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BIOLOGICAL EFFECTS OF NON-IONIZING RADIATIONS: CELLULAR PROPERTIES AND INTERACTIONS

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The Lauriston Taylor lectures honor the founder of the National Committee on Radiation Protection and Measurement, soon to be followed by the corresponding international organization. These standard setting bodies had a vast influence on proper recognition of radiation hazards. The 10th Taylor lecture is the first to deal with nonionizing radiations and may be, therefore, of particular interest to the bioengineer. During early history biophysics and bioengineering were primarily concerned with ionizing radiation bioeffects and electrophysiology. The nonionizing part of the radiation field and electrophysiology are closely related. Biomedical observation, biophysical and bioengineering efforts in the nonionizing radiation field are defined and complement each other. Topics concentrate on the relevant biophysical and bioengineering efforts of the author and his colleagues. They include: electrical properties of biological systems; established electrical field interactions (excitation, macromolecular responses and cellular responses); problems of dosimetry (macroscopic and microscopic considerations); conclusions about relative merits of various research approaches.

INTRODUCTION AND SOME HISTORICAL REMARKS

It is a signal honor to have been chosen to present the 10th Lauriston S. Taylor Lecture, and I am grateful to the NCRP for having chosen me. My appreciation is the greater, because I am the first to present a lecture on non-ionizing electromagnetic radiations under the banner of this distinguished man. My introduction to

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Lauriston Taylor came early, while I was still a student. He had already established himself in the ionizing-radiation discipline as an important scientist, and shortly thereafter he was instrumental in creating the organizations that eventuated in the NCRP. His unremitting efforts and concerns have had a marked influence, both nationally and internationally, on standards for radiation safety and on safeguards of the public's health.

My training took place in a biophysical institute that was dedicated primarily to research on ionizing radiations, but my interests lay in electrical and acoustic properties of biological materials. I am delighted, therefore, that these properties are now a significant part of the broad spectrum of interests of the NCRP.

During the early emergence of the discipline of biophysics, there was a preponderance of work in the areas of electrophysiology, electrical properties of cells and tissues, and biophysical properties of ionizing radiations. Later came recognition that the electrical properties of biological materials and the interaction of electromagnetic fields with these materials are interrelated, and that both are of importance to the study of the biological response to radiation. The same held true for the discipline of bioacoustics, which greatly profited from studies of acoustic properties of biological materials and of the interaction of acoustic fields with these materials.

My early studies were undertaken at Goettingen and Frankfurt in the areas of mathematics and physics. Misfortune in my family—my father lost his position as a teacher in 1934 because of his political and liberal convictions—forced interruption of my studies. I spent some time working for electronics firms, such as Siemens, until a former professor of mine found me a job at the Oswalt Foundation Institute for Physical Foundations of Medicine in Frankfurt. This institute had been founded in the early 1920s by Friedrich Dessauer, an early pioneer in the study of ionizing radiations. The Oswalt Foundation was one of the first formally established institutes of biophysics. When I joined the foundation, it was headed by Boris Rajewsky, who shared Dessauer's interests, and who succeeded him when Dessauer left Germany for political reasons.

A small, but important effort among the Oswalt Institute's activities was given to study of electrical properties of cells and to applications of the newly emerging technology of ultrashortwave diathermy. Rajewsky gave me reprints of papers by Hugo Fricke and Kenneth Cole, whose contributions have had a lasting influence on my work. Hugo Fricke made significant contributions to the radiobiology of ionizing radiations. He also investigated electrical properties of cells, which he elevated from a qualitative to a quantitative science. His work on ionizing radiations is well known. His dielectric work is equally renowned, and he and Cole are considered, by experts in the field, as pioneers of a development that was to have a most significant influence on electrophysiology and on the radio-biology of non-ionizing radiations. Hugo Fricke exemplifies to me an outstanding scientist's ability to combine equal interests in properties and interactions that involve seemingly unrelated disciplines. That the disciplines are related in the biological response to non-ionizing radiations is the central theme of my lecture.

Shortly after I emigrated to the United States, I established contacts with Fricke and Cole. I profited much from frequent visits with both men, particularly those with Fricke. It was at this time that I decided to undertake a broad program in the study of properties, mechanisms, interactions, and applications of non-ionizing radiations. The program was to include:

- assessment of electrical properties of biological materials over an extended range of frequencies, including those of VLF and microwave fields not previously explored
- determination of acoustic properties of biological materials at ultrasonic frequencies
- clarification of biophysical principles
- determination of the mode of propagation of radiant energy in biological tissues
- applications in theoretical electrocardiography and in therapy based on ultrasound and on radio-frequency electromagnetic fields
- evaluation of potential hazards of non-ionizing radiations.

Initially, mine was a lonely effort. But the proposed program of research was funded and, in short order, an increasing number of scientists with similar interests joined the effort, first at my university and then at many other institutions.

There are several approaches that characterize the interdisciplinary interplay between the physical and engineering sciences and the biomedical disciplines. I list them as:

- the biophysical approach, which primarily is given to microscopic and submicroscopic observations at the cellular, membrane, and macromolecular levels
- the macroscopic approach of bioengineering, physiology, and experimental psychology, as exemplified in development and application of dosimetry, and in characterization of thermoregulatory responses
- the observational approach of the biomedical and biosocial sciences, as exemplified by case and epidemiological studies.

These approaches to the study of non-ionizing radiations reflect, to some extent, earlier developments in the arena of ionizing radiations. Witness the early mathematical modeling of dose-response relations in concert with statistical theories and the target-hit concept. These developments were followed by the revelation of the molecular nature of radiation insult, the formation of radicals. In parallel, there was the biomedical effort to assemble data on dose-response relations, which led to the still provocative question, *is there a threshold?* Similar questions, not yet formulated, probably will emerge in the study of non-ionizing radiations.

The three approaches complement each other. Experimental observations, of course, are the most important. But without an understanding of basic mechanisms and of pertinent biophysical and physiological principles, the utility of empirical data is limited because in isolation they do not provide the means for extrapolation and generalization. The biophysical approach can provide these means but, unfortunately, has been the least utilized. In contrast, the macroscopic approach of the bioengineers, which largely has been focused on problems of dosimetry, has been very successful and has contributed significantly to the formulation of protective standards. Important, too, is the *in vivo*, intact-organism approach by which the effects of non-ionizing radiations are evaluated at the complex levels of physiology and behavior.

I turn now to a summary of the efforts by which my colleagues and I have attempted to contribute to the biophysics of non-ionizing radiations.

ELECTRICAL PROPERTIES OF BIOLOGICAL SYSTEMS

The bioelectric properties of paramount interest are the dielectric constant (or permittivity) and conductivity. Magnetic properties are not included because magnetic

susceptibilities are extremely small for nearly all biological materials. My summary concentrates also on linear properties, because nonlinearities normally are encountered only at the relatively high field strengths that produce measurable thermalization of biological materials. (An exception to the rule of linearity may lie in membrane processes, such as excitation of neurons; more to this possibility later.)

Figure 1 presents data on electrical properties of muscular tissue as a function of frequency. The figure indicates two unusual features. First, the frequency response includes three distinct inflections or dispersions that are labeled α, β, and γ. They are sufficiently well separated to permit identification of differing underlying mechanisms. Second, the dielectric constant e relative to that of free space reaches enormous values—in excess of one million. These general features are typical for most tissues and for cells in suspension, although the characteristic frequencies of the various dispersions and their magnitudes vary greatly.

Appendix A lists the mechanisms that have been identified. Water, which is abundant in most soft tissues, displays dispersive behavior above 1 GHz and accounts for the γ-effect. The β-effect results from electrical charging of cell membranes via intra- and extra-cellular pathways; this is the Maxwell-Wagner effect, which typically occurs in inhomogeneous materials. The low frequency α-effects are associated with a variety of entirely different processes. These include the relatively slow charging of the internal-cellular membranes that ramify with the cell's outer membrane, the polarization of the counter-ion cloud that surrounds the surface of charged membranes and, perhaps, contributions from the double-layer capacitance beyond the surface of the membrane. The various contributions of the cell's surface to dispersive phenomena have yet to be fully sorted out, which underscores the complexity of the extended surface coat (the glycocaly) and its unknown distribution of fixed charges.

After a lapse of almost two decades, many efforts are underway to improve the

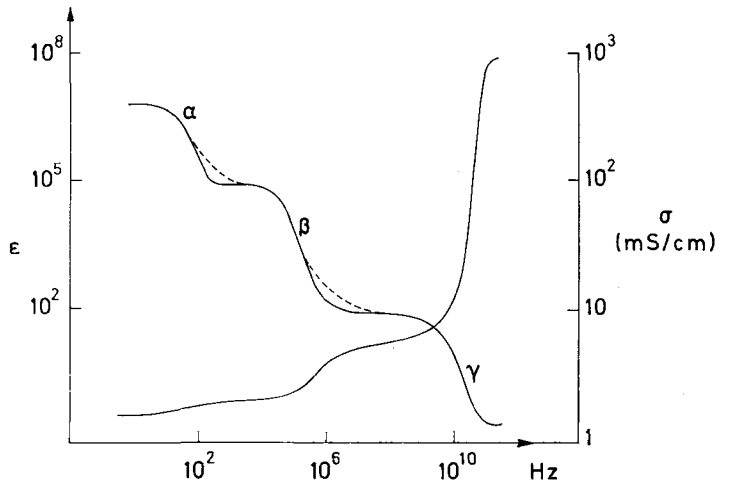

FIGURE 1. The dielectric constant ϵ and the conductivity σ of muscle are shown as a function of frequency of electromagnetic radiation. The three major dispersions, α, β, and γ, are typical for all tissues and cells in suspension, although magnitudes and dispersion frequencies vary. Additional, smaller, relaxation effects contribute to the high frequency-tails of the α- and β- dispersions, as indicated by the dashed curves. Additional information on dispersions is given in Appendix A.

theory of counter-ion relaxation and to understand more fully the electrical properties of the cell's surface. These efforts should be good news to all who believe that the cell's surface is an important site in the interaction of electromagnetic fields with biological materials. Studies based on dielectric and electrophoretic techniques have yielded data that reveal interesting surface properties. They emphasize the importance of these tools in advancing an understanding of the field interactions with cells.

Identification of the mechanisms listed in Appendix A was fairly easy for suspensions of cells and for proteins in solution—at least for the γ- and β-dispersions. In studies of tissue preparations, some difficulties arise because: (a) dielectric-mixture theories fail to give a precise account of highly concentrated populations of cells; (b) the complex geometry of cells *in situ* presents theoretical problems; and (c) the effects of cellular inclusions and connections, such as gap junctions, are difficult to contain, but eventually they must be taken into account. However, the general features of the dielectric frequency response of tissues are similar to those of cells in suspension, which indicates that interactions with the field are probably mediated by the same mechanisms.

Techniques by which to study the electrical properties summarized above include the following: (a) bulk measurements based on many cells in solution, which permit evaluation of their composite dielectric properties; (b) single-cell studies based on bipolar electrodes that contact a given cell's interior and surface (an approach usually limited to evaluation of low-frequency fields because of the high impedance of microelectrodes); and (c) studies of cells during exposure to alternating electric fields. In connection with this last-named technique, it was recognized in recent years that cells can be manipulated by application of electric fields. The response may be movement of cells in an inhomogeneous field or rotation in a rotating field. These field-induced response effects will be discussed below. The dispersive response as a function of frequency has yielded important information about membrane and cytoplasmic properties.

Work is underway to evaluate more fully the relative advantages and potentialities of the various techniques. Bulk measurements now profit from the recent introduction of automated impedance analyzers, which are fast and accurate. Less accurate, but extremely fast, is time-domain spectroscopy. The single-cell technique has profited enormously from the introduction of the Neher-Sackmann patch, a voltage-clamp technique that permits detection and study of single-membrane channels. Finally, the rotating field technique has recently yielded significant advances in the precision of technical measurements and in the rigor of relevant theory. It permits the study of single cells over a much broader range of frequencies than does the microelectrode technique. The scientific community can anticipate significant advances in all these areas.

Considered next are some established field interactions and their equivalent dielectric responses.

ESTABLISHED INTERACTIONS

1. Excitation

The first interaction of interest involves membrane excitation and cellular contractile phenomena. Excitation phenomena in irritable tissues have been of intense scientific interest for the past 200 years, and they continue to attract the attention of many scientists. However, the excitation response is a "strong" one—in the sense that sub-

stantial electric fields near or above the thermogenic level are required to evoke neuronal conduction or muscular contraction.

Excitation of biological membranes is the basis of the nervous system's ability to transmit signals and of a muscle's ability to contract. In both cases, excitation is brought about by electric fields that evoke shifts of membrane potentials on the order of millivolts. Corresponding values of field strengths in tissues, or in the medium surrounding the excited cell, are on the order of 1 V/cm, but they can be larger or smaller, depending on circumstance. Because the thickness of the membrane is only about 100 Å, the induced shift in strength of the field across the excited membrane and its surface structure approximates 10 kV/cm. This means that an external field of 1 V/cm is amplified by a factor of 1000 to 10,000. The amplification factor for a spherical cell is simply given by the ratio of a cell's radius to its membrane thickness. It is this amplification that permits fairly small electric fields in the medium to become so effective in the membrane. However, the amplification process operates only at low frequencies.

The membrane's electrical properties can be nonlinear in the presence of fields at high strengths. The well known Hodgkin-Huxley equations were developed to express nonlinear properties of membranes, and membrane channels have been identified that control the exchange of ions. The operation of individual channels can now be observed with the voltage-patch-clamp technique, which was recently introduced, and much work is underway with this technique to provide a better understanding of channel operation and conformational properties of membrane proteins.

An integral part of the work on irritable tissues has been efforts to determine membrane capacitances and, more important, changes of membrane conductance. Much data are available, including those on nonlinear responses. The internal consistency of these data is fairly good. More data will be obtained by the various techniques summarized above. The data on membranes of the squid's giant axon, which are based on a fast, pseudo-random-signal technique, revealed admittance values essentially as predicted by the linearized version of the Hodgkin-Huxley equations. However, the Hodgkin-Huxley model does not contain properties of the membrane's surface coat, of the potential gradients near the membrane's surface, or of the influence of these gradients on the time course of the action potential as measured by the voltage-clamp technique.

2. Macromolecular Responses

The Debye theory of molecular orientation has had a pronounced influence on the discipline of physical chemistry. In consequence of this theory, which achieved greater sophistication in the works of Onsager, Kirkwood, Fuoss, and many others, much work has been done on the molecular response to time-varying electric fields. Appendix B summarizes some of this work as it relates to dielectric properties of proteins and other biological macromolecules.

Linear dielectric properties are observed at field strengths of interest in this context, and these properties are largely understood. The dielectric response of proteins is consistent with a molecular dipole moment of several hundred Debye units and with the rotation of protein molecules when a time-varying field is applied. Relaxation frequencies are on the order of a few megahertz. Some unresolved questions relate to the relative contributions of induced-dipole effects, such as those associated with counter-ion movement and polar-dipole effects in nucleic acids. A variety of

interesting field effects such as formation of micelles and field-induced changes in conformation of macromolecules has been reported by Schwarz and others.

Less work has been performed on macromolecules at the higher electric-field strengths at which dielectric-saturation effects are anticipated. Dielectric saturation occurs at field strengths near 10 kV/cm. The experimental data for the cases in which significant molecular orientation overcomes thermal motion are in fairly good agreement with the Langevin criterion. The data indicate that significant orientation of macromolecules in solution can only be achieved by very intense fields. I note that the dielectric-saturation response near 10 kV/cm occurs in fields that induce the membrane responses mentioned earlier. This agreement probably is not accidental. At the field strengths of interest (i.e., a few volts per centimeter), the Langevin likelihood factor for preferential orientation of proteins is extremely small, as indicated in Appendix B. I conclude that the macromolecular properties revealed, so far, by dielectric spectroscopy indicate that significant interactions occur only at very high strengths of the electric field. However, more work should be done that addresses the extent to which complex biological reactions at the molecular level may be sensitive to time-varying electric fields.

3. Cellular Responses

Time-varying electric fields can exert demonstrable effects on biological cells at field strengths that are much weaker than those associated with the macromolecular responses so far investigated. In large part, this sensitivity is present because induced-dipole moments are proportional to R^3, where R is the cell's radius. The forces associated with these moments emerge above the thermal threshold at field strengths on the order of one volt per centimeter (Fig. 2). Randomly distributed cells undergo a *phase transition* to form *pearl chains*, and cells may become deformed as indicated in Fig. 3.

Dielectrophoresis is the movement of cells in an inhomogeneous electric field, and it has been used as a means of separating cells. Cells in the field may rotate under certain circumstances or they may fuse. Such responses have led to important new techniques in biotechnology. Earlier theoretical work provided a fair understanding of the physics involved, but a rigorous treatment of the forces acting on cells, and on the rotational behavior, has been achieved only recently.

Appendix C gives equations for the force acting on a spherical cell in a inhomogeneous field, the force between two cells, and the torque acting on a cell in a rotating electric field. Also listed is the optimal frequency of rotation, which is obtained as one measures the speed of rotation as a function of frequency while the strength of the field is held constant. This frequency-dependent rotational response can be shown to be identical to that of the imaginary part of the complex dielectric constant of a dilute suspension of cells. In all equations cited in Appendix C, the ratio u appears, which reflects a transformation of the cellular property ϵ_c^*. But note that the magnitude of the real and imaginary parts of this ratio are involved. Hence, the frequency responses of the three effects are different. The dielectric constant ϵ_m in the medium surrounding the cell may become large at low frequencies with higher concentrations of cells because of the α-dispersion properties of the bulk preparation. This, possibly, may explain the decrease in the threshold of field strength that occurs at low frequencies (Fig. 4).

Investigation of the field effects I have described is presently of interest to a rap-

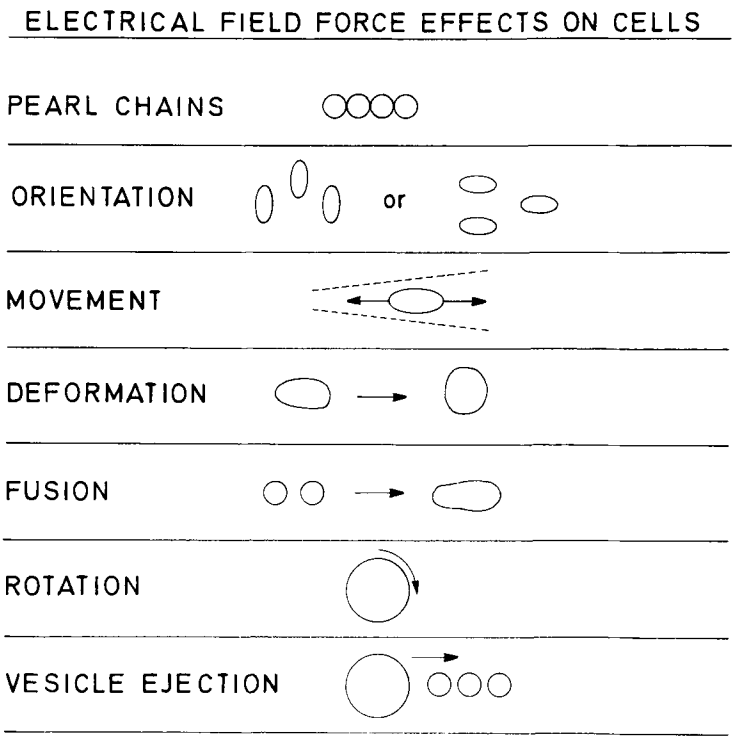

FIGURE 2. Several athermal (field-force) effects can be induced in cells and biologically simulating particles by time-varying electric fields. These effects are summarized pictorially.

idly growing number of scientists, and fairly rapid progress is being made. Thresholds of field strength are on the order of a few volts per centimeter and therefore, they are comparable to those needed at low frequencies to stimulate cells through membrane interaction. However, the lowest possible thresholds of biological significance have not been established.

The athermal mechanisms of interaction I have described are well established. I have excluded from my discussion other, more speculative concepts of athermal interaction because they are largely qualitative in nature and are not easily tested in the laboratory. As for thermal interactions, I note that they are even more firmly established than are the athermal mechanisms, and they are better and more widely understood.

Next I shall consider briefly two important applications of dielectric data to problems of dosimetry.

PROBLEMS OF DOSIMETRY

Two dosimetric problems have been presented to investigators of the biological response to non-ionizing electromagnetic radiations. The first is that of macrodosimetry—of the whole-body-averaged and part-body strengths of fields, and of specific absorption rates (SARs), in human beings and in models of human beings. The second is that of microdosimetry—of the distribution of fields and their strengths in

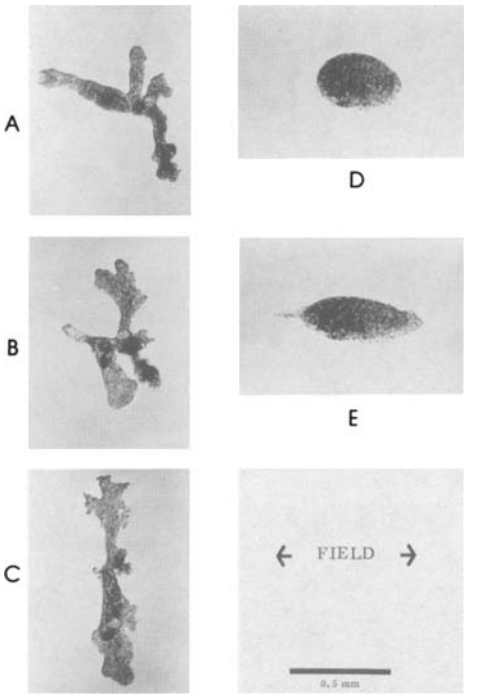

FIGURE 3. Perpendicular and parallel extension of the fresh-water amoeba *Chaos chaos* can be induced by time-varying electric fields. In the sequence a, b, and c, *Chaos* is first shown in the absence of the field, then as partially extended perpendicularly by a 316-Hz field at ~15 V/cm, and finally, as fully extended by the same field. Segment d shows a quiescent amoeba under control conditions, and segment e shows it extended parallel to a 1-MHz field at a strength of 250 V/cm (courtesy of A. W. Friend, Jr., see Ref. 5 for details).

cells and in their constituent parts. The first problem has been studied intensely and with great thoroughness. This study led to a significant revision of the ANSI standard in 1982, and its influence is strongly manifest in the NCRP's recently published volume on non-ionizing radiations. The second problem has barely been recognized but will assume great importance when definite, field-specific interactions at the macromolecular, membrane, and cellular levels have been identified. A prerequisite to the solution of both problems is knowledge of the dielectric properties of tissues, cells, and subcellular organelles.

1. Macroscopic Considerations

Appendix D summarizes important contributions to the dosimetry of non-ionizing radiations. Early efforts by me and my colleagues were given to collection of dielectric data and to their conversion into absorption coefficients or their inverse equivalent (i.e., depth-of-penetration values). Typical examples of penetration depth are shown in Fig. 5; these values are characteristic of tissues of high water content, and two major ranges are displayed. At lower frequencies, the depth of penetration D slowly decreases with increasing frequency. At higher frequencies, a more rapid change in depth of penetration takes place.

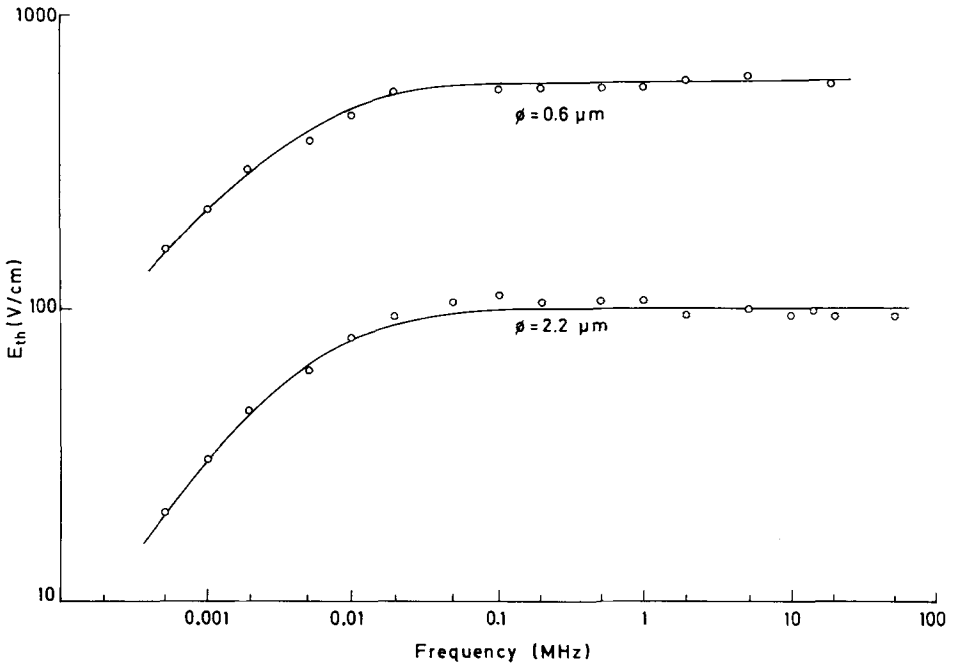

FIGURE 4. Field-strength thresholds of pearl-chain formation are shown as a function of frequency for suspensions of silicon particles of differing diameter, as indicated (see Ref. 5 for more details). The sharply reduced thresholds at lower frequencies are probably associated with a corresponding increase in the bulk dielectric constant of the particulate suspension (α-dispersion). Extrapolation of these data to larger cells may implicate sensitivities at or below the 1 V/cm level.

Reflection phenomena also are important in studies of the mode of propagation of electromagnetic waves in tissue, as demonstrated in Fig. 6.

Finally, SAR-distributions were obtained in tissue configurations, as indicated in Appendix D. (I note parenthetically that the same approach was taken in dosimetric studies of ultrasonic fields.)

The results of efforts to determine effects of non-ionizing radiations on biological systems were pertinent to early work on localized applications of radiant energy, such as clinical treatment by ultrashortwave and microwave diathermy. The mode of propagation in tissues by these higher-frequency fields was found to be strongly frequency dependent, and absorption was shown largely to be at the body's surface at frequencies above a few gigahertz. Reflection of fields from the body's surface and determination of SAR distributions are complex functions of frequency, especially at frequencies in the range between 0.5 and 5 GHz. Some of the *resonance* phenomena observed earlier in my laboratories were found, more recently, to be responsible for the wavelength dependencies of the human being's whole-body-averaged SARs at frequencies above primary resonance for the entire body.

As interest turned from applications of diathermy to concerns for health, efficiency of absorption of non-ionizing radiations during part- and whole-body exposures became of interest to me. Studies of the absorption cross-section were first

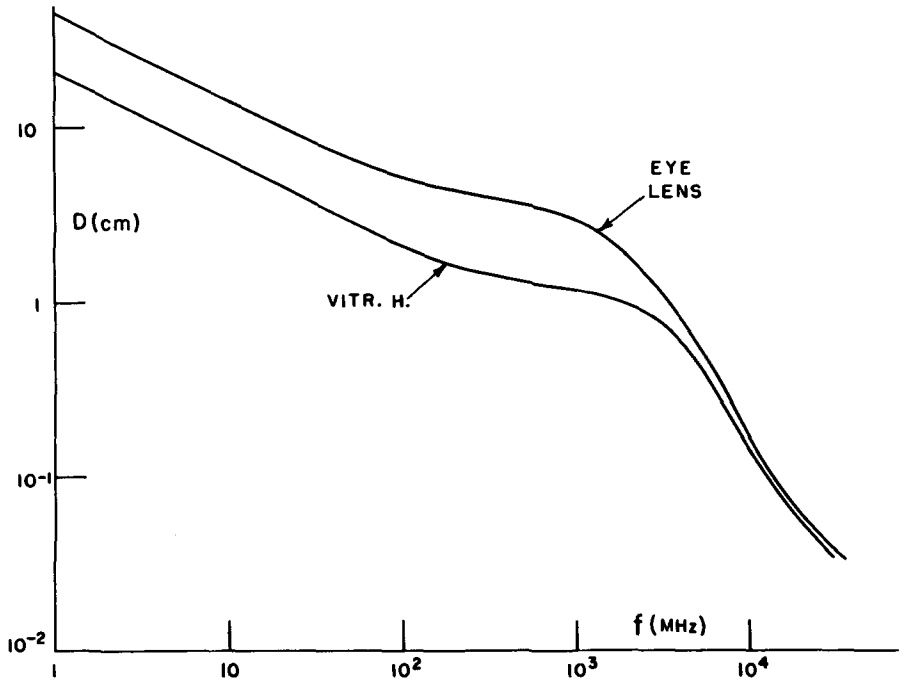

FIGURE 5. Depth of electric-field penetration *D* as a function of frequency is shown for the lens of the eye and for the eye's vitreous humor, the former of which has a much higher protein content. Fields in most tissues of high-water content (e.g., muscle) penetrate to depths that are intermediate between values displayed by the two curves. *D* is defined as the distance needed to reduce the flux of radiant energy by *e*.

carried out experimentally and theoretically on spherical models of man and on complete-body mannequins, both of which were filled with solutions that stimulated conductivities and dielectric properties of human tissues. An example of early work that was performed in my laboratories is given in Fig. 7. This work was followed by studies of cylindrical models and of live, unrestrained animals under conditions in which dosing with microwave energy was both controlled and determinate; these pioneering studies, which laid the empirical grounds and rationale for exposure standards later recommended by the ANSI, the NCRP, and the EPA, were performed by Justesen and King. Their work was followed in turn by work of ever-increasing sophistication in theoretical insight and elegant experimentation, first by Guy and his co-workers, then by Gandhi, Johnson, Durney, and their colleagues. Other scientists contributed experimentally and analytically, which has led to the current advanced state of the dosimetry of non-ionizing radiations.

Early on in my laboratories, my colleagues and I recognized that *electrical hot spots* may be induced by fields under certain conditions. The underlying mechanisms include quasi-optical focusing and part-body resonance, as demonstrated by Kritikos et al. Simple thermal models were next used in my laboratory to estimate elevations of temperature associated with the electrical hot spots. Subsequently, modern, com-

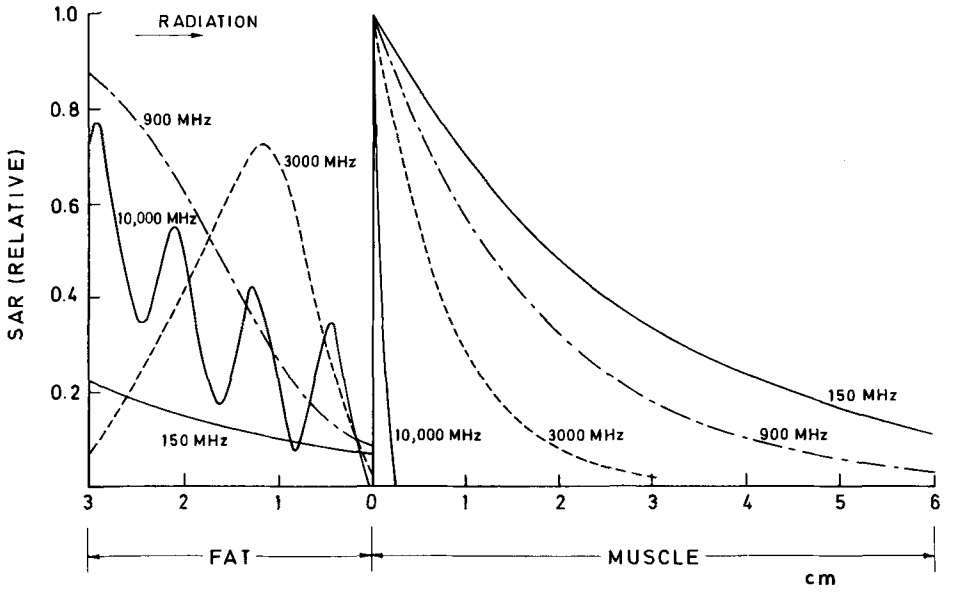

FIGURE 6. Relative distribution of SARs in a fat-muscle model exposed to plane-wave electromagnetic radiations. The arrow indicates the direction of flow of the radiant energy. The complexity of the SAR distribution results from standing-wave patterns that are produced by reflections at the fat-muscle interface.

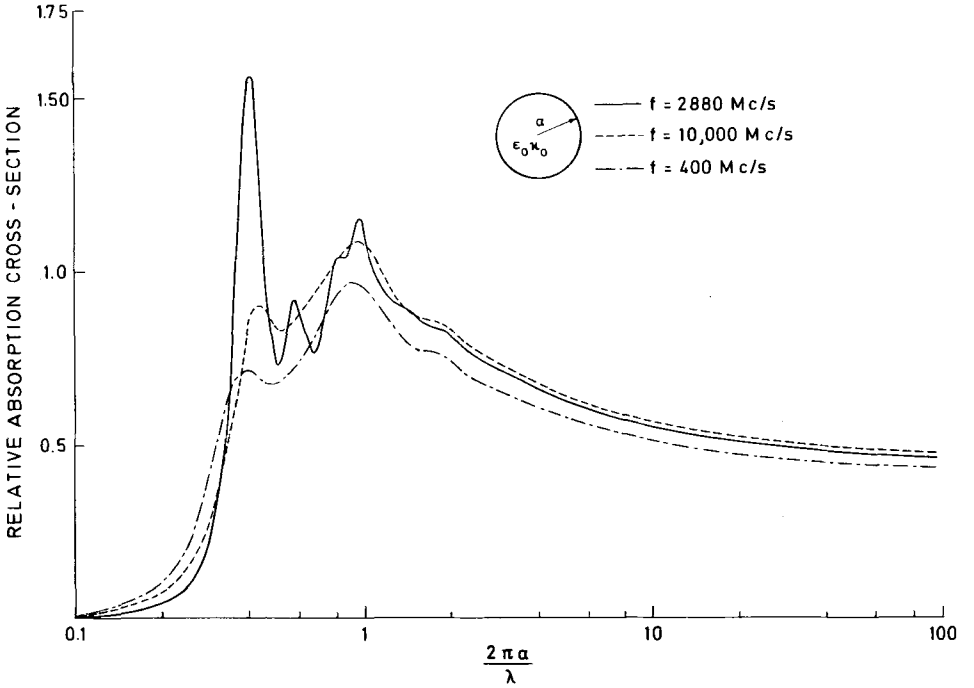

FIGURE 7. Relative absorption cross-section of a tissue-simulating sphere as a function of the ratio of radius *a* and free-space wavelength λ.

partmental thermoregulatory models were introduced to emulate the human thermal response to non-ionizing radiations. More recently, primates as models of the human thermal and thermoregulatory responses to microwave radiation have been used most effectively by Adair and her colleagues.

It is fair to conclude that substantial progress has been made in all areas pertinent to the macrodosimetry of non-ionizing radiations.

2. Microscopic Considerations

Knowledge of field strengths and of frequency dependencies at the microscopic level—in cellular membranes, in cytoplasmic membranes, and in the interior of organelles, such as the mitochondria and the cell nucleus—is needed to apply any fully, predictive theory of electrical field interactions with biological materials. Do fields interact primarily with cell membranes? with the cell membrane's surface? with the genetic apparatus in the nucleus? with the cytoskeleton? or with what else? Or, to be more precise, at what frequencies or pulse durations may one expect fields to be optimal in any one of these cellular constituents?

Two examples illustrate the insight gained into locus of interaction; both arose from analysis of the mechanism responsible for the β-dispersion. The analytical approach to this dispersion was that of solving the Laplace potential equation for a spherical or an elliptical cell the cytoplasm of which is surrounded by a poorly conducting membrane. The solution and the use of an appropriate mixture theory yielded the effective, dielectric constant and conductivity of a suspension of cells. This treatment resulted in the recognition that the β-dispersion of cells can be modeled simply, but precisely as a series of membrane capacities in combination with resistive values that characterize the internal and external fluid compartments. The emerging theory has been applied successfully to many cells by many investigators, and it has withstood the test of time. It is now an integral part of the knowledge of the cell's electrical properties as the cell is exposed to a time-varying electric field.

The Laplacian treatment of the cell also provides the induced potential and field strength on the membrane, and the field strength in the cytoplasm. The membrane's field strength has two components, radial and tangential, as noted in the following equations:

$$E(\text{rad}) = 1.5\ E\ R/d \cos\delta$$

$$E(\text{tan}) = 1.5\ E \sin\delta$$

The lateral-field component is therefore much smaller than that of the radial field. Nevertheless, modest external fields of a few volts per centimeter have been demonstrated to produce lateral movement of membrane proteins. Figure 8 exemplifies the frequency dependence of the membrane potential and the strength of the internal field. At low frequencies, the membrane potential reaches its optimal value, but the interior of the cell is effectively shielded. At high frequencies, the cell's interior is fully exposed. I conclude that membrane interactions can be more readily anticipated at low frequencies, and that interactions with the cell's interior are more probable at high frequencies. It follows that strong interactions of microwave fields with membranes are unlikely. If such interactions exist, they must be much more subtle than the excitation phenomena observed at low frequencies, because they do not benefit from the spatial-amplification process mentioned earlier.

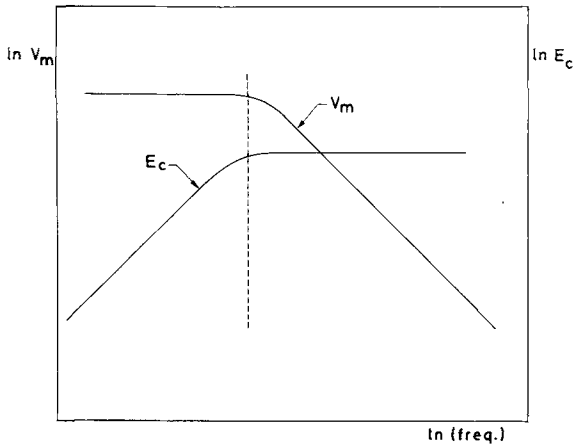

FIGURE 8. Frequency dependence of the cell-membrane potential, V_m, and the cytoplasmic field strength, E_c. A frequency-independent field is assumed outside the cell.

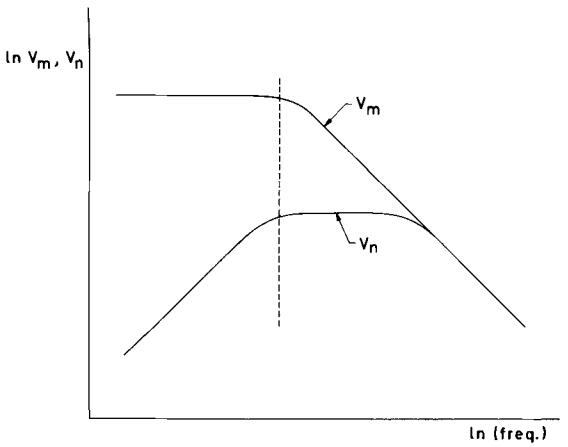

FIGURE 9. Frequency dependence of the cell-membrane potential, V_m, and of the nuclear-membrane potential, V_n. A frequency-independent field is assumed outside the cell. The V_n values may be higher or lower than the V_m values at high frequencies, depending on electrical conductivities inside and outside the cell.

Figure 9 presents comparative information on the frequency dependence of the nuclear membrane's potential and that of the cell membrane. Significant nuclear-membrane potentials can be generated only over a limited range of frequencies. Thus, dielectric breakthrough, a prerequisite for the fusion of cell nuclei in a large fused cell, can be accomplished only within a narrow range of frequencies or of pulse durations, findings that may well be of importance to biotechnology. In Fig. 9, the curves for potentials of the nuclear and cell membranes are seen to merge at high frequencies. This merging holds only if resistivities inside the nucleus, in the cytoplasm, and outside the cell, are equal. Resistivities will differ in most cases, and the curve of the

nuclear-membrane potential may rise above or fall below the curve for the cell membrane's potential at high frequencies, depending on circumstance. That interactions of the field with the mitotic apparatus inside the nucleus are possible only at higher frequencies is an observation of potentially great importance.

The data presented in Figs. 8 and 9 illustrate the importance of microdosimetry. Because relatively little effort has been spent so far in this domain, its promise for enhancing an understanding of mechanisms is justification for much additional study.

SOME CONCLUSIONS

I have chosen excitation, macro- and micro-dosimetry, and the athermal, field-force effects as examples that demonstrate the dependence of the biological response to non-ionizing radiations on dielectric properties. Only linear and dispersive properties need be discussed in this context. This does not rule out the existence of resonant interactions. Indeed, membrane-admittance behavior, at low frequencies, does display indications of resonance, as indicated earlier. Quite recently, dielectric data at gigahertz frequencies have been published by Edwards, Davis, and Swicord, and their data are indicative of resonances, which are unexpected because water, so effectively, dampens resonance in this range of frequencies. In addition, sharp resonances in the growth of yeast cells exposed to millimeter waves have been extensively reported, but these findings still await independent confirmation.

It has been stated that biologically significant field interactions may occur that are not reflected in observable changes in dielectric properties. This could be true in all cases in which an interaction involves only a small fraction of a sample preparation so that it does not measurably affect the dielectric response of the sample as a whole. However, this part-whole problem of measurement does not pertain to the effects and dielectric data noted above. To the contrary, methods of measurement, based on modern techniques, are now sufficiently refined to detect single-membrane channel operation and single-cell responses to high-frequency fields. In addition, sensitivity to conformational properties of macromolecules has been demonstrated by dielectric techniques. Given these technical advances, I believe that dielectric techniques will continue to be important in the future.

I mentioned earlier in this lecture that approaches to the study of non-ionizing radiations can be conveniently subdivided into several categories, at least so far as electromagnetic fields are concerned. Macroscopic aspects such as details of the field configuration within or as incident on the human subject have been treated largely by engineers that are knowledgeable about pertinent field theories. I called this the bioengineering approach. The work at the cellular and macromolecular levels has been primarily of interest to physicists, biophysicists and physical chemists. I called this the biophysical approach. Finally, I mentioned the biomedical observational approach, which is based on exposure data from living subjects. I have discussed activities in selected areas of bioengineering and biophysics, but have neglected biomedical endeavors. One reason for doing so is that this selection reflects my own efforts and knowledge. The other reason is that much emphasis in biomedical endeavors is placed on the purely observational approach. In the final analysis, medical and biological data are decisive. But I contend that valid interpretation and extrapolation of data—for example, from lower animals to man—are highly dependent on physiological, bioengineering, and biophysical principles.

By far, most reports published these days are based on studies of clinical end points and how these end points respond to a given field. A large amount of work has been done on macroscopic dosimetry, which has had a profound impact on recently promulgated standards of safety. Closely related and also of a more macroscopic orientation are the reports performed in physiological and behavioral laboratories. Here, the complex system of man, or that of the experimental animal under test, is studied as the intact subject reacts to radiation. As an example of the close relation between thermoregulatory physiology and dosimetry, I noted the modeling approach that combines thermoregulatory compartmental models with SAR distributions.

In contrast to the studies with a macroscopic orientation, the biophysical effort is small. One reason may be that biophysical data for the greater part have not yielded evidence of weak and subtle interactions. Perhaps this difference arises from a greater sensitivity of the integrated nervous and endocrine systems of the intact animal. On the other hand, the noise-to-signal ratio—the probability of a spurious datum—may be higher at the purely observational level at which experimental control is frequently lacking. Fortunately, activity at the biophysical level is on the rise, although often in contexts unrelated to the non-ionizing radiations.

APPENDIX A

Dielectric Characteristics of Cells and Tissues

The dielectric response of the cell to time-varying electric fields is characterized by several dispersions. Responses to extremely-low-frequency (ELF), medium radio-frequency (RF), and higher radio-frequency microwave (MW) fields are labeled α, β and γ. The γ response is that of water dispersion, β represents the charging response of the cell's external membrane. β_1 is a weaker protein response, and β_2 is the response of membranes of intracellular organelles. The α_1 and α_2 responses are respectively contributed by membrane systems that ramify with the cell's outer membrane, and by counter-ion displacement near the fixed-surface charges of the membrane's glycocalix. The weaker δ-response at ultra-high frequencies (UHF) is associated with protein-bound water and with partial rotation of submacromolecules.

Tisssue and Cellular Constituents	Dispersions Participating in Dielectric Response
Electrolytes	γ
Macromolecules	
Amino Acids	$\delta + \gamma$
Proteins	$\beta_1 + \delta - \gamma$
Cells	
Uncharged, no Protein	$\beta + \delta$
With Proteins	$\beta + \gamma$
No Surface Charge	$\beta + \beta_1 + \delta + \gamma$
Surface Charge Only	$\alpha_2 + \beta + \beta_1 + \delta + \gamma$
Subcellular Organelles Only	$\beta + \beta_1 + \beta_2 + \delta + \gamma$
Connecting Membranes Only	$\alpha_1 + \beta + \beta_1 + \delta + \gamma$

APPENDIX B

Linear and Non-Linear Responses to Non-Ionizing Radiations

Linear dielectric properties of macromolecules, such as proteins, have been well investigated. Significant orientation effects are expected when the Langevin function L is greater than unity. These effects require field strengths in the kV/cm range. Fields at comparable strengths are required for all phenomena based on induced dipoles. At kV/cm strengths, fields are readily induced in biological membranes by V/cm fields in the medium external to the cell.

Bipolymer Response

Linear Dielectric Properties
  Well Investigated by Arrhenius, Onley, Takashima, Schwarz and others
"Significant" Nonlinear Responses
  Few Confirmed Data
  Langevin Function Criterion

$$L(\mu E/kT) = \coth(\mu E/kT) - kT/\mu E \rightarrow \mu E/3kT$$

Examples are:

$$2 \text{ V/cm and } 400 \text{ D (protein)} \rightarrow L = 2 \cdot 10^{-5}$$

$$100 \text{ kV/cm and ind. moment of } 20 \text{ Å} \rightarrow L \sim 1$$

APPENDIX C

Equations for Non-Linear (Athermal) Responses to Time-Varying Fields

In the equations shown, F is the force acting on a charged particle exposed to an inhomogeneous electric field, E_{th} is the field-strength threshold for pearl-chain formation, and L is the torque acting on a spherical particle in a rotating electric field. In the β-dispersion range, L peaks at frequency f_0, which is given in Eq. 4. Equations 1, 2, and 3 are derived from equations in Refs. 11 and 12, and the derivation of Eq. 4 is provided in Refs. 1 and 10. The radius of a particle is R, p is a particle's volume concentration, and the asterisk designates the complex dielectric constant of which ϵ'_m is the real part of ϵ^*_m. Field strength is designated by E, C_m is membrane capacitance per cm^2, and ρ_i and ρ_m are resistivities inside and outside the cell. Subscripts c and m denote a cellular particle and its surrounding medium.

$$F = \pi \epsilon'_m R^3 \operatorname{Re}(u) \nabla |E|^2 \tag{C1}$$

$$E^2_{\text{th}} \epsilon'_m R^3 |u|^2 = kT/6p \tag{C2}$$

$$L = 4\pi \epsilon'_m R^3 E^2 Im(u) \tag{C3}$$

with

$$u = (\epsilon_c^* - \epsilon_m^*)/(\epsilon_c^* + 2\epsilon_m^*)$$

$$1/f_0 = 2\pi RC_m(\rho_1 + 0.5\rho_m) \tag{C4}$$

APPENDIX D

Contributions of Bioengineering to Non-Ionizing Macrodosimetry

Topics are listed that have been of interest in the development of macrodosimetry. The topics reflect concerns of bioengineers and other biological investigators for modes of propagation of electromagnetic energy in the human body and that of animals, in electrical hotspots, and in resultant elevations of temperature, both part- and whole-body. Data that have emerged in response to these concerns have figured prominently in the development of exposure standards for non-ionizing electromagnetic fields.

1. Determination of depth of penetration by radiant fields in static and living models
2. Assessment of reflections of radiant energy from air-to-tissue and tissue-tissue interfaces
3. Determination of absorption cross sections of the human body
4. Measurement of energy absorption by skin, fat, and muscle and by whole-body models via analytical and calormetric techniques
5. Analytical and experimental assessment of electromagnetic and thermal "hot-spots"
6. Measurement of temperature elevations, part- and whole-body
7. Studies of autonomic and behavioral thermoregulation in irradiated subjects, human and infrahuman
8. Determination of SAR thresholds of behavioral impairment
9. Interpretation of the analytical and experimental data in development of exposure standards for general and occupational populations

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