes (39°C) cell swelling observing cell boundaries, more large processes (42°C) clumps and cell processes covering cell surfaces (45°C leading to rough fibrous cell surfaces, with many holes, after exposure to 50°C. Stromal changes, leading to stromal disorganization and separation into many fine individual strands at 50°C also occurred with increasing temperature.
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