



HANDBOOK OF BIOLOGICAL EFFECTS OF  
ELECTROMAGNETIC FIELDS

THIRD EDITION

# Biological and Medical Aspects of Electromagnetic Fields

EDITED BY

Frank S. Barnes  
Ben Greenebaum



Taylor & Francis  
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## *Preface*

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We are honored to have been asked to carry on the tradition established by Dr. Postow and the late Dr. Polk in the first two editions of the *Handbook of Biological Effects of Electromagnetic Fields*. Their editions of this handbook were each recognized as the authoritative standards of their time for scientists working in bioelectromagnetics, the science of electromagnetic field effects on biological systems, and for others seeking information about this field of research.

In revising and updating this edition of the *Handbook of Biological Effects of Electromagnetic Fields*, we have expanded the coverage to include more material on diagnostic and therapeutic applications. At the same time, in updating and expanding the previous editions' coverage of the basic science and studies related to the possible biological effects of the electromagnetic fields, we have added new material on the related physics and chemistry as well as reviews of the recent developments in the setting standards for exposure limits. Following the previous edition's lead, we have charged the authors of the individual chapters with providing the reader, whom we imagine is fairly well founded in one or more of the sciences underlying bioelectromagnetics but perhaps not in the others or in the interdisciplinary subject of bioelectromagnetics itself, with both an introduction to their topic and a basis for further reading. We asked the chapter authors to write what they would like to be the first thing they would ask a new graduate student in their laboratory to read. We hope that this edition, like its two predecessors, will be useful to many as a reference book and to others as a text for a graduate course that introduces bioelectromagnetics or some of its aspects.

As a "handbook" and not an encyclopedia, this work does not intend to cover all aspects of bioelectromagnetics. Nevertheless, taking into account the breadth of topics and growth of research in this field since the last edition, we have expanded the number of topics and the number of chapters. Unavoidably, some ideas are duplicated in chapters, sometimes from different viewpoints that could be instructive to the reader; and different aspects of others are presented in different chapters. The increased amount of material has led to the publication of the handbook as two separate, but inter-related volumes: *Biological and Medical Aspects of Electromagnetic Fields (BMA)* and *Bioengineering and Biophysical Aspects of Electromagnetic Fields (BBA)*. Because there is no sharp dividing line, some topics are dealt with in parts of both volumes. The reader should be particularly aware that various theoretical models, which are proposed for explaining how fields interact with biological systems at a biophysical level, are distributed among a number of chapters. No one model has become widely accepted, and it is quite possible that more than one will in fact be needed to explain all observed phenomena. Most of these discussions are in the *Biological and Medical* volume, but the *Bioengineering and Biophysics* volume's chapters on electroporation and on mechanisms and therapeutic applications, for example, also have relevant material. Similarly, the chapters on biological effects of static magnetic fields and on endogenous electric fields in animals could equally well have been in the *Biological and Medical* volume. We have tried to use the index and cross-references in the chapters to direct the reader to the most relevant linkages, and we apologize for those we have missed.

Research in bioelectromagnetics stems from three sources, all of which are important; and various chapters treat both basic physical science and engineering aspects and the

biological and medical aspects of these three. Bioelectromagnetics first emerged as a separate scientific subject because of interest in studying possible hazards from exposure to electromagnetic fields and setting exposure limits. A second interest is in the beneficial use of fields to advance health, both in diagnostics and in treatment, an interest that is as old as the discovery of electricity itself. Finally, the interactions between electromagnetic fields and biological systems raise some fundamental, unanswered scientific questions and may also lead to fields being used as tools to probe basic biology and biophysics. Answering basic bioelectromagnetic questions will not only lead to answers about potential electromagnetic hazards and to better beneficial applications, but they should also contribute significantly to our basic understanding of biological processes. Both strong fields and those on the order of the fields generated within biological systems may become tools to perturb the systems, either for experiments seeking to understand how the systems operate or simply to change the systems, such as by injecting a plasmid containing genes whose effects are to be investigated. These three threads are intertwined throughout bioelectromagnetics. Although any specific chapter in this work will emphasize one or another of these threads, the reader should be aware that each aspect of the research is relevant to a greater or lesser extent to all three.

The reader should note that the chapter authors have a wide variety of interests and backgrounds and have concentrated their work in areas ranging from safety standards and possible health effects of low-level fields to therapy through biology and medicine to the fundamental physics and chemistry underlying the biology. It is therefore not surprising that they have different and sometimes conflicting points of view on the significance of various results and their potential applications. Thus authors should only be held responsible for the viewpoints expressed in their chapters and not in others. We have tried to select the authors and topics so as to cover the scientific results to date that are likely to serve as a starting point for future work that will lead to the further development of the field. Each chapter's extensive reference section should be helpful for those needing to obtain a more extensive background than is possible from a book of this type.

Some of the material, as well as various authors' viewpoints, are controversial, and their importance is likely to change as the field develops and our understanding of the underlying science improves. We hope that this volume will serve as a starting point for both students and practitioners to come up-to-date with the state of understanding of the various parts of the field as of late 2004 or mid-2005, when authors contributing to this volume finished their literature reviews.

The editors would like to express their appreciation to all the authors for the extensive time and effort they have put into preparing this edition, and it is our wish that it will prove to be of value to the readers and lead to advancing our understanding of this challenging field.

**Frank S. Barnes**  
**Ben Greenebaum**

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## *Editors*

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**Frank Barnes** received his B.S. in electrical engineering in 1954 from Princeton University and his M.S., engineering, and Ph.D. degrees from Stanford University in 1955, 1956, and 1958, respectively. He was a Fulbright scholar in Baghdad, Iraq, in 1958 and joined the University of Colorado in 1959, where he is currently a distinguished professor. He has served as chairman of the Department of Electrical Engineering, acting dean of the College of Engineering, and in 1971 as cofounder/director with Professor George Codding of the Political Science Department of the Interdisciplinary Telecommunications Program (ITP).

He has served as chair of the IEEE Electron Device Society, president of the Electrical Engineering Department Heads Association, vice president of IEEE for Publications, editor of the *IEEE Student Journal* and the *IEEE Transactions on Education*, as well as president of the Bioelectromagnetics Society and U.S. Chair of Commission K—International Union of Radio Science (URSI). He is a fellow of the AAAS, IEEE, International Engineering Consortium, and a member of the National Academy of Engineering.

Dr. Barnes has been awarded the Curtis McGraw Research Award from ASEE, the Leon Montgomery Award from the International Communications Association, the 2003 IEEE Education Society Achievement Award, Distinguished Lecturer for IEEE Electron Device Society, the 2002 ECE Distinguished Educator Award from ASEE, The Colorado Institute of Technology Catalyst Award 2004, and the Bernard M. Gordon Prize from National Academy of Engineering for Innovations in Engineering Education 2004. He was born in Pasadena, CA, in 1932 and attended numerous elementary schools throughout the country. He and his wife, Gay, have two children and two grandchildren.

**Ben Greenebaum** retired as professor of physics at the University of Wisconsin–Parkside, Kenosha, WI, in May 2001, but was appointed as emeritus professor and adjunct professor to continue research, journal editing, and university outreach projects. He received his Ph.D. in physics from Harvard University in 1965. He joined the faculty of UW–Parkside as assistant professor in 1970 following postdoctoral positions at Harvard and Princeton Universities. He was promoted to associate professor in 1972 and to professor in 1980. Greenebaum is author or coauthor of more than 50 scientific papers. Since 1992, he has been editor in chief of *Bioelectromagnetics*, an international peer-reviewed scientific journal and the most cited specialized journal in this field. He spent 1997–1998 as consultant in the World Health Organization's International EMF Project in Geneva, Switzerland. Between 1971 and 2000, he was part of an interdisciplinary research team investigating the biological effects of electromagnetic fields on biological cell cultures. From his graduate student days through 1975, his research studied the spins and moments of radioactive nuclei. In 1977 he became a special assistant to the chancellor and in 1978, associate dean of faculty (equivalent to the present associate vice chancellor position). He served 2 years as acting vice chancellor (1984–1985 and 1986–1987). In 1989, he was appointed as dean of the School of Science and Technology, serving until the school was abolished in 1996.

On the personal side, he was born in Chicago and has lived in Racine, WI, since 1970. Married since 1965, he and his wife have three adult sons.





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# Introduction

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Charles Polk\*

*Revised for the 3rd Edition by Ben Greenebaum*

Much has been learned since this handbook's first edition, but a full understanding of biological effects of electromagnetic fields has to be achieved. The broad range of what must be studied has to be a factor in the apparent slow progress toward this ultimate end. The broad range of disciplines involved includes basic biology, medical science and clinical practice, biological and electrical engineering, basic chemistry and biochemistry, and fundamental physics and biophysics. The subject matter ranges over characteristic lengths and timescales from, at one extreme, direct current (dc) or  $\sim 10^4$  km-wavelengths, multimillisecond ac fields and large, long-lived organisms to, at the other extreme, submillimeter wavelength fields with periods below  $10^{-12}$  s and subcellular structures and molecules with subnanometer dimensions and characteristic times as short as the  $10^{-15}$  s or less of biochemical reactions.

This chapter provides an introduction and overview of the research and the contents of this handbook.

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## 0.1 Near Fields and Radiation Fields

In recent years it has become, unfortunately, a fairly common practice—particularly in nontechnical literature—to refer to the entire subject of interaction of electric ( $E$ ) and magnetic ( $H$ ) fields with organic matter as biological effects of nonionizing radiation, although fields that do not vary with time and, for most practical purposes, slowly time-varying fields do not involve radiation at all. The terminology had its origin in an effort to differentiate between relatively low-energy microwave radiation and high-energy radiation, such as UV and x-rays, capable of imparting enough energy to a molecule or an atom to disrupt its structure by removing one or more electrons with a single photon. However, when applied to dc or extremely low-frequency (ELF), the term “nonionizing radiation” is inappropriate and misleading.

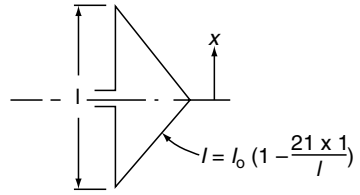
A structure is capable of efficiently radiating electromagnetic waves only when its dimensions are significant in comparison with the wavelength  $\lambda$ . But in free space  $\lambda = c/f$ , where  $c$  is the velocity of light in vacuum ( $3 \times 10^8$  m/s) and  $f$  is the frequency in hertz (cycles/s); therefore the wavelength at the power distribution frequency of 60 Hz, e.g., is 5000 km, guaranteeing that most available human-made structures are much smaller than one wavelength.

The poor radiation efficiency of electrically small structures (i.e., structures whose largest linear dimension  $L \ll \lambda$ ) can be illustrated easily for linear antennas. In free space the radiation resistance,  $R_r$  of a current element, i.e., an electrically short wire of length  $\ell$  carrying uniform current along its length [1], is

$$R_r = 80\pi^2 \left(\frac{\ell}{\lambda}\right)^2 \quad (0.1)$$

---

\*Deceased.



**FIGURE 0.1**  
Current distribution on short, thin, center-fed antenna.

whereas the  $R_r$  of an actual center-fed radiator of total length  $\ell$  with current going to zero at its ends, as illustrated in Figure 0.1, is

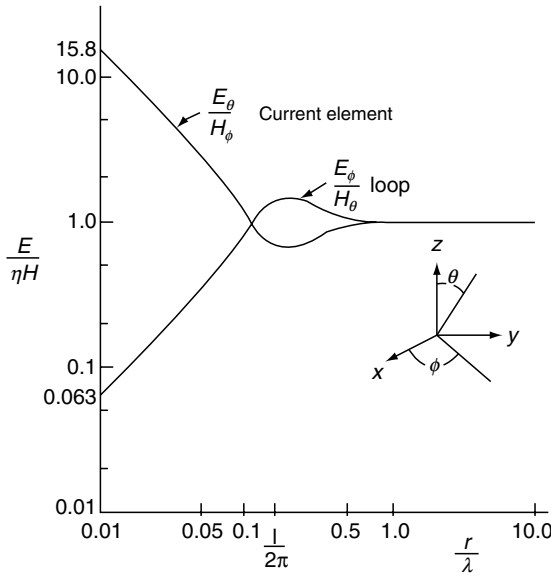
$$R_r = 20\pi^2 \left( \frac{\ell}{\lambda} \right)^2 \quad (0.2)$$

Thus, the  $R_r$  of a  $0.01 \lambda$  antenna, 50 km long at 60 Hz, would be  $0.0197 \Omega$ . As the radiated power  $P_r = I^2 R_r$  where  $I$  is the antenna terminal current, whereas the power dissipated as heat in the antenna wire is  $I^2 R_d$ ; when  $I$  is uniform, the  $P_r$  will be very much less than the power used to heat the antenna, given that the ohmic resistance  $R_d$  of any practical wire at room temperature will be very much larger and  $R_r$ . For example, the resistance of a 50-km long, 1/2-in. diameter solid copper wire could be  $6.65 \Omega$ . At dc, of course, no radiation of any sort takes place, as acceleration of charges is a condition for radiation of electromagnetic waves.

The second set of circumstances, which guarantees that any object subjected to low-frequency  $E$  and  $H$  fields usually does not experience effects of radiation, is that any configuration that carries electric currents sets up  $E$  and  $H$  field components which store energy without contributing to radiation. A short, linear antenna in free space (short electric dipole) generates, in addition to the radiation field  $E_r$ , an electrostatic field  $E_s$  and an induction field  $E_i$ . Neither  $E_s$  nor  $E_i$  contribute to the  $P_r$  [2,3]. Whereas  $E_r$  varies as  $1/r$ , where  $r$  is the distance from the antenna,  $E_i$  varies as  $1/r^2$ , and  $E_s$  as  $1/r^3$ . At a distance from the antenna of approximately one sixth of the wavelength ( $r = \lambda/2\pi$ ), the  $E_i$  equals the  $E_r$ , and when  $r \ll \lambda/6$  the  $E_r$  quickly becomes negligible in comparison with  $E_i$  and  $E_s$ . Similar results are obtained for other antenna configurations [4]. At 60 Hz the distance  $\lambda/2\pi$  corresponds to about 800 km and objects at distances of a few kilometers or less from a 60-Hz system are exposed to nonradiating field components, which are orders of magnitude larger than the part of the field that contributes to radiation.

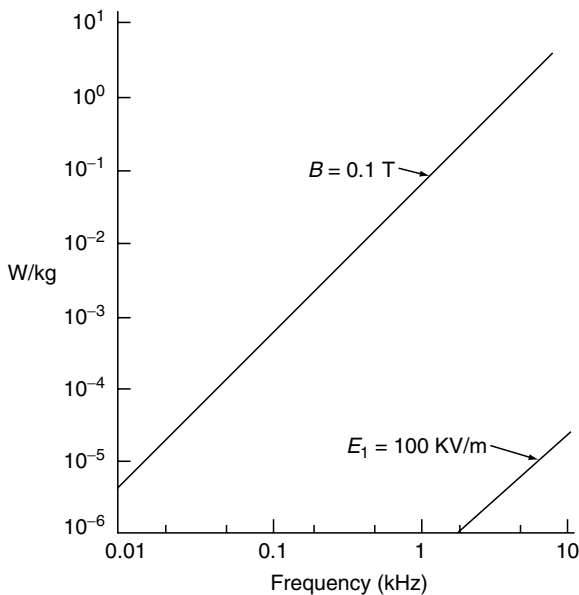
A living organism exposed to a static (dc) field or to a nonradiating near field may extract energy from it, but the quantitative description of the mechanism by which this extraction takes place is very different than at higher frequencies, where energy is transferred by radiation:

1. In the near field the relative magnitudes of  $E$  and  $H$  are a function of the current or charge configuration and the distance from the electric system. The  $E$  field may be much larger than the  $H$  field or vice versa (see Figure 0.2).
2. In the radiation field the ratio the  $E$  to  $H$  is fixed and equal to 377 in free space, if  $E$  is given in volt per meter and  $H$  in ampere per meter.
3. In the vicinity of most presently available human-made devices or systems carrying static electric charges, dc, or low-frequency ( $<1000$  Hz) currents, the  $E$  and  $H$  fields will only under very exceptional circumstances be large enough to produce heating effects inside a living object, as illustrated by Figure 0.3. (This statement assumes that the living object does not form part of a conducting path



**FIGURE 0.2** Ratio of  $E$  to  $H$  field (divided by wave impedance of free space  $\eta = 377 \Omega$ ) at  $\theta = 90^\circ$  for electric current element at origin along  $z$ -axis and for electrically small loop centered at the origin in  $x$ - $y$  plane.

that permits direct entrance of current from a wire or conducting ground.) However, nonthermal effects are possible; thus an  $E$  field of sufficient magnitude may orient dipoles, or translate ions or polarizable neutral particles (see Chapter 3 and Chapter 4 in *BBA\**).



**FIGURE 0.3** *Top line:* Eddy current loss produced in cylinder by sinusoidally time-varying axial  $H$  field. Cylinder parameters are conductivity  $\sigma = 0.1 \text{ S/m}$ , radius  $0.1 \text{ m}$ , density  $D = 1100 \text{ kg/m}^3$ , RMS magnetic flux density  $0.1 \text{ T} = 1000 \text{ G}$ . Watt per kilogram =  $\sigma B^2 r^2 w^2 / 8D$ ; see Equation 0.15 and use power per volume =  $j^2 / \sigma$ . *Lower line:* loss produced by 60-Hz  $E$  field in Watt per kilogram =  $\sigma E_{\text{int}}^2 / D$ , where external field  $E_1$  is related to  $E_{\text{int}}$  by Equation 0.9 with  $\epsilon_2 = \epsilon_0 \times 10^5$  at 1 kHz and  $\epsilon_0 = 8 \times 10^4$  at 10 kHz.

\**BBA: Bioengineering and Biophysical Aspects of Electromagnetic Fields* (ISBN 0-8493-9539-9); *BMA: Biological and Medical Aspects of Electromagnetic Fields* (ISBN 0-8493-9538-0).



4. With radiated power it is relatively easy to produce heating effects in living objects with presently available human-made devices (see Chapter 10 in *BBA* and Chapter 5 in *BMA*). This does not imply, of course, that all biological effects of radiated radio frequency (RF) power necessarily arise from temperature changes.

The results of experiments involving exposure of organic materials and entire living organisms to static  $E$  and ELF  $E$  fields are described in *BBA*, Chapter 3. Various mechanisms for the interaction of such fields with living tissue are also discussed there and in *BBA*, Chapter 5. In the present introduction, we shall only point out that one salient feature of static (dc) and ELF  $E$  field interaction with living organisms is that the external or applied  $E$  field is always larger by several orders of magnitude than the resultant average internal  $E$  field [5,6]. This is a direct consequence of boundary conditions derived from Maxwell's equations [1–3].

## 0.2 Penetration of Direct Current and Low-Frequency Electric Fields into Tissue

Assuming that the two materials illustrated schematically in Figure 0.4 are characterized, respectively, by conductivities  $\sigma_1$  and  $\sigma_2$  and dielectric permittivities  $\epsilon_1$  and  $\epsilon_2$ , we write  $E$ -field components parallel to the boundary as  $E_P$  and components perpendicular to the boundary as  $E_{\perp}$ . For both static and time-varying fields

$$E_{P1} = E_{P2} \quad (0.3)$$

and for static (dc) fields

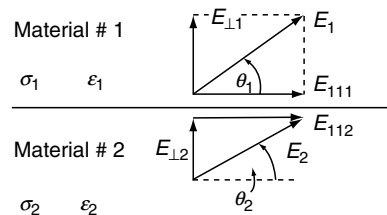
$$\sigma_1 E_{\perp 1} = \sigma_2 E_{\perp 2} \quad (0.4)$$

as a consequence of the continuity of current (or conservation of charge). The orientations of the total  $E$  fields in media 1 and 2 can be represented by the tangents of the angles between the total fields and the boundary line

$$\tan \theta_1 = \frac{E_{\perp 1}}{E_{P1}}, \quad \tan \theta_2 = \frac{E_{\perp 2}}{E_{P2}} \quad (0.5)$$

From these equations it follows that

$$\tan \theta_1 = \frac{\sigma_2}{\sigma_1} \frac{E_{\perp 1}}{E_{P1}} = \frac{\sigma_2}{\sigma_1} \frac{E_{\perp 2}}{E_{P2}} = \frac{\sigma_2}{\sigma_1} \tan \theta_2 \quad (0.6)$$



**FIGURE 0.4**  
Symbols used in description of boundary conditions for  $E$ -field components.

If material 1 is air with conductivity [7]  $\sigma_1 = 10^{-13}$  S/m and material 2 a typical living tissue with  $\sigma_2 \approx 10^{-1}$  S/m (compare [Chapter 3](#) in *BBA*),  $\tan \theta_1 = 10^{12} \tan \theta_2$ , and therefore even if the field in material 2 (the inside field) is almost parallel to the boundary so that  $\theta_2 \cong 0.5^\circ$  or  $\tan \theta_2 \approx (1/100)$ ,  $\tan \theta_1 = 10^{10}$  or  $\theta_1 = (\pi/2 - 10)^{-10}$  radians. Thus an electrostatic field in air, at the boundary between air and living tissue, must be practically perpendicular to the boundary. The situation is virtually the same at ELF although [Equation 0.4](#) must be replaced by

$$\sigma_1 E_{\perp 1} - \sigma_2 E_{\perp 2} = -j\omega\rho_s \quad (0.7)$$

and

$$\varepsilon_1 E_{\perp 1} - \varepsilon_2 E_{\perp 2} = \rho_s \quad (0.8)$$

where  $j = \sqrt{-1}$ ,  $\omega$  is the radian frequency ( $= 2\pi \times$  frequency), and  $\rho_s$  is the surface charge density. In [Chapter 3](#) in *BBA* it is shown that at ELF the relative dielectric permittivity of living tissue may be as high as  $10^6$  so that  $\varepsilon_2 = 10^6 \varepsilon_0$ , where  $\varepsilon_0$  is the dielectric permittivity of free space  $(1/36\pi) 10^{-9}$  F/m; however, it is still valid to assume that  $\varepsilon_2 \leq 0^{-5}$ . Then from [Equation 0.7](#) and [Equation 0.8](#)

$$E_{\perp 1} = \frac{\sigma_2 + j\omega\varepsilon_2}{\sigma_1 + j\omega\varepsilon_1} E_{\perp 2} \quad (0.9)$$

which gives at 60 Hz with  $\sigma_2 = 10^1$  S/m,  $\sigma_1 = 10^{-13}$  S/m,  $\varepsilon_2 \approx 10^{-5}$  F/m, and  $\varepsilon_1 \approx 10^{-11}$  F/m

$$E_{\perp 1} = \frac{10^{-1} + j_4 10^{-3}}{10^{-13} + j_4 10^{-9}} E_{\perp 2} \approx \frac{\sigma_2}{j\omega\varepsilon_1} = -j(2.5 \times 10^7) E_{\perp 2} \quad (0.10)$$

This result, together with [Equation 0.3](#) and [Equation 0.5](#), shows that for the given material properties, the field in air must still be practically perpendicular to the boundary of a living organism:  $\tan \theta_1: 2.5(10^7) \tan \theta_2$ .

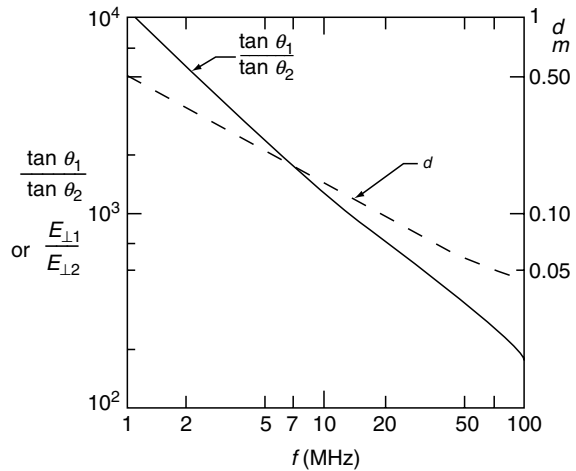
Knowing now that the living organism will distort the  $E$  field in its vicinity in such a way that the external field will be nearly perpendicular to the boundary surface, we can calculate the internal field by substituting the total field for the perpendicular field in [Equation 0.4](#) (dc) and [Equation 0.9](#) (ELF). For the assumed typical material parameters we find that in the static (dc) case

$$\frac{E_{\text{internal}}}{E_{\text{external}}} \approx 10^{-12} \quad (0.11)$$

$$\rho_f = \frac{3(\sigma_2\varepsilon_1 - \sigma_1\varepsilon_2)E_0}{2\sigma_1 + \sigma_2} \cos \vartheta \text{ C/m}^2$$

and for 60 Hz

$$\frac{E_{\text{internal}}}{E_{\text{external}}} \approx 4(10^{-8}) \quad (0.12)$$



**FIGURE 0.5**

Orientation of  $E$ -field components at air–muscle boundary (or ratio of fields perpendicular to boundary); depth ( $d$ ) at which field component parallel to boundary surface decreases by approximately 50% ( $d = 0.693\delta$ ).

Thus, a 60-Hz external field of 100 kV/m will produce an average  $E_{\text{internal}}$  field of the order of 4 mV/m.

If the boundary between air and the organic material consists of curved surfaces instead of infinite planes, the results will be modified only slightly. Thus, for a finite sphere (with  $\varepsilon$  and  $\sigma$  as assumed here) embedded in air, the ratios of the internal field to the undisturbed external field will vary with the angle  $\theta$  and distance  $r$  as indicated in Figure 0.5, but will not deviate from the results indicated by Equation 0.7 and Equation 0.8 by more than a factor of 3 [3,8]. Long cylinders ( $L \ll r$ ) aligned parallel to the external field will have interior fields essentially equal to the unperturbed external field, except near the ends where the field component perpendicular to the membrane surface will be intensified approximately as above (see Chapter 9 and Chapter 10 in this volume).

### 0.3 Direct Current and Low-Frequency Magnetic Fields

Direct current  $H$  fields are considered in more detail in the Chapter 3, Chapter 5, and Chapter 8 in *BBA*. ELF  $H$  fields are considered in various places, including Chapter 5 and Chapter 7 in *BBA* and Chapter 2 and Chapter 11 in *BMA*. As the magnetic permeability  $\mu$  of most biological materials is practically equal to the magnetic permeability  $\mu_0$  of free space,  $4\pi(10^{-7})$  H/m, the dc or ELF  $H$  field “inside” will be practically equal to the  $H$  field “outside.” The only exceptions are organisms such as the magnetotactic bacteria, which synthesize ferromagnetic material, discussed in Chapter 8 of *BBA*. The known and suggested mechanisms of interaction of dc  $H$  fields with living matter are:

1. Orientation of ferromagnetic particles, including biologically synthesized particles of magnetite.
2. Orientation of diamagnetically or paramagnetically anisotropic molecules and cellular elements [9].
3. Generation of potential differences at right angles to a stream of moving ions (Hall effect, also sometimes called a magnetohydrodynamic effect) as a result of the magnetic force  $F_m = qvB \sin \theta$ , where  $q$  is the electric charge,  $v$  is the

velocity of the charge,  $B$  is the magnetic flux density, and  $\sin \theta$  is the sine of the angle  $\theta$  between the directions  $v$  and  $B$ . One well-documented result of this mechanism is a “spike” in the electrocardiograms of vertebrates subjected to large dc  $H$  fields.

4. Changes in intermediate products or structural arrangements in the course of light-induced chemical (electron transfer) reactions, brought about by Zeeman splitting of molecular energy levels or effects upon hyperfine structure. (The Zeeman effect is the splitting of spectral lines, characteristic of electronic transitions, under the influence of an external  $H$  field; hyperfine splitting of electronic transition lines in the absence of an external  $H$  field is due to the magnetic moment of the nucleus; such hyperfine splitting can be modified by an externally applied  $H$  field.) The magnetic flux densities involved not only depend upon the particular system and can be as high as 0.2 T (2000 G) but also  $<0.01$  mT (100 G). Bacterial photosynthesis and effects upon the visual system are prime candidates for this mechanism [10,11].
5. Induction of  $E$  fields with resulting electrical potential differences and currents within an organism by rapid motion through a large static  $H$  field. Some magnetic phosphenes are due to such motion [12].

Relatively slow time-varying  $H$  fields, which are discussed in the basic mechanisms and therapeutic uses chapters ([Chapter 5](#) of *BBA* and [Chapter 11](#) in *BMA*), among others, may interact with living organisms through the same mechanisms that can be triggered by static  $H$  fields, provided the variation with time is slow enough to allow particles of finite size and mass, located in a viscous medium, to change orientation or position where required (mechanism 1 and 2) and provided the field intensity is sufficient to produce the particular effect. However, time-varying  $H$  fields, including ELF  $H$  fields, can also induce electric currents into stationary conducting objects. Thus, all modes of interaction of time-varying  $E$  fields with living matter may be triggered by time-varying, but not by static,  $H$  fields.

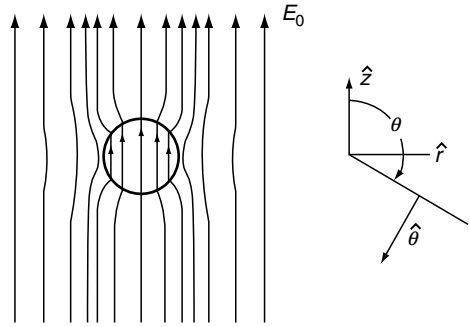
In view of Faraday’s law, a time-varying magnetic flux will induce  $E$  fields with resulting electrical potential differences and “eddy” currents through available conducting paths. As very large external ELF  $E$  fields are required (as indicated by [Equation 0.9](#) through [Equation 0.12](#)) to generate even small internal  $E$  fields, many human-made devices and systems generating both ELF  $E$  and  $H$  fields are more likely to produce physiologically significant internal  $E$  fields through the mechanism of *magnetic* induction.

The induced voltage  $V$  around some closed path is given by

$$V = \oint E \cdot d\ell = - \iint \frac{\partial B}{\partial t} ds \quad (0.13)$$

where  $E$  is the induced  $E$  field. The integration  $\oint E \cdot d\ell$  is over the appropriate conducting path,  $\partial B/\partial t$  is the time derivative of the magnetic flux density, and the “dot” product with the surface element,  $ds$ , indicates that only the component of  $\partial B/\partial t$  perpendicular to the surface, i.e., parallel to the direction of the vector  $ds$ , enclosed by the conducting path, induces an  $E$  field. To obtain an order-of-magnitude indication of the induced current that can be expected as a result of an ELF  $H$  field, we consider the circular path of radius  $r$ , illustrated by [Figure 0.6](#). Equation 0.13 then gives the magnitude of the  $E$  field as

$$E = \frac{\omega Br}{2} \quad (0.14)$$



**FIGURE 0.6**

$E$  field when sphere of radius  $R$ , conductivity  $\sigma_2$ , and dielectric permittivity  $\epsilon_2$  is placed into an initially uniform static field ( $E = 2E_0$ ) within a medium with conductivity  $\sigma_1$  and permittivity  $\epsilon_1$ . The surface charge density is  $\rho_r = \frac{3(\sigma_2\epsilon_1 - \sigma_1\epsilon_2)E_0}{2\sigma_1 + \sigma_2} \cos \theta$  C/m<sup>2</sup>.

$$\begin{aligned}
 r < R \quad \vec{E} &= \frac{3\sigma_1 E_0}{2\sigma_1 + \sigma_2} \hat{z} & \epsilon_2, \sigma_2 \\
 r < R \quad \vec{E} &= E_0 \cos \theta \left[ 1 + \frac{2R^3(\sigma_2 \epsilon_1 \sigma_1)}{r^3(2\sigma_1 + \sigma_2)} \right] \hat{r} & \epsilon_1, \sigma_1 \\
 & - E \sin \theta \left[ 1 - \frac{R^3(\sigma_2 \epsilon_1 \sigma_1)}{r^3(2\sigma_1 + \sigma_2)} \right] \hat{\theta}
 \end{aligned}$$

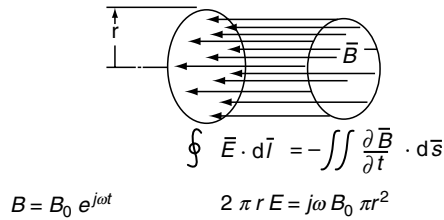
where  $\omega$  is the  $2\pi f$  and  $f$  is the frequency. The magnitude of the resulting electric current density  $J$  in ampere per square meter is\*

$$J = \sigma E = \frac{\sigma \omega B r}{2} \tag{0.15}$$

where  $\sigma$  is the conductivity along the path in Siemens per meter. In the SI (Systeme Internationale) units used throughout this book,  $B$  is measured in tesla ( $T = 10^4$  G) and  $r$  in meters. Choosing for illustration a circular path of 0.1 m radius, a frequency of 60 Hz, and a conductivity of 0.1 S/m, Equation 0.14 and Equation 0.15 give  $E = 18.85$  B and  $J = 1.885$  B. The magnetic flux density required to obtain a current density of 1 mA/m<sup>2</sup> is 0.53 mT or about 5 G. The  $E$  field induced by that flux density along the circular path is 10 mV/m. To produce this same 10 mV/m  $E_{\text{internal}}$  field by an external 60 Hz  $E_{\text{external}}$  field would require, by Equation 0.12, a field intensity of 250 kV/m.

As the induced voltage is proportional to the time rate of change of the  $H$  field (Equation 0.13), implying a linear increase with frequency (Equation 0.14), one would expect that the ability of a time-varying  $H$  field to induce currents deep inside a conductive object would increase indefinitely as the frequency increases; or conversely, that the magnetic flux density required to induce a specified  $E$  field would decrease linearly with frequency, as indicated in Figure 0.7. This is not true however, because the displacement current density  $\partial D/\partial t$ , where  $D = \epsilon E$ , must also be considered as the frequency increases. This leads to the wave behavior discussed in Part III, implying that at sufficiently high frequencies the effects of both external  $E$  and  $H$  fields are limited

\*Equation 0.15 neglects the  $H$  field generated by the induced eddy currents. If this field is taken into account, it can be shown that the induced current density in a cylindrical shell of radius  $r$  and thickness  $\Delta$  is given by  $\Delta r < 0.01 \text{ m}^2/[1 + j\Delta r/\delta^2]$ , where  $H_0 = B_0/\mu_0$  and  $\delta$  is the skin depth defined by Equation 0.17 below. However, for conductivities of biological materials ( $\sigma < 5$  s/m) one obtains at audio frequencies  $\delta > 1$  m and as for most dimensions of interest  $\Delta r < 0.01 \text{ m}^2$  the term  $j\Delta r/\delta^2$  becomes negligible. The result  $-jrH_0/\delta^2$  is then identical with Equation 0.15.



**FIGURE 0.7**

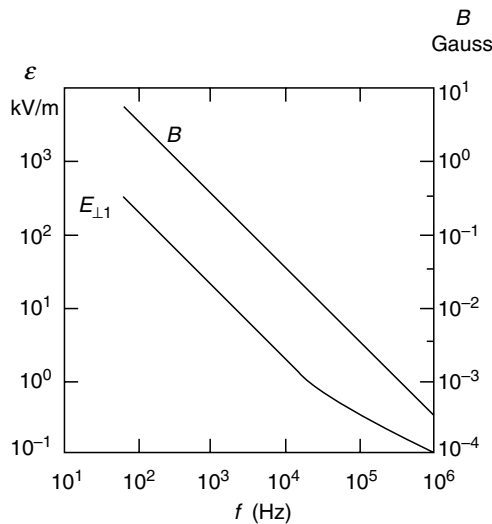
Circular path (loop) of radius  $r$  enclosing uniform magnetic flux density perpendicular to the plane of the loop. For sinusoidal time variation  $B = B_0 e^{j\omega t}$ .

by reflection losses (Figure 0.8 through Figure 0.10) as well as by skin effect [13], i.e., limited depth of penetration  $d$  in Figure 0.5.

## 0.4 RF Fields

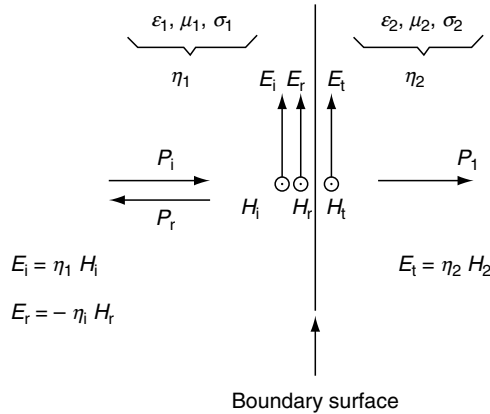
At frequencies well below those where most animals and many field-generating systems have dimensions of the order of one free space wavelength, e.g., at 10 MHz where  $\lambda = 30$  m, the skin effect limits penetration of the external field. This phenomenon is fundamentally different from the small ratio of internal to external  $E$  fields described in Equation 0.4 (applicable to dc) and Equation 0.9.

Equation 0.9 expresses a “boundary condition” applicable at all frequencies, but as the angular frequency  $\omega$  increases (and in view of the rapid decrease with frequency of the dielectric permittivity  $\epsilon_2$  in biological materials—see Chapter 3 of *BBA*, the ratio of the normal component of the external to the internal  $E$  field at the boundary decreases



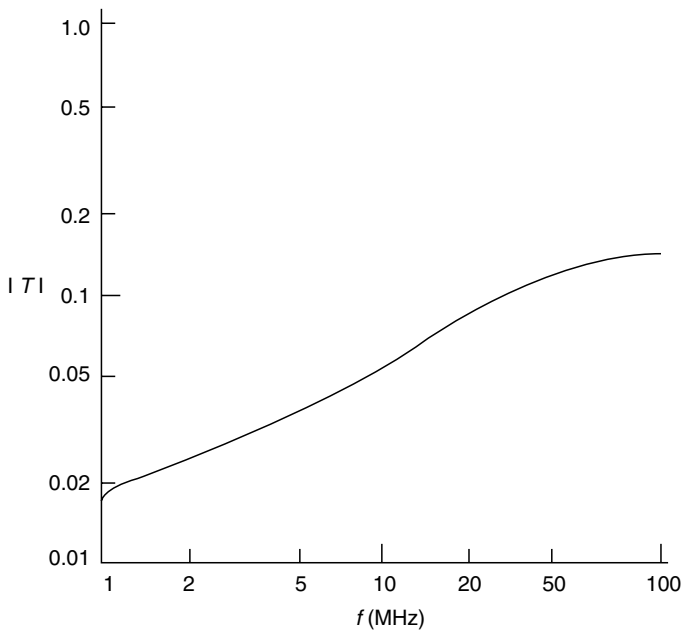
**FIGURE 0.8**

External  $E$  and  $H$  field required to obtain an internal  $E$  field of 10 mV/m (conductivity and dielectric permittivity for skeletal muscle from Foster, K.R., Schepps, J.L., and Schwan, H.P. 1980. *Biophys. J.*, 29:271–281.  $H$ -field calculation assumes a circular path of 0.1-m radius perpendicular to magnetic flux).



**FIGURE 0.9** Reflection and transmission of an electromagnetic wave at the boundary between two different media, perpendicular incidence;  $P_i$  = incident power,  $P_r$  = reflected power,  $P_t$  = transmitted power.

with increasing frequency. This is illustrated by Figure 0.10 where  $\tan \theta_1 / \tan \theta_2$  is also equal to  $E_{\perp 1} / E_{\perp 2}$  in view of Equation 0.3, Equation 0.5, and Equation 0.9. However, at low frequencies the total field inside the boundary can be somewhat larger than the perpendicular field at the boundary; and any field variation with distance from the boundary is not primarily due to energy dissipation, but in a homogeneous body is a consequence of shape. At RF, on the other hand, the  $E$  and  $H$  fields of the incoming



**FIGURE 0.10** Magnitude of transmission coefficient  $T$  for incident  $E$  field parallel to boundary surface.  $T = E_t/E_i$ ; reflection coefficient  $r = E_r/E_i = T-1$ .  $\Gamma$  and  $T$  are complex numbers;  $\epsilon_r$  and  $\sigma$  for skeletal muscle from Chapter 3 in BBA.

electromagnetic wave, after reflection at the boundary, are further decreased due to energy dissipation. Both  $E$  and  $H$  fields decrease exponentially with distance from the boundary

$$g(z) = Ae^{-\frac{z}{\delta}} \quad (0.16)$$

where  $g(z)$  is the field at the distance  $z$  and  $A$  is the magnitude of the field just *inside* the boundary.

As defined by Equation 0.16 the skin depth  $\delta$  is the distance over which the field decreases to  $1/e$  ( $= 0.368$ ) of its value just *inside* the boundary. (Due to reflection, the field  $A$  just inside the boundary can already be very much smaller than the incident external field; see Figure 0.8 and Figure 0.9.)

Expressions for  $\delta$  given below were derived [2,3,13,14] for plane boundaries between infinite media. They are reasonable accurate for cylindrical structures if the ratio of radius of curvature to skin depth ( $r_0/\delta$ ) is larger than about five [13]. For a good conductor

$$\delta = \frac{1}{\sqrt{\pi f \mu \sigma}} \quad (0.17)$$

where a good conductor is one for which the ratio  $p$  of conduction current,  $J = \sigma E$ , to displacement current,  $\partial D/\partial t = \varepsilon (\partial E/\partial t) = j\omega\varepsilon E$  is large:

$$p = \frac{\sigma}{\omega\varepsilon} \gg 1 \quad (0.18)$$

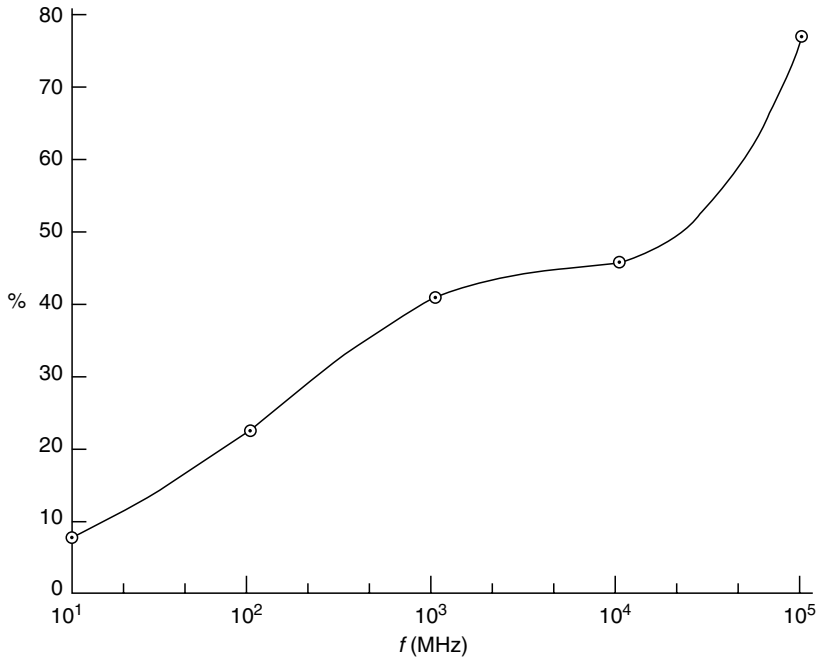
Since for most biological materials  $p$  is of the order of one ( $0.1 < p < 10$ ) over a very wide frequency range (see Chapter 3 of *BBA*), it is frequently necessary to use the more general expression [13]

$$\delta = \frac{1}{\omega \left[ \frac{\mu\varepsilon}{2} (\sqrt{1+p^2} - 1) \right]^{1/2}} \quad (0.19)$$

The decrease of field intensity with distance from the boundary surface indicated by Equation 0.16 becomes significant for many biological objects at frequencies where  $r_0/\delta \geq 5$  is not satisfied. However, the error resulting from the use of Equation 0.16 and Equation 0.17 or Equation 0.19 with curved objects is less when  $z < \delta$ . Thus at  $z = 0.693 \delta$ , where  $g(z) = 0.5 A$  from Equation 0.16 and Equation 0.17, the correct values of  $g(z)$ , obtained by solving the wave equation in cylindrical coordinates, differs only by 20% (it is 0.6 A) even when  $r_0/\delta$  is as small as 2.39 [14]. Therefore, Figure 0.10 shows the distance  $d = 0.693 \delta$ , at which the field decreases to half of its value just inside the boundary surface, using Equation 0.19 with typical values for  $\sigma$  and  $\varepsilon$  for muscle from Figure 0.11. It is apparent that the skin effect becomes significant for humans and larger vertebrates at frequencies  $> 10$  MHz.

Directly related to skin depth, which is defined for fields varying sinusoidally with time, is the fact that a rapid transient variation of an applied magnetic flux density constitutes an exception to the statement that the dc  $H$  field inside the boundary is equal to the  $H$  field outside. Thus, from one viewpoint one may consider the rapid application or removal of a dc  $H$  field as equivalent to applying a high-frequency field during the switching period, with the highest frequencies present of the order of  $1/\tau$ , where  $\tau$  is the rise time of the applied step function. Thus, if  $\tau < 10^{-8}$  s, the skin effect will be important during the transient period, as  $d$  in Figure 0.5 is  $< 5$  cm above 100 MHz. It is also possible to calculate directly the magnetic flux density inside a conducting cylinder as a function of radial position  $r$  and time  $t$  when a magnetic pulse is applied in the axial





**FIGURE 0.11**

Ratio of transmitted to incident power expressed as percent of incident power. Air–muscle interface, perpendicular incidence (Equation 0.31, Table 0.1).

direction [15,16]. Assuming zero rise time of the applied field  $B_0$ , i.e., a true step function, one finds that the field inside a cylinder of radius  $a$  is

$$B = B_0 \left[ 1 - \sum_{k=1}^{\infty} J_0 \left( r \frac{v_k}{a} \right) e^{-t/T_k} \right] \quad (0.20)$$

where  $J_0 (r v_k/a)$  is the zero-order Bessel function of argument  $r v_k/a$  and the summation is over the nulls of  $J_0$  designated  $v_k$  (the first four values of  $v_k$  are 2.405, 5.520, 8.654, and 11.792).\*  $T_k$  is the rise time of the  $k$ th term in the series and is given by

$$T_k = \frac{\mu_0 \sigma a^2}{v_k} \quad (0.21)$$

As  $v_k$  increases, the rise time decreases and therefore the longest delay is due to the first term in the summation with  $k = 1$

$$T_1 = \frac{\mu_0 \sigma a^2}{2.405} \quad (0.22)$$

For a cylinder with 0.1 m radius and a conductivity  $\sigma \approx 1$  S/m, which is a typical value for muscle between 100 and 1000 MHz, Equation 0.22 gives  $T_1 = 2.6 \times 10^{-8}$  s. This finite rise time (or decay time in case of field removal) of the internal  $H$  field may be of some importance when pulsed  $H$  fields are used therapeutically [17]. It might also be used

\*This result is based on solution of  $\partial B/\partial t = (1/\mu_0)\nabla^2 B$ , which is a consequence of Ampere's and Faraday's laws when displacement is disregarded. Equations 0.20 to 0.22 are therefore only correct when  $p \gg 1$ .

to measure noninvasively the conductivity of biological substances *in vivo* through determination of the final decay rate of the voltage induced into a probe coil by the slowly decaying internal field after the applied field is removed [16].

The properties of biological substances in the intermediate frequency range, above ELF (>300 Hz), and below the higher RFs, where wave behavior and skin effect begin to be important (~20 MHz), are discussed in [Chapter 3](#) of *BBA*. However, many subsequent chapters are concerned with biological effects at dc and ELF frequencies below a few kilohertz, while others deal primarily with the higher RFs, >50 MHz. One reason for this limited treatment of the intermediate frequency range is that very little animal data are available for this spectral region in comparison with the large number of experiments performed at ELF and microwave frequencies in recent years.\* Another reason is that most electrical processes known to occur naturally in biological systems—action potentials, EKG, EEG, ERG, etc.—occur at dc and ELF frequencies. Therefore, one might expect some physiological effects from external fields of appropriate intensity in the same frequency range, even if the magnitude of such fields is not large enough to produce thermal effects. As illustrated by [Figure 0.3](#) and [Figure 0.7](#), most *E* fields below 100 kHz set up by currently used human-made devices, and most *H* fields below 10 kHz except the very strongest, are incapable of producing thermal effects in living organisms, excluding, of course, fields accompanying currents directly introduced into the organism via electrodes. Thus, the frequencies between about 10 and 100 kHz have been of relatively little interest because they are not very likely to produce thermal or other biological effects. On the other hand, the higher RFs are frequently generated at power levels where enough energy may be introduced into living organisms to produce local or general heating. In addition, despite skin effect and the reflection loss to be discussed in more detail below, microwaves modulated at an ELF rate may serve as a vehicle for introducing ELF fields into a living organism of at least the same order of magnitude as would be introduced by direct exposure to ELF. Any effect of such ELF-modulated microwaves would, of course, require the existence of some amplitude-dependent demodulation mechanism to extract the ELF from the microwave carrier.

Among the chapters dealing with RF, [Chapter 10](#) and [Chapter 11](#) of *BBA* give the necessary information for establishing the magnitude of the fields present in biological objects: (1) experimental techniques and (2) analytical methods for predicting field intensities without construction of physical models made with “phantom” materials, i.e., dielectric materials with properties similar to those of living objects which are to be exposed. As thermal effects at microwave frequencies are certainly important, although one cannot assume *a priori* that they are the only biological effects of this part of the spectrum, and as some (but not all) thermal effects occur at levels where the thermoregulatory system of animals is activated, thermoregulation in the presence of microwave fields is discussed in [Chapter 5](#) of *BMA*, as well as in [Chapter 10](#) of *BBA*. Not only are the therapeutic applications of microwaves based upon their thermal effects, but also the experimental establishment of possible nonthermal effects at the threshold of large scale tissue heating in particular living systems and also requires thorough understanding of thermoregulatory mechanisms. The vast amount of experimental data obtained on animal systems exposed to microwave is discussed in [Chapter 3](#) and [Chapter 4](#) in *BMA*. Both nonmodulated fields and modulated fields, where the type of modulation had no apparent effect other than modification of the average power level, are considered. These chapters and the [Chapter 9](#) in *BMA* are considered to be very new extension of experiments into exposures to ultra-short and to ultra-high power pulses.

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\*Though this statement was written in for the second edition in 1995, it continues to be true in 2005—Ben Greenebaum.

At the higher RFs, the external  $E$  field is not necessarily perpendicular to the boundary of biological materials (see Figure 0.4 and Figure 0.10), and the ratio of the total external  $E$  field to the total internal field is not given by Equation 0.9. However, the skin effect (Equation 0.16 through Equation 0.19) and reflection losses still reduce the  $E$  field within any biological object below the value of the external field. As pointed out in Chapter 3, dielectric permittivity and electrical conductivity of organic substances both vary with frequency. At RF, most biological substances are neither very good electrical conductors nor very good insulators, with the exception of cell membranes, which are good dielectrics at RF but at ELF can act as intermittent conductors or as dielectrics and are ion-selective [18–20]). The ratio  $p$  (Equation 0.18) is neither much smaller nor very much larger than values shown for typical muscle tissue [21,22] in Table 0.1.

Reflection loss at the surface of an organism is a consequence of the difference between its electrical properties and those of air. Whenever an electromagnetic wave travels, from one material to another with different electrical properties, the boundary conditions (Equation 0.3 and Equation 0.8) and similar relations for the  $H$  field require the existence of a reflected wave. The expressions for the reflection coefficient

$$\Gamma = \frac{E_r}{E_i} \quad (0.23)$$

and the transmission coefficient

$$T = \frac{E_t}{E_i} \quad (0.24)$$

become rather simple for loss-free dielectrics ( $p \ll 1$ ) and for good conductors ( $p \gg 1$ ). As biological substances are neither the most general expressions for  $\Gamma$  and  $T$ , applicable at plane boundaries, are needed [3,13]. For perpendicular incidence, illustrated by Figure 0.8,

$$\Gamma = \frac{\eta_2 - \eta_1}{\eta_2 + \eta_1} \quad (0.25)$$

$$T = \frac{2\eta_2}{\eta_2 + \eta_1} = 1 + \Gamma \quad (0.26)$$

**TABLE 0.1**

Ratio  $p$  of Conduction Current to Displacement as a Function of Frequency

$f$ (MHz)	$\sigma$	$\epsilon_r$	$p = \frac{\sigma}{\omega\epsilon_0\epsilon_r}$
1	0.40	2000	3.6
10	0.63	160	7.1
100	0.89	72	2.2
$10^3$	1.65	50	0.59
$10^4$	10.3	40	0.46
$10^5$	80	6	2.4

where  $\eta_1$  and  $\eta_2$  are the wave impedances, respectively, of mediums 1 and 2. The wave impedance of a medium is the ration of the  $E$  to the  $H$  field in a plane wave traveling through that medium; it is given by [13]

$$\eta = \left( \frac{j\omega\mu}{\sigma + j\omega\epsilon} \right)^{1/2} \quad (0.27)$$

Clearly  $\Gamma$  and  $T$  are in general complex numbers, even when medium 1 is air for which Equation 0.27 reduces to the real quantity  $\eta_0 = \sqrt{\mu_0/\epsilon_0}$ , because medium 2, which here is living matter, usually has a complex wave impedance at RFs.

The incident, reflected, and transmitted powers are given by [13]

$$P_i = R_1 |E_i|^2 \frac{1}{\eta_1^*} = \frac{|E_i|^2}{|\eta_1|^2} R_1 \quad (0.28)$$

$$P_r = R_1 |E_r|^2 \frac{1}{\eta_1^*} = \frac{|E_r|^2}{|\eta_1|^2} R_1 \quad (0.29)$$

$$P_t = R_1 |E_t|^2 \frac{1}{\eta_2^*} = \frac{|E_t|^2}{|\eta_2|^2} R_2 \quad (0.30)$$

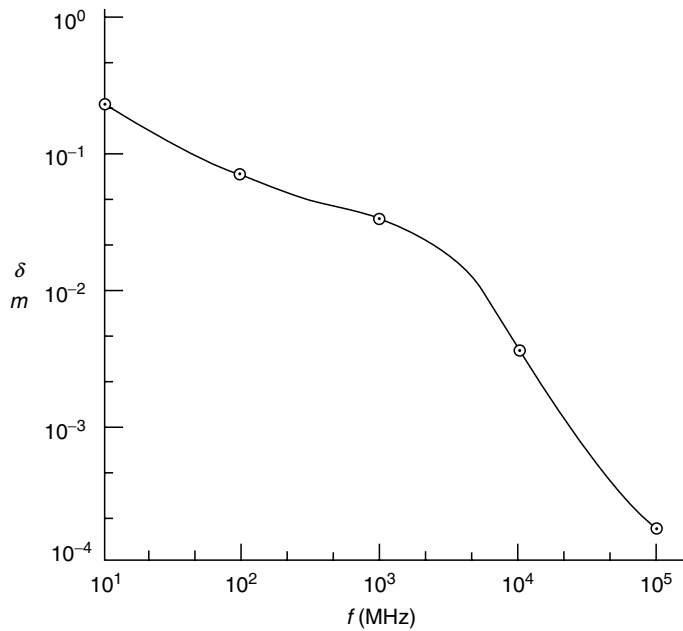
where the  $E$  fields are effective values ( $E_{\text{eff}} = E_{\text{peak}}/\sqrt{2}$ ) of sinusoidal quantities,  $R_1$  signifies “real part of,”  $\eta^*$  is the complex conjugate of  $\eta$ , and  $R_1$  and  $R_2$  are the real parts of  $\eta_1$  and  $\eta_2$ . If medium 1 is air,  $\eta_1 = R_1 = 377 \Omega$ , it follows from Equation 0.23, Equation 0.24, and Equation 0.28 through Equation 0.30 and conservation of energy that the ratio of the transmitted to the incident real power is given by

$$\frac{P}{P_1} = |T|^2 \frac{\eta_1 \eta_2^* + \eta_1^* \eta_2}{2|\eta_2|^2} = 1 - \frac{P_r}{P_1} = 1 - |\Gamma|^2 \quad (0.31)$$

The magnitude of the transmission coefficient  $T$  for the air–muscle interface over the 1- to 100-MHz frequency range is plotted in Figure 0.9, which shows that the magnitude of the transmitted  $E$  field in muscle tissue is considerably smaller than the  $E$  field in air. The fraction of the total incident power that is transmitted (Equation 0.31) is shown in Figure 0.11, indicating clearly that reflection loss at the interface decreases with frequency. However, for deeper lying tissue this effect is offset by the fact that the skin depth  $\delta$  (Equation 0.19) also decreases with frequency (Figure 0.12) so that the total power penetrating beyond the surface decreases rapidly.

In addition to reflection at the air–tissue boundary, further reflections take place at each boundary between dissimilar materials. For example, the magnitude of the reflection coefficient at the boundary surface between muscle and organic materials with low-water content, such as fat or bone, is shown in Table 0.2.

The situation is actually more complicated than indicated by Figure 0.9 and Figure 0.11, because the wave front of the incident electromagnetic wave may not be parallel to the air–tissue boundary. Two situations are possible: the incident  $E$  field may be polarized perpendicular to the plane of incidence defined in Figure 0.13 (perpendicular polarization, Figure 0.13a) or parallel to the plane of incidence (parallel polarization, Figure 0.13b). The transmission and reflection coefficients [8] are different for the two types of polarization and also become functions of the angle of incidence  $\alpha_1$ :



**FIGURE 0.12** Electromagnetic skin depth in muscle tissue from plane wave expression (Equation 0.19, Table 0.1).

$$\text{Perpendicular polarization} \begin{cases} T_{\perp} = \frac{2\eta_2 \cos \alpha_1}{\eta_2 \cos \alpha_1 + \eta_1 \cos \alpha_2} \\ \Gamma_{\perp} = \frac{\eta_2 \cos \alpha_1 - \eta_1 \cos \alpha_2}{\eta_2 \cos \alpha_1 + \eta_1 \cos \alpha_2} \end{cases} \quad (0.32),(0.33)$$

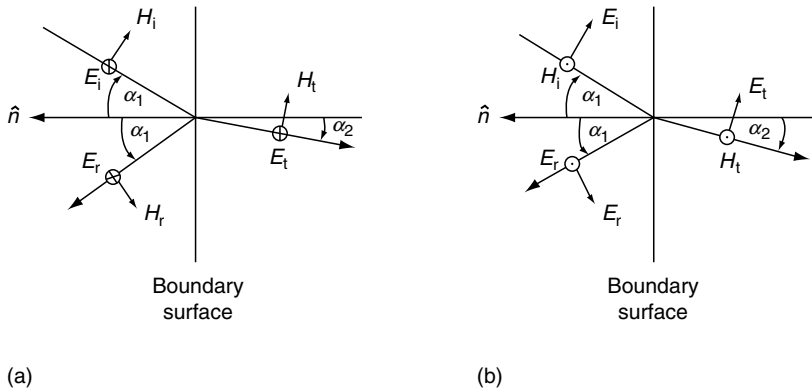
$$\text{Parallel polarization} \begin{cases} T_{\text{P}} = \frac{2\eta_2 \cos \alpha_1}{\eta_2 \cos \alpha_2 + \eta_1 \cos \alpha_1} \\ \Gamma_{\text{P}} = \frac{\eta_1 \cos \alpha_1 - \eta_2 \cos \alpha_2}{\eta_2 \cos \alpha_2 + \eta_1 \cos \alpha_1} \end{cases} \quad (0.34),(0.35)$$

where  $\alpha_2$  is given by the generalized Snell's law (when both the media have the magnetic permeability of free space) by

**TABLE 0.2** Reflection Coefficient "Capital Gamma" for Low-Water-Content Materials

f (MHz)	Fat or Bone		Muscle <sup>a</sup> -Fat ( $\Gamma$ )
	$\sigma$ (S/m)	$\epsilon_r$	
10 <sup>2</sup>	0.048	7.5	0.65
10 <sup>3</sup>	0.101	5.6	0.52
10 <sup>4</sup>	0.437	4.5	0.52

<sup>a</sup> $\sigma$  and  $\epsilon_r$  for muscle from Table 0.1.



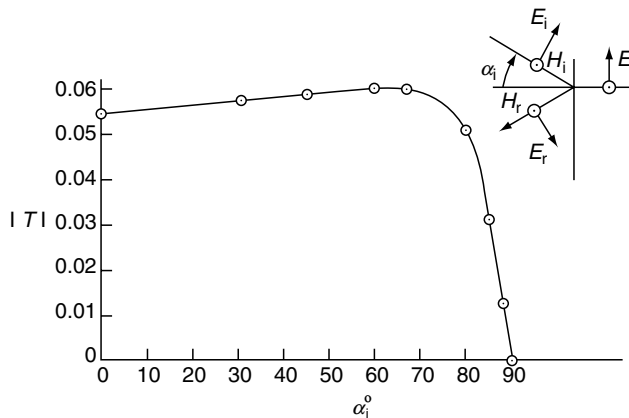
**FIGURE 0.13**

Oblique incidence of an electromagnetic wave at the boundary between two different media. (a) Perpendicular polarization ( $E$  vector perpendicular to plane of incidence); (b) parallel polarization ( $E$  vector parallel to plane of incidence). The plane of incidence is the plane formed by the surface normal (unit vector  $\hat{n}$  and the direction of the incident wave);  $\otimes$  indicates a vector into the plane of the paper;  $\odot$  indicates a vector out of the plane of the paper. The orientation of the field vectors in the transmitted field is shown for loss-free dielectrics. For illustration of the transmitted wave into a medium with finite conductivity, where the wave impedance  $\eta_2$  becomes a complex number, see Stratton, J.A., *Electromagnetic Theory*, McGraw-Hill, New York, 1941, p. 435.

$$\sin \alpha_2 = \frac{\sqrt{\epsilon_1}}{\sqrt{\epsilon_2 - j\frac{\sigma_2}{\omega}}} \quad (0.36)$$

so that  $\cos \alpha_2 = \sqrt{1 - \sin^2 \alpha_2}$  is a complex number unless  $\rho_2 = (\sigma_2/\omega\epsilon_2) = 1$ .

As illustration, the variation with angle of incidence of the transmission coefficient for parallel polarization at the air–muscle interface at 10 MHz, is shown in Figure 0.14. It is apparent that the transmitted field is not necessarily maximized by perpendicular incidence in the case of parallel polarization. Furthermore, whenever  $p \approx 1$  or  $p > 1$  (see Table 0.1, above),  $\alpha_2$  is complex, which causes the waves entering the tissue to be inhomogeneous—they are not simple plane waves, but waves where surfaces of constant phase and constant amplitude do not coincide [3,23]; only the planes of constant amplitude are parallel to the boundary surface.



**FIGURE 0.14**

Magnitude of complex transmission coefficient for parallel polarization versus angle of incidence  $\alpha_1$  at 10 MHz ( $E$  field in plane of incidence,  $H$  field parallel to boundary plane;  $\sigma_2 = 0.7\text{S/m}$ ,  $\epsilon_{r2} = 150$ ,  $T = E_t/E_i$ ).

Analytical solutions for nonplanar structures taking into account size and shape of entire animals have been given [24] and are also described in the RF modeling [Chapter 10](#) of *BBA*.

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## 0.5 Biophysical Interactions of Fields: Ionization, Ionizing Radiation, Chemical Bonds, and Excitation

RF fields can be characterized as nonionizing radiation. By this we mean that there is not enough energy in a single quantum of energy,  $hf$ , to ionize an atom or a molecule at RFs, where  $h$  is Planck's constant and  $f$  is the frequency. By comparison radiation in the UV or x-rays often lead to ionization. It is desirable to begin by reviewing the differences between ionizing and nonionizing radiations, to explain ionization phenomena and also to discuss related excitation phenomena, which require less energy than ionization. Then a number of the proposed models concerning atomic or molecular-level interactions of fields will be introduced. A number of these theories will be discussed and their predictions compared with experimental results in later chapters, including [Chapter 5](#) through [Chapter 7](#) and [Chapter 9](#) in *BBA*; Chapter 9 and [Chapter 11](#) in *BMA*. Heating, cell excitation, electroporation, and other results of high-intensity fields have been accepted as explanations for many bioelectromagnetic phenomena. For low-intensity exposure, however, no theory is widely accepted as a general explanation for bioelectromagnetic phenomena, and few specific phenomena have accepted explanations. It is quite possible that no general explanation exists and that more than one mechanism of interaction between fields will be found to be operating, depending on the situation. Binhi's book [25] contains a good summary of most recent theoretical proposals, including comparisons with data and critiques of their strong and weak points, as well as his own theory.

We note first that the energy of electromagnetic waves is quantized with the quantum of energy (in joules) being equal to Planck's constant ( $h = 6.63 \times 10^{-34}$  J s) times the frequency. This energy can also be expressed in electron volts, i.e., in multiples of the kinetic energy acquired by an electron accelerated through a potential difference of 1 eV ( $1 \text{ eV} \approx 1.6 \times 10^{-19}$  J). Energy quanta for a few frequencies are listed in [Table 0.3](#).

Quantized energy can "excite" molecules; appropriate frequencies can couple to vibrational and rotational oscillation; and if the incident energy quantum has sufficient magnitude it can excite other changes in the electron configuration, such as changing an electron to another (unoccupied) energy level or tearing an electron away from one of the constituent atoms, the latter process called as ionization. The energy required to remove one electron from the highest energy orbit of a particular chemical element is called its "ionization potential." Typical ionization potentials are of the order 10 eV; for example, for the hydrogen atom it is 13.6 eV and for gaseous sodium it is 5.1 eV. As chemical binding forces are essentially electrostatic, ionization implies profound chemical changes. Therefore ionization by any outside agent of the complex compounds that make up a living system leads to profound and often irreversible changes in the operation of that system.

Table 0.3 shows that even the highest RF (millimeter waves) has quantum energies well below the ionization potential of any known substance; thus one speaks of nonionizing radiation when referring to electromagnetic waves below UV light frequencies. Ionizing radiation includes UV and higher frequency electromagnetic waves (x-rays,  $\gamma$ -rays).

**TABLE 0.3**

Wave and Quantum Characteristics of Various Types of Radiation

Name of Radiation or Application	Frequency (Hz)	Wavelength (m)	Energy of 1 Quantum of Radiation (eV)
UHF TV	$7 \times 10^8$	0.43	$2.88 \times 10^{-6}$
Microwave radar	$10^{10}$	$3 \times 10^{-2}$	$4.12 \times 10^{-5}$
Millimeter wave	$3 \times 10^{11}$	$1 \times 10^{-3}$	$1.24 \times 10^{-3}$
Visible light	$6 \times 10^{14}$	$5 \times 10^{-7}$	2.47
Ionizing UV	$10^{16}$	$3 \times 10^{-4}$	41.2
Soft x-ray	$10^{18}$	$3 \times 10^{-10}$	4120
Penetrating x-ray	$10^{20}$	$3 \times 10^{-12}$	$4.12 \times 10^5$

This explanation of the difference between ionizing and nonionizing radiation should not imply that nonionizing electromagnetic radiation cannot have profound effects upon inorganic and organic substances. As excitation of coherent vibrational and rotational modes requires considerably less energy than ionization, it could occur at RF; this will be discussed in later chapters. In addition, many other possible biological effects require energies well below the level of ionizing potentials. Examples are tissue heating, dielectrophoresis, depolarization of cell membranes, mechanical stress due to piezoelectric transduction, or dielectric saturation, resulting in the orientation of the polar side chains of macromolecules and leading to the breaking of hydrogen bonds. These and other mechanisms will be discussed by the authors of several chapters (see especially [Chapter 5](#) through [Chapter 7](#) of *BBA* and [Chapter 9](#) of *BMA*), who will also give estimates of rates at which energy must be delivered to produce particular effects.

Returning to the discussion of ionization, it is important to note that ionization of a chemical element can be brought about not only by absorption of electromagnetic energy, but also by collision either with foreign (injected) atoms, molecules, or subatomic particles of the requisite energy, or by sufficiently violent collision among its own atoms. The latter process constitutes ionization by heating, or thermal breakdown of a substance, which will occur when the kinetic energy of the colliding particles exceeds the ionization potential. As the average thermal kinetic energy of particles is related to temperature [26] by  $W = kT$  where  $k$  is Boltzmann's constant ( $= 1.38 \times 10^{-23}$  J/K), we find that the required temperature is

$$1.38(10^{-23})T \approx 5 \text{ eV} \approx (5)1.6(10^{-19})\text{J}$$

$$T \approx 5(10^4)\text{K}$$

which is about twice the temperature inside a lightning stroke [27] and orders of magnitude higher than any temperature obtainable from electromagnetic waves traveling through air.

Actually, initiation of lightning strokes is an example of ionization by collision with injected energetic particles. The few free electrons and ions always present in the air due to ionization by cosmic rays are accelerated by the  $E$  fields generated within clouds to velocities corresponding to the required ionization energy. Only when the field is large enough to impart this energy over distances shorter than the mean free path of the free electrons or ions at atmospheric pressure can an avalanche process take place: an accelerated electron separates a low-energy electron from the molecule with which it collides and in the process loses most of its own energy; thus, one high-energy free electron is exchanged for two free low-energy electrons and one positive ion. Both the



electrons are in turn accelerated again by the field, giving them high kinetic energy before they collide with neutral molecules; their collision produces four free electrons and the multiplication process continues. The breakdown field strength for air at atmospheric pressure is approximately  $3 \times 10^6$  V/m, implying a mean free path of electrons

$$\Delta\ell \approx [5 \text{ eV}/3 \times 10^6 \text{ V/m}] \approx 10^{-6} \text{ m}$$

However, this model is not entirely accurate because the actual mean free path corresponds to energies of the order of 0.1 eV, which is only sufficient to excite vibrational modes in the target molecule. Apparently such excitation is sufficient to cause ionization if the collision process lasts long enough [28].

Except for some laboratory conditions where a sufficiently high potential difference can be applied directly across a biological membrane to bring about its destruction, collisional ionization is generally not a factor in the interaction of electromagnetic waves with tissue: The potential difference required for membrane destruction [29] is between 100 nV and 300 mV, corresponding to a field strength of the order of  $2 \times 10^7$  V/m, assuming a membrane thickness ( $d = 100$  Å;  $E = V/d$ ). However, there is a third mechanism of ionization that is particularly important in biological systems. When a chemical compound of the type wherein positive and negative ions are held together by their electrostatic attraction, such as the ionic crystal NaCl, is placed in a suitable solvent, such as H<sub>2</sub>O, it is separated into its ionic components. The resulting solution becomes an electrolyte, i.e., an electrically conducting medium in which the only charge carriers are ions.

In this process of chemical ionization, the Na<sup>+</sup> cations and Cl<sup>-</sup> anions are separated from the original NaCl crystal lattice and individually surrounded by a sheet of solvent molecules, the “hydration sheath.” If the solvent is H<sub>2</sub>O, this process is called “hydration,” or more generally, for any solvent, “solvation.”

A dilute solution of NaCl crystals in H<sub>2</sub>O is slightly cooler than the original constituents before the solvation process, indicating that some internal energy of the system was consumed. Actually energy is consumed in breaking up the original NaCl bonds and some, but less, is liberated in the interaction between the dipole moment of the solvent molecule (H<sub>2</sub>O in our example) and the electric charges on the ions. Thus, solvents with higher relative dielectric constant  $\epsilon_r$ , indicating higher inherent electric dipole moment per unit volume ( $P$ ), solvate ions more strongly ( $\epsilon_r = 1 + P/[\epsilon_0 E]$ , where  $E$  is the electric field applied during the measurement of  $\epsilon_r$ ). For example, H<sub>2</sub>O with  $\epsilon_r \approx 80$  solvates more strongly than methanol with  $\epsilon_r \approx 33$ . For biological applications it is worth noting that solvation may affect not only ionic substances, but also polar groups, i.e., molecular components which have an inherent dipole moment, such as—C=O, —NH, or —NO<sub>2</sub>. Details of the process are discussed in texts on electrochemistry [30,31].

In biological processes not only chemical ionization and solvation of ionic compounds, but also all kinds of chemical reaction take place. One of the central questions in the study of biological effects of  $E$  and  $H$  fields is therefore not only whether they can cause or influence ionization, but also whether they can affect—speed up, slow down, or modify—any naturally occurring biologically important chemical reaction.

In Table 0.4 typical energies for various types of chemical bonds are listed. For comparison the thermal energy per elementary particle at 310 K is also shown. Complementing the numbers in Table 0.4 one should also point out that:

1. The large spread in the statistical distribution of energies of thermal motion guarantees that at physiological temperatures some molecules always have sufficient energy to break the strongest weak bonds [32].

**TABLE 0.4**

Bond and Thermal Energies

Type of Bond	Change in Free Energy (Binding Energy) kcal/mol	eV/Molecule
Covalent	50–100	2.2–4.8
Van der Waals	1–2	0.04–0.08
Hydrogen	3–7	0.13–0.30
Ionic <sup>a</sup>	5	0.2
Avg. thermal energy at 310 K	0.62	0.027

<sup>a</sup>For ionic groups of organic molecules such as COO<sup>-</sup>, NH<sub>3</sub><sup>+</sup> in aqueous solution.

2. The average lifetime of a weak bond is only a fraction of a second.
3. The weak binding forces are effective only between the surfaces in close proximity and usually require complementary structures such as a (microscopic) plug and hole, such as are thought to exist, for instance, between antigen and antibody [33].
4. Most molecules in aqueous solution form secondary bonds.
5. The metabolism of biological systems continuously transforms molecules and therefore also changes the secondary bonds that are formed.

Comparison of the last columns in Table 0.3 and Table 0.4 shows that millimeter waves have quantum energies, which are only about one order of magnitude below typical Van der Waals energies (waves at a frequency of 10<sup>12</sup> Hz with a quantum energy of 0.004 eV have a wavelength of 0.3 mm and can still be classified as millimeter waves). One might expect therefore that such waves could initiate chemically important events, such as configurational changes, by e.g., multiple transitions between closely spaced vibrational states at successively high-energy levels [46].

Energies associated with transition from one to another mode of rotation of a diatomic molecule are given by  $W = \ell(\ell + 1)A$  [26,33], where  $\ell = 0, 1, 2, 3 \dots$  and  $A = 6 \times 10^{-5}$  eV; thus an electromagnetic wave with a frequency as low as 29 GHz—still in the microwave region—can excite a rotational mode. Vibrational modes of diatomic molecules [26,33] correspond to energies of the order of 0.04 eV, requiring excitation in the IR region. Vibrational frequencies in a typical H-bonded system [34] are of the order of 3000 GHz; however, attenuation at this frequency by omnipresent free H<sub>2</sub>O may prevent any substantial effect [34].

Kohli et al. [34] predict that longitudinal and torsional modes of double helical DNA should not be critically damped at frequencies >1 GHz, although relaxation times are of the order of picoseconds, and Kondepudi [36] suggests the possibility of an influence of millimeter waves at approximately  $5 \times 10^{11}$  Hz upon oxygen affinity of hemoglobin due to resonant excitation of heme plane oscillations. Although Furia et al. [37] did not find resonance absorption at millimeter waves in yeast, such was reported by Grundler et al. [38,47]. The latter experiment has been interpreted [39,40] as supporting Fröhlich's theory of cooperative phenomena in biological systems. That theory postulates "electric polarization waves" in biological membranes which are polarized by strong biologically generated [18] fields (10<sup>7</sup> V/m). Fröhlich [41,42] suggests that metabolically supplied energy initiates mechanical vibrations of cell membranes. The frequency of such vibrations is determined by the dimensions and the elastic constants of the membranes;

based on an estimate of the sound velocity in the membrane of  $10^3$  m/s and a membrane thickness of 100 Å (equal to one half wavelength) one obtains a frequency of  $5(10^{10})$  Hz. Individual molecules within and outside the membrane may also oscillate, and frequency estimates vary between  $10^9$  Hz for helical RNA [43] and  $5 \times 10^{13}$  Hz for hydrogen-bonded amide structures [44]. As the membranes and molecules involved are strongly polarized, the mechanically oscillating dipole electromagnetic fields that are able to transmit energy, at least in some situations, over distances much larger than the distance to the next adjacent molecule.

Electromagnetic coupling of this type may produce long-range cooperative phenomena. In particular, Fröhlich [45] has shown that two molecular systems may exert strong forces upon each other when their respective oscillation frequencies are nearly equal, provided the dielectric permittivity of the medium between them is strongly dispersive or excitation is supplied by pumping, i.e., by excitation at the correct frequency from an external source. The mechanism is nonlinear in the sense that it displays a steplike dependence on excitation intensity. Possible long-range effects may be, for example, attraction between enzyme and substrate [42]. These and related topics have been discussed in detail by Illinger [34] and are reviewed in the present volume in [Chapter 11](#) and [Chapter 5](#) of *BBA*.

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## *Effects of Radiofrequency and Extremely Low-Frequency Electromagnetic Field Radiation on Cells of the Immune System*

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### 1.1 Introduction

Establishing the biological effects of exposure to electromagnetic fields (EMF), particularly extremely low-frequency (ELF, 0 to ~3000 Hz) fields and radiofrequency (RF) radiation, remains an elusive goal to this day. Over the last 20 to 30 y, hundreds of scientists

across the world have used different types of EMF and RF sources and exposure regimens on different biological experimental models, and different types of data analyses have been applied to both experimental and epidemiologic data. Despite the breadth of these studies (or perhaps because of it), no universally accepted hypotheses exist in this field of research. In this chapter, we attempt to present selected examples from the plethora of available material that best emphasize positive and negative findings from the literature pertinent to the immune system and discuss possible causes for the disparity of the published data.

Before going further into an exploration of the effects of RF and ELF EMF on the immune system, we emphasize some unique aspects of this view on the biological effects of EMF. First of all, many studies were done on the cells of the immune system because it is relatively simple to obtain these cells in a noninvasive way from humans and whole organisms; second, many chose to study effects of EMF on the immune system because this system is one of the first to interact with the environment and, it is generally believed, the most likely to be affected by stimuli from the environment. Clearly, cells of the immune system are well attuned to a broad variety of perturbations in the environment. Nevertheless, it is also true that immune cells are very difficult to study for prolonged periods since their lifetime in culture is relatively short; and any stimulus that these cells are exposed to is, of necessity, added to an already long list of stimuli to which the immune cells respond. Therefore, the immune system may be a difficult subject of study when one examines the effects of an agent without a direct target such as EMF exposure.

A broad range of different exposure conditions has been used for studies on the immune system, and often it is difficult to compare studies using such different exposure conditions. Some of the RF radiation qualities used in the studies reviewed here include continuous-wave (CW) or pulsed-wave RF radiation at 1.748, 1.9, or 2.45 GHz with power densities up to 5 mW/cm<sup>2</sup> and calculated specific absorption rates (SARs) in biological material between 0.1 and 12 W/kg. Some studies involved microwave (MW) with a frequency of 42.2 GHz, peak power density of  $31 \pm 5$  mW/cm<sup>2</sup>, and peak SARs of  $622 \pm 100$  W/kg. Another large group of RF studies included variations of different possible mobile phone type RF—plane wave or CW with frequencies around values of 900 and 800 MHz, power densities up to 950 W/m<sup>2</sup>, and SARs between 0.008 and 8.8 W/kg.

Electric and magnetic fields were, most often, tested with low-frequency (50 to 60 Hz) and magnetic field intensities between 0.2 and 500  $\mu$ T. Static electric and magnetic fields were tested as well, often with magnetic intensities as high as 10 T. Furthermore, different investigators used continuous and/or intermittent exposures; according to some authors intermittent exposures are more efficient in causing biological effects, [1] while some case report studies concentrated on exposures that included peaked EMF exposures, such as when a surge occurs as a power line is severed. Homogeneous and gradient static MF exposures were compared, e.g., Hirose and others found the gradient MF to be more biologically effective [2]. EMF pre-, co-, or post-exposures were combined with exposures to chemical (mitomycin C [MMC], vinblastine, and others) and/or physical (gamma- and x-ray ionizing radiation, UVB, and UVA radiation) mutagens or substances that are responsive to magnetic fields but are not genotoxic on their own (FeCl<sub>2</sub>). Experimental exposure lengths used varied between 5 min and 350 d, while case report studies included exposures that were years in duration.

In addition to case report studies and testing of blood samples from humans occupationally exposed to RF or ELF EMF, planned experiments were set up with biological targets that included human or rodent peripheral blood cells (PBC) exposed *ex vivo*, cultured cells of lymphoblast and monocyte lineages (Jurkat, HL-60, Mono Mac-6, K-562, U-937, and DG-75), or blood cells derived from exposed animals (Sprague-Dawley rats or mice: Swiss, C57BL/6, BALB/c, DDY, pKZ1, E mu-Pim1).

Different endpoints were tested by these studies to estimate the effects of EMF and RF radiation exposures on the immune system. We have broadly grouped the studies reviewed here into several groups based on the most significant endpoints of the study.

The effects of any type of stress on the immune system include multiple events in different cell types, and it is very rare that any study of a stress factor includes more than several endpoints. These always represent only a fraction of all the ongoing events. Cells of the immune system interact intensely with each other and their surroundings, and the more elaborate the conditions of exposure, e.g., *in vivo* exposure compared to less complex *in vitro* exposure, the more numerous these interactions will be. Therefore, it is not really possible to extrapolate results of studies involving PBC *in vitro* to the possible fate of the same population of cells exposed *in vivo*. In different studies presented here, the same endpoint was often pursued in systems with different complexities.

At present, many groups are using recently developed tools for studies of gene expression such as microarrays or different proteomics approaches; unfortunately, at the moment, these data are still largely unpublished. One rare example of this type is work by Olivares-Banuelos on chromaffin cells [3]. In these studies, chromaffin cells were allowed to differentiate in the EMF before RNA harvesting, which may limit the applicability of the data. It is clear that the wealth of information obtained from microarray and proteomics studies will have a great impact on the field.

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## 1.2 Proliferation, Activity, and Function of Lymphocytes

There are several recent studies that have quantified the number of lymphocytes and their subtypes in humans exposed to whole-body RF or ELF EMF. In some cases, comparisons between exposed and control individuals were made, while in others, the results obtained on the same individuals were compared over time. Data obtained were not consistent from one study to another and often were not consistent even within the same study. While this may be explained by insufficiently controlled exposure conditions in some cases, it is far more likely that the differences in the results obtained are based on the different experimental conditions and individual differences among the subjects of these studies.

### 1.2.1 Human Studies

An interesting but small study compared six individuals, occupationally exposed for 8 h/d for 5 y to low-frequency (50 Hz) EMFs of 0.2 to 6.6  $\mu\text{T}$ , with six control subjects [4]. Total lymphocytes, CD4+, and CD3+ cell counts decreased, while natural killer (NK) cells increased at statistically significant levels. Six months after cessation of exposure, total lymphocyte counts had significantly increased, particularly CD4+, CD3+, and CD19+ counts (+28, +22, and +17%, respectively), in the same human subjects. The authors proceeded to expose 12 Swiss male mice to identical conditions for 109 d, comparing them to 12 additional mice as unexposed controls. Similar to the human data, total lymphocytes, leukocytes, polymorphonuclear neutrophils, and CD4+ cells were significantly lower in exposed animals. However, unlike the human studies, the NK counts were lower in the exposed mice at the time of completion of the exposure. In their earlier work, the same authors investigated 13 subjects occupationally exposed for 5 y to low-frequency (50 Hz) EMF of 0.2 to 6.6  $\mu\text{T}$  [5]. In comparison to 13 controls matched for gender, age, and socioeconomic status, the exposed group showed a significant decrease in total lymphocytes,



CD4+, CD3+, and CD2+ lymphocytes and a rise in NK cells. Contradictory results were published by others who counted lymphocyte subsets and found that the number of NK cells showed statistically significant differences compared to controls in workers occupationally exposed to magnetic fields generated at induction heaters [6]. Another similar study was done on subjects exposed to ELF EMF during electric arc welding (field intensities: 0.10 and 0.25 mT) [7]. Comparisons were done between 16 male welders and 14 healthy males who were 20 to 40 y of age, from the same geographic area, and with similar lifestyles. It was found that while the hematocrit levels of the welders had elevated, their CD4+ and CD8+ cells had decreased.

### 1.2.2 Peripheral Blood Cells Cultured *Ex Vivo*

PBC were obtained from a number of individuals and in some cases pooled together. This led to an increase in variability within individual exposure samples, while differences from one sample to another could be, in essence, disregarded. The effect of 60 Hz sinusoidal magnetic fields (MF) at magnetic flux densities of 1.0, 1.5, and 2.0 mT on human PBC cultures was studied at an exposure time of 72 h and, in some cases, with MMC treatment [8]. MF treatment was shown to increase cell proliferation; and even cells treated with MMC and MF showed a higher proliferation index—a reversal of the decrease in proliferation induced by MMC treatment alone. The same was true for the mitotic index as well.

Capri and others exposed peripheral blood mononuclear cells (PBMC) *ex vivo* to a 900 MHz global system of mobile communication (GSM) mobile telephone or CW RF field for 1 h/d for 3 d in a transverse electromagnetic mode (TEM) cell system (70–76 mW/kg of average SAR) [9]. A slight decrease in cell proliferation was found following stimulation of PBMC with the lowest mitogen concentration, while a slight increase in the number of cells with an altered distribution of phosphatidylserine across the membrane was observed following exposure to GSM, but not CW RF field.

### 1.2.3 Animal Studies

Many experiments have been done using animal systems. In these experiments, contradictory data were obtained. Often a decrease in the number of lymphocytes was noted, similar to the data obtained in human exposures; however, in some cases an increase in proliferation was observed as well, occasionally in the same experiments [10].

C57BL/6 mice, exposed for 2 h/d for 1, 2, or 4 weeks in a TEM cell of 900 MHz GSM-modulated radiation and a SAR of 1 or 2 W/kg, were monitored for effects on spleen cells. The authors tested splenic cellularity, the percentages of B and T cells, and the distribution of CD4+ and CD8+ populations and found that none of these parameters were altered by the exposure. Nevertheless, at 1 week of exposure to 1 or 2 W/kg, an increase in IFN-gamma production was observed, which was lost after prolonged exposure for 2 or 4 weeks [11].

Continuous exposure of twelve 6-week-old Swiss mice for 350 d to ELF EMF of 5.0  $\mu$ T was generated by a transformer station and high current bus bars [10]. When compared on different days, the exposed animals sometimes showed a decrease in the numbers of leukocytes and/or neutrophils in comparison to controls (days 20, 90), while at other times they did not (days 43, 63, 350). Individual differences among the mice were also observed, and two of the exposed animals showed a significant increase in leukocyte and polymorphonuclear neutrophil counts compared to the unexposed mice.

Murabayashi and others investigated the effect of ELF-pulsed magnetic fields on proliferation (measured by bromo-2'-deoxy-uridine incorporation) of mitogen concanavalin

A (ConA)-stimulated spleen lymphocytes from DDY mice for 72 h [12]. Exposure times varied between 20 min and 2 h. The authors found that TVMF exposure augmented proliferation if the exposure took place within the first 40 min following ConA stimulation, but not afterward. They speculated that  $\text{Ca}^{2+}$  influx may be responsible for this early ConA response.

Another study concentrated on the effects of 25–150 mT (250–1500 Gauss) static magnetic fields' (SMF) exposure on the cellular immune functions of macrophages, splenic lymphocytes, and thymic cells isolated from the C57BI/6 mice and cultured *ex vivo* [13]. A decrease in phagocytic uptake of fluorescent latex microspheres and increased intracellular  $\text{Ca}^{2+}$  levels were found in macrophages; mitogenic responses in lymphocytes measured by [ $^3\text{H}$ ]thymidine incorporation were decreased, while Con A-stimulated lymphocytes showed an increased  $\text{Ca}^{2+}$  influx. Thymocytes showed enhanced levels of apoptosis, as determined by flow cytometry.

Exposure of the nasal area of BALB/c mice for 30 min/d for 3 d to low-power electromagnetic millimeter waves (frequency: 42.2 GHz; peak incident power density:  $31 \pm 5 \text{ mW/cm}^2$ , and peak SAR at the skin surface:  $622 \pm 100 \text{ W/kg}$ ) combined with treatment with cyclophosphamide (CPA) at 100 mg/kg, acting as an immunosuppressant, was studied [14]. The mice exposed to combination treatment showed statistically significant proliferation recovery of splenocytes and altered activation of CD4+ T cells.

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### 1.3 Cell Death

A large number of studies examined potential detrimental effects of EMF exposure on lymphocytes.

#### 1.3.1 Cell Culture and Peripheral Blood Cells *Ex Vivo* Studies

PBMC from human volunteers were exposed to a 10-T SMF [15]. In the absence of lymphocyte stimulation, there were no significant differences in the viability of the exposed and unexposed T cells, B cells, and NK cells. However, when T cells were activated by mitogen phytohemagglutinin (PHA), both the CD4+ and CD8+ T cell populations expressed enhanced death due to apoptosis. Another report on the exposure of cultured human lymphoblast cancer cells lines predominantly of the myeloid lineage (K-562, U-937, DG-75, and HL-60) to a pulsating EMF (sinusoidal wave form, 35 mT peak, 50 Hz) for 4 h tested the cells for cell death at the conclusion of exposure or 24 h later [16]. Necrosis was induced in these experimental conditions, but the extent of the reaction depended also on the medium composition, its pH value, conductivity, and temperature.

Jajte and others exposed rat lymphocytes for 3 h to 7 mT SMF and 10  $\mu\text{g/ml}$  of  $\text{FeCl}_2$ . This resulted in an increase in the percentage of apoptotic and necrotic cells [17].

Negative results were also obtained from these types of experiments. For example, Ikeda and others exposed human PBMC to three different magnetic field polarizations at 50 and 60 Hz: linearly polarized (vertical) with flux densities of 500, 100, 20, or 2  $\mu\text{T}$ ; circularly polarized; and elliptically polarized with flux density of 500  $\mu\text{T}$  [18]. The authors used the cytotoxicity assay and found no effects of ELF MFs on cytotoxicity of human PBMC.

### 1.3.2 Studies in Animals

Cell death of thymocytes during thymus involution has been shown to be accelerated by continuous light exposure. Studies on the effects of long-term exposure to extremely low-frequency (50 Hz) sinusoidal electric and magnetic fields at two levels of field strength on thymocytes of rats housed in a regular dark/light cycle or under continuous light were done to test thymus involution-promoting effects of EMF [19]. The concurrent exposure to continuous light and ELF EMF significantly increased the number of cell deaths, in comparison with animals exposed to continuous light only or with animals exposed to EMF in a 12 h dark–light cycle. The authors concluded that the long-term exposure to ELF EMF in animals experiencing a photic stress leads to a more rapid thymus involution.

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## 1.4 Micronucleus Formation

A large number but not all studies that examined micronucleus formation following RF or ELF EMF exposure resulted in acquisition of positive data.

### 1.4.1 Cell Culture and Peripheral Blood Cells *Ex Vivo* Studies

Human PBC were exposed *ex vivo* for 15, 30, or 60 min to different EMF frequencies (2.45 and 7.7 GHz) and power densities (10, 20, and 30 mW/cm<sup>2</sup>) [20]. Exposures for 30 min or more to a power density of 30 mW/cm<sup>2</sup> led to formation of an increased number of micronuclei. On the other hand, another group of investigators studied the effect of 15 min *ex vivo* exposure to 1.748-GHz, continuous (CW) or phase-only modulated wave (GMSK) on human peripheral blood lymphocytes (PBL) [21]. The maximum SAR was 5 W/kg. No changes were found in cell proliferation kinetics after exposure to either field; but the micronucleus frequency showed a statistically significant increase following exposure to GMSK, but not CW. Tice and others studied the effects of RF signals emitted by four different types of cellular telephones: analog signal generator with voice modulated at 837 MHz, time division multiple access (TDMA) voice modulated at 837 MHz, 837 MHz code division multiple access (CDMA) without voice modulation, and 1909.8 MHz GSM-type personal communication systems (PCS) with voice modulation [22]. Exposure of PBL for 24 h at an average SAR of 5.0 or 10.0 W/kg to any one of the four RF sources produced a significant and reproducible increase of micronucleated lymphocytes.

Human PBC from 20 donors were exposed to EM field at a frequency used for mobile telephones (900 Hz) using CW-intermittent exposure (SAR: 1.6 W/kg) for 14 cycles with a 6 min exposure and a 3 h pause, a GSM signal and the same type of intermittent exposure, a GSM signal and the same type of intermittent exposure 24 h before stimulation with PHA (8 on/off cycles), and a GSM signal, with intermittent exposure (SAR: 0.2 W/kg) for 1 h/d for 3 d [23]. No statistically significant changes in micronucleus frequency or cell cycle kinetics under any of the experimental conditions were found.

Lymphocytes from 18 individuals were exposed to 50 Hz ELF EMF of 80 and 800  $\mu$ T for a 72 h period and challenged with vinblastine in concentrations of 0, 5, 10, or 15 ng/ml during the last 48 h of this period [24]. Vinblastine- and ELF EMF-exposed groups expressed a higher proportion of micronuclei than the group with vinblastine treatment alone. On the other hand, an increase in the nuclear division index (NDI) was observed in the ELF EMF 800  $\mu$ T treated group.

### 1.4.2 Studies in Animals

Rats exposed for 24 h to 2450 MHz CW RF radiation at an average whole-body SAR of 12 W/kg showed no increase of micronuclei in PB or bone marrow cells [25].

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## 1.5 Chromosome Damage (Sister Chromatid Exchange, Aberrations, etc.)

Understanding whether EMF causes chromosomal damage would play a large role in determining any possible role exposure might play in the onset or progression of cancers, particularly those of the immune system. Several studies have examined the role of EMF exposure on the development of chromosomal abnormalities in lymphoid cells.

### 1.5.1 Studies on Human Subjects

Skyberg and others performed a study on 24 employees engaged in high-voltage testing and generator soldering and 24 controls from a Norwegian transformer factory [26]. Blood samples taken from the subjects were either analyzed immediately or the lymphocytes were briefly cultured and treated with hydroxyurea and caffeine to inhibit DNA synthesis and repair. In the latter type of cultures, samples from the high-voltage laboratory testers showed twice the median number of sister chromatid breaks (5 vs. 2.5 per 50 cells) compared to controls. The median number of aberrant cells was 5 vs. 3.5 for the high-voltage laboratory testers compared to controls.

### 1.5.2 Cells in Culture and Peripheral Blood Cells Cultured *Ex Vivo*

In another experiment, PBL were exposed to continuous 830 MHz EMFs at average SARs between 1.6 and 8.8 W/kg [27]. The exposure occurred for 72 h in a parallel plate resonator at temperatures between 34.5 and 37.5°C. The authors found an increase in chromosome 17 aneuploidy as a function of the increased SAR value.

Cho and Chung used human lymphocytes to investigate combined effects of ELF EMF and benzo(a)pyrene (BP) on the frequency of micronuclei and sister chromatid exchange (SCE) [28]. BP co-exposure with a 60 Hz field of 0.8 mT for 24 h, followed by BP exposure for 48 h led to significant increases in the frequencies of micronuclei and SCE compared to BP-alone treatment for 72 h. At the same time, no significant difference was observed between ELF EMF and sham-exposed control cells. The authors concluded from these findings that low-density ELF EMF is not an initiator, but a promoter of carcinogenesis.

Another group investigated 50 Hz magnetic fields alone or in combination with either MMC or x-rays, and found that magnetic fields up to 2500  $\mu$ T did not change the frequencies of the chromosome aberrations and SCE, either alone or in combination with the mutagens [29]. Nevertheless, there were exceptions—504  $\mu$ T MF treatment before and during cultivation showed a significant decrease in SCE frequency, while cells cultivated at 88.4  $\mu$ T showed a significant increase in chromosome aberration frequency following x-ray exposure when compared to x-ray exposure alone.

Vijayalaxmi and others tested the effect of exposure to 835.62 MHz RF radiation, a frequency employed in customer-to-base station transmission of cellular telephone communications, on PB samples [30]. An analog signal generator and the frequency division multiple access (FDMA, CW) technology were used; a forward power of 68 W was used,

with a power density of 860 W/m<sup>2</sup>; and the mean SAR was 4.4 or 5.0 W/kg. Following a 24 h exposure, the lymphocytes were stimulated with the mitogen PHA and cultured for an additional 48 or 72 h to assess the frequencies of chromosomal aberrations and micronuclei. No evidence of either was found.

The same group also tested the effect of exposure to 847.74 MHz CW RF on PB samples from four individuals [31]. CDMA technology was used, with a nominal net forward power of 75 W, a nominal power density of 950 W/m<sup>2</sup>, and a mean SAR of 4.9 or 5.5 W/kg. No differences between test and control samples were found for cellular mitotic indices; frequencies of exchange aberrations; or presence of excess fragments, binucleate cells, or micronuclei.

Another group exposed human blood lymphocytes simultaneously to 2.0 Gy of  $\gamma$  rays and a 50 Hz power frequency magnetic field of 0.23, 0.47, or 0.7 mT [32]. No differences in the frequency of chromosomal aberrations were observed between cells not exposed to or co-exposed to EMF.

### 1.5.3 Studies in Animals

In one of the recent articles dealing with chromosome aberrations in RF-exposed animals, the authors have concentrated on comparing 4 W/kg pulsed 900 MHz RF delivered daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms (GSM pulsing pattern) for 1, 5, or 25 d. They studied somatic intrachromosomal recombination in the spleen in recombination mutagenesis model beta-galactosidase transgenic mouse pKZ1 [33]. While somatic intrachromosomal recombination inversion events are usually found in spleen cells of these transgenic mice, a decrease in the frequency of inversions was observed in the 25 d exposure group.

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## 1.6 DNA Damage as Single-Strand and Double-Strand Breaks

A variety of studies have been done to examine the effects of EMF on single-strand (SSB) and double-strand breaks (DSB), predominantly as an assessment of the ability of the exposure to induce DNA damage and therefore have mutagenic potential. Most of the results demonstrated little effect of EMF exposure on DNA damage.

PBC were used to study the effect of a 2 h-long exposure to pulsed-wave 2450 MHz RF radiation with a net forward power of 21 W, transmitted from a standard gain rectangular antenna horn in a vertically downward direction [34]. The average power density at the position of the cells in the flask was 5 mW/cm<sup>2</sup>, while the SAR was 2.135  $\pm$  0.005 (SE) W/kg. To estimate primary DNA damage (SSB and alkali-labile lesions), the authors used the alkaline comet assay performed immediately after the exposures or 4 h post exposure—a sensitive method for monitoring DNA strand breaks. No RF exposure-related increase in DNA damage was observed.

Another group investigated the effect of a CW 1.9 GHz RF on PBC using a series of six circularly polarized, cylindrical waveguides [35]. Mean SARs of 0.0, 0.1, 0.26, 0.92, 2.4, and 10 W/kg were achieved, while the temperature was maintained steadily at 37.0°C. Exposures lasted 2 h, and no increase of primary DNA damage or micronuclei was found under any of the experimental conditions. The same investigators also studied the effect of a pulse-modulated 1.9 GHz RF field on human leukocytes [36]. Again, six circularly polarized cylindrical wave guides were used. Mean SARs ranged from 0 to

10 W/kg, and the temperature was maintained at 37°C. Exposures lasted 2 h, and no primary DNA damage was detected by the alkaline comet assay, nor were there any changes in the frequency of binucleated or micronucleated cells in comparison to sham-treated controls.

Maes and others studied effects of a 900 MHz RF radiation with different modes of exposure (continuous, pseudorandom, and dummy burst) and different power outputs (0, 2, 8, 15, 25, 50 W) on human blood cells collected from six donors [37]. The SAR varied between 0 and 10 W/kg. The authors investigated the effects of the 900 MHz radiation alone or in combination with a subsequent exposure to the chemical or physical mutagens, MMC (0.1 µg/ml) and x-rays (1 Gy exposure). A transverse electromagnetic transmission line (TEM) cell was used and PB cells were exposed for 2 h. No indication was found for mutagenic and/or co-mutagenic/synergistic effects between EMF and mutagen exposure under these experimental conditions.

Nevertheless, some reports suggest that EMF can serve as a co-mutagen and increase the amount of damage accumulated, most likely by slowing down repair processes. This was also reported recently in some nonlymphoid cell types, e.g., ELF EMF potentiates x-ray-induced DNA strand breaks in glioma MO54 cells [38]. Also, Nindl and others tested the combined effects of a low-frequency, low-intensity EMF of 100 Hz, 1 mT and UVB irradiation on Jurkat cell line [38]. When the UVB exposure was accompanied by EMF exposure, a decrease in DNA repair synthesis of  $34 \pm 13\%$  was observed, compared to UVB treatment alone.

Zhang and others studied exposure to 2450 MHz MW radiation (5.0 mW/cm<sup>2</sup>) concomitantly with MMC (0.0125, 0.025, or 0.1 µg/ml) and found synergistic genotoxic effects using single-cell gel electrophoresis comet assay and the cytokinesis-blocked micronucleus test *in vitro* [39]. Pooled whole blood cells from a male human donor and a female human donor were exposed to MW for 2 h, MMC only, or MMC for 24 h after exposure to MW. The last of these led to significant increase in DNA damage.

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## 1.7 Leukemia and Lymphoma

Leukemia and lymphoma of the immune system, just as any other cancer, develop following accumulation of several mutations in the same cell clone. Therefore, a potential mutagenic influence of EMF on the immune system could result in the development of these types of cancer. For reasons listed in [Section 1.1](#), and because of the intrinsic complications with evaluation of the epidemiologic data, we limit ourselves to work done in animal models. Recent results obtained in experimental systems targeted at studying links between RF radiation or EMF exposure and the development of leukemia and lymphoma have often been negative.

For example, in 1997 Repacholi and others found increased odds' ratio (OR) of 2.4,  $P = 0.006$ , 95% confidence interval (CI) of 1.3–4.5 for the development of lymphoma in lymphoma-prone E mu-Pim1 transgenic mice exposed to pulse-modulated RF fields twice for 30 min/d for up to 18 months (plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz, pulse width of 0.6 ms; incident power densities were 2.6 to 13 W/m<sup>2</sup>, and SAR of 0.008 to 4.2 W/kg, averaging 0.13–1.4 W/kg) [40]. However, no such increased risk of lymphomas was found when Utteridge and others exposed 120 E mu-Pim1 heterozygous mice and 120 wild-type mice to GSM-modulated 898.4 MHz radiation for 1 h/d, 5 d/week at SARs of 0.25, 1.0, 2.0, and 4.0 W/kg in "Ferris-wheel" exposure systems for up to 104 weeks (26 months) [41]. As controls, 120 heterozygous and

120 wild-type mice were sham exposed, and some additional animals served as an unrestrained negative control group.

Other recent animal studies have involved 4 groups of 50 female CBA/S mice, 3 to 5 weeks of age, to test the effects of combined exposure to ionizing radiation and RF radiation [42]. At the beginning of the study, the mice were irradiated and then exposed to RF radiation for 1.5 h/d, 5 d a week for 78 weeks. Two sources of RF were used: a continuous NMT (Nordic Mobile Telephones)-type frequency-modulated RF radiation at a frequency of 902.5 MHz and SAR of 1.5 W/kg, and a pulsed GSM-type RF radiation with a carrier wave frequency of 902.4 MHz, pulse frequency of 217 Hz, and SAR of 0.35 W/kg. No change in cancer incidence was found in association with RF exposure. Nevertheless, the same group found that co-exposure to low-level RF radiation and ultraviolet (UV) light slightly accelerated skin cancer development [43]. The same was true for co-exposure to UV and 50 Hz magnetic fields [44].

Therefore, for RF radiation exposures, the consensus at this moment is that they have no lymphoid cancer-initiating or -promoting capacity (see, e.g., the review by Elwood) [45].

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## 1.8 Possible Mechanisms of Action of Radiofrequency Radiation and Electromagnetic Field

As evidence accumulates that direct DNA damage is not induced by either RF radiation or EMF exposure and yet at the same time cellular damage such as micronuclei formation frequently arises from these exposures, many different avenues are being pursued in the search for possible mechanisms of action of RF and ELF EMF. Some more recent ideas have proposed that RF and ELF EMF interact with electric charges in proteins and nucleic acids at the level of the molecules themselves, perturbing their electron clouds. Blank and Goodman found DNA sequences, which they call *electromagnetic field response elements* in the promoters of c-myc and HSP70 genes [46]. They maintain that EMF exposure-dependent activation of these genes relies on destabilization of hydrogen bonds in these DNA response elements under the influence of the field [47]. On the other hand experimental proofs of local changes in protein conformation, other than for *in vitro* experiments with isolated proteins, are still largely unavailable. For example, protein aggregation and the formation of amyloid fibers are increased by RF exposures to 1 GHz at SAR of 0.5 W/kg [48]. Several of the “older” ideas and some supporting, as well as contradictory findings, especially in the cells of the immune system, are presented below, including Ca<sup>2+</sup> homeostasis, nonthermal heat shock effects, gene/protein activation, and induction of reactive oxygen species (ROS).

### 1.8.1 Ca<sup>2+</sup> Homeostasis

Mixed results were obtained from studies investigating changes in Ca<sup>2+</sup> as a potential biochemical pathway that mediates biological effects of EMF. The assumption was that the magnetic fields increase the cytosolic calcium concentration in lymphocytes in the same manner as a physiological stimulus, e.g., interaction with antibodies directed against the CD3 complex. Nevertheless, in some cases, nearly identical experimental conditions were used with different outcomes, e.g., the work by Lindstrom and others in 1993 and 1995 and that by Wey and others in 2000 using Jurkat T-cells exposed to 50 Hz MF. [49–51] Below are several such studies as an illustration of the breadth of the results obtained.

Studies showing no effect of RF exposure on  $\text{Ca}^{2+}$  flux were obtained by Cranfield and others who tested the effect of continuous-wave and pulsed-wave GSM mobile phone signals (915 MHz, 2 W/kg) on calcium concentration, using Fluo-3 fluorescence, in the Jurkat human lymphocyte cell line treated (or not) with PHA [52]. The only changes were found in PHA-activated cells treated by the pulsed wave.

Positive effects of exposure were obtained in another group of studies where a 2–150 mT SMF exposure of macrophages and ConA-stimulated lymphocytes from the C57BL/6 mice cultured *ex vivo* led to increased intracellular  $\text{Ca}^{2+}$  levels [13]. This was accompanied by a decrease in phagocytic uptake of fluorescent latex microspheres by macrophages. Aldinucci and others tested the effects of a combination of a static EMF on Jurkat cells and human lymphocytes at a flux density of 4.75 T and a pulsed EMF with a flux density of 0.7 mT generated by an NMR apparatus [53]. The authors monitored movements of  $\text{Ca}^{2+}$ , cell proliferation, and the production of cytokines following 1 h exposure in cells treated by the EMF fields alone or cells additionally activated by 5 mg/ml of PHA immediately before the exposure. EMF induced movements of  $\text{Ca}^{2+}$ , but had no other effect, be it proliferative, activating, or proinflammatory, on nonstimulated or on PHA-stimulated lymphocytes. In Jurkat cells (T-cell lineage), exposure to EMF led to a decreased intracellular  $\text{Ca}^{2+}$  concentration and proliferation.

Another group of investigators studied the effects of low-intensity 60-Hz electric fields (E: 0.5 V/m; current density: 0.8 A/m<sup>2</sup>) on agonist-induced  $\text{Ca}^{2+}$  flux in HL 60 cells (myeloid lineage) [54]. The 60 min exposure to the E field was accompanied by the addition of 1  $\mu\text{M}$  ATP or 100  $\mu\text{M}$  histamine. ATP-activated and E field-exposed cells showed a 20–30% decrease in intracellular  $\text{Ca}^{2+}$  concentration compared to ATP alone, while histamine and E field-exposed cells showed a 20–40% increase of intracellular  $\text{Ca}^{2+}$ .

McCreary and others examined the influence of the cell cycle, pH of the medium, and response to a calcium agonist to changes in  $\text{Ca}^{2+}$  in Jurkat E6.1 cells treated by 60 Hz, 100  $\mu\text{T}$  (peak) sinusoidal AC magnetic field (MF), 78  $\mu\text{T}$  static MF (DC), and the combination of both MFs [55]. When the effect of the cell cycle and the quality of the alpha-CD3 monoclonal antibody response were taken into account, all exposure groups were significantly different from the control.

### 1.8.2 Nonthermal Heat Shock Effects

Some of the earliest literature on EMF-related biological effects implicated heat shock proteins in biological response to EMF; however, due to possible “contamination” of EMF effects with thermal effects, it took long to prove that the nonthermal effect of EMF also induced heat shock proteins and cell behaviors [56]. In recent years more articles have been published on the nonthermal EMF effects [57]; listed below are a few that focused predominantly on cells of the immune system.

Sarimov and others investigated GSM MWs at different frequencies ranging between 895 and 915 MHz in experiments with PBL from seven healthy persons [58]. Exposures were done in transverse electromagnetic transmission line cell (TEM cell) using a GSM test-mobile phone, 2 W output power in the pulses, and SAR of 5.4 mW/kg. The authors measured changes in chromatin conformation by anomalous viscosity time dependencies’ method and found that 30 min exposure to all tested MW qualities led to chromosome condensation similar to the effects of heat shock in lymphocytes of at least some (1 in 5 to 4 in 5) of the tested individuals.

Belyaev and Alipov studied the effect of ELF EMF of 21  $\mu\text{T}$  rms on human lymphocytes, using anomalous viscosity time dependencies to measure chromosome condensation [59]. The authors observed two resonance-type frequency windows with strong effects



on human lymphocytes at 8 and 58 Hz and proceeded to compare these frequency windows with predictions at frequencies of harmonics and subharmonics for natural isotopes of Na, K, Ca, Mg, and Zn ions, based on the ion cyclotron resonance model and the magnetic parametric resonance model. The overall good overlap between these frequencies suggests that the effective resonance-type frequency windows may interfere with most abundant cellular ions.

On the other hand, human PBC exposed to 900-MHz CW and GSM fields at three average SARs of 0.4, 2.0, and 3.6 W/kg for 20 min, 1 h, or 4 h in a TEM cell at 37°C and 5% CO<sub>2</sub> showed no changes in heat shock proteins, hsp70 and hsp27, using flow cytometry approaches [60]. Similarly, exposure of PBMC from young and old donors to RF radiation (1800-MHz RF; three signal modulations of the GSM-type: GSM Basic, discontinuous transmission, and Talk; at SARs of 1.4 and 2.0 W/kg) does not affect apoptosis or hsp70 level [61].

### 1.8.3 Gene and Protein Activation

In a number of studies, different proteins have been reported as activated by EMF or RF radiation. Some of the more recent examples are listed below.

Cultured human monocytes (Mono Mac-6) were exposed to high peak power pulsed RF at a frequency of 8.2 GHz. The peak to average power ratio was 455:1; 2.2 μs pulse width pulse repetition rate of 1000 pulses/s; average power density was 50 W/m<sup>2</sup>; SAR was 10.8 ± 7.1 W/kg at the position where the cells were located [62]. Following a 90 min exposure and 4 h incubation, the cells were harvested and an increase of 3.6-fold was detected in the DNA-binding activity of NF-κB transcription factor from nuclear extracts.

DNA-binding activity of the transcription factor cyclic-AMP responsive element-binding protein (CREB) changes following exposure of HL60 cells to a 0.1 mT 50 Hz ELF sinusoidal MF [63]. Magnetic field exposure induced increased binding for CREB *cis* element after 10 min, reached a peak at 1 h, and returned to basal level 4 h after exposure. Moreover, a new ATF2/ATF2 homodimer was formed after MF exposure for 30 min, 1, and 2 h. The authors argue that this activity was dependent on both extracellular and intracellular Ca<sup>2+</sup>. Romano-Spica and others used an exposure system generating 50 MHz RF nonionizing radiation modulated with a 16 Hz frequency (field intensity of 0.2 μT), magnetic field parallel to the ground, and a 60 V/m electric field orthogonal to the earth's magnetic field and found overexpression of the *ets1* mRNA in Jurkat cells [64]. This occurred only at 16 Hz modulation; according to the authors, this is due to correspondence with the resonance frequency for calcium ions with a DC magnetic field of 45.7 μT. A static MF exposure system with a spatially inhomogeneous 6 T strong MF having a gradient of 41.7 T/m or that with a spatially homogeneous 10 T static MF was compared as well [2]. Exposure to the gradient MF induced c-Jun protein expression in HL-60 cells exposed for 24 to 72 h.

### 1.8.4 Induction of Reactive Oxygen Species

Some investigators believe that EMF or RF exert its effects on the biological systems by enhancing (or creating) ROS. ROS are known to mediate a large variety of biological consequences, including DNA damage and possibly mutation induction. In a recent article, Simko and Mattsson have provided some lines of evidence suggesting that ELF EMF exposure increases the level of free radicals in cells through several routes mainly relying on the activation of macrophages (or other ROS-producing cell type) [65].

Zmyslony and others tested combined effects of 930 MHz CW RF, a carrier frequency for signals emitted by cellular phones, and FeCl<sub>2</sub> on creation of ROS in cells [66]. ROS were measured by the fluorescent probe—dichlorofluorescein diacetate. Exposure of rat lymphocytes was done in a GTEM cell, and the power density was 5 W/m<sup>2</sup> and the calculated SAR was 1.5 W/kg. RF radiation exposures alone of 5 and 15 min did not alter production of ROS, but combined with addition of 10 μg/ml FeCl<sub>2</sub> a significant increase of ROS occurred. The same authors also tested the effects of co-exposure to UVA (150 J/m<sup>2</sup>) and EMF (50 Hz, 40 μT rms) on rat lymphocytes [67]. UVA exposure of 5 min accompanied by 5 or 60 min of exposure to EMF showed that 60, but not 5, minutes' exposure to EMF coupled with UVA increased damage to DNA, which the authors attributed to increased ROS presence in doubly exposed cells. Similarly, for lymphocytes exposed to 7 mT static SMF and FeCl<sub>2</sub>, a combined 3 h exposure to SMF and 10 μg/ml FeCl<sub>2</sub> led to an increase in the percentage of apoptotic and necrotic cells [17]. This was accompanied by a significant increase in the amount of lipid peroxidation end products, MDA+4 HNE.

Moreover, Ding and others exposed HL-60 cells simultaneously to an ELF magnetic field (60 Hz, 5 mT) and H<sub>2</sub>O<sub>2</sub> (85 or 100 μM) [68]. A 24 h co-exposure significantly decreased the number of viable cells in comparison to cells treated with H<sub>2</sub>O<sub>2</sub> alone, thereby establishing ELF EMF as an apoptosis promoter. Interestingly, while no changes in Bax and Bcl-2 protein levels or caspase-3 activation were observed, activation of caspase-7 as well as poly (ADP-ribose) polymerase cleavage increased.

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## 1.9 Conclusions—Possible Explanations of Contradictory Results

Brusick and others reviewed 100 different studies and concluded that the vast majority of adverse effects from exposure to high frequencies and high-power intensities of RF radiation are the result of hyperthermia; nevertheless, the authors indicate that there may be some subtle and indirect effects on the DNA replication and/or gene regulation under restricted exposure conditions [69]. Black and Heynick reviewed more recent literature on RF EMF and concluded that leukocytes are much more sensitive to RF EMF than erythrocytes, but that these effects are consistent with systemic temperature fluctuation [70]. Moreover, lifetime studies of RF EMF-exposed animals did not show cumulative adverse effects on their immune systems. Meltz emphasized similar points about RF, claiming that for all tested frequencies and modulations regardless of length of exposure, there is no induction of DNA damage on any scale (from mutations to chromosome aberrations) or promotion of DNA damage caused by chemical or physical mutagens; in other words no evidence that RF is either a carcinogen, cocarcinogen, tumor inducer, or tumor promoter [71]. The only biological effect of RF with acknowledged mixed evidence is that certain RF exposure conditions cause micronuclei formation. Reviewing the epidemiologic data, Elwood similarly emphasized on the inconsistency of data obtained from the effects of RF radiation exposures [45].

Perhaps the best summary of the controversy in the field of RF-related biological research is the review done by Vijayalaxmi [72]. She listed findings from the period 1990–2003 and calculated percentages of studies that showed or did not show biological effects of exposure to RF radiation. Negative reports comprised 58% of RF-related literature, positive 23%, and inconclusive 19%.

Research related to biological effects of ELF EMF is even more riddled by conflicting findings. Some reviews attempted to deal with it by limiting the literature available to that describing a specific experimental setup. McCann and others reviewed studies concerning

potential genotoxicity of electric and magnetic fields and ascribed the highest merit to studies fulfilling one of the following criteria: ELF magnetic fields, 150  $\mu$ T to 5 mT; combined ELF electric and ELF magnetic fields, 0.2 mT and 240 mV/m; and SMFs, 1–3.7 T [73]. The authors believed that some of the studies showing positive data met these criteria, but nevertheless wanted independent repetition before they could be admitted as truly positive data [74–80].

Miyakoshi reviewed findings on the effects of static MF at the cellular level and summarized that most but not all studies suggested that this type of MF has no effect on growth rate or cell cycle distribution [81]. On the other hand, (co-)genotoxicity of static MF following exposure to known mutagens, e.g., X-irradiation, or coupled with treatment with trace amounts of ferrous ions, appears to be firmly established.

At this juncture, it is also relevant to mention studies of Marino and others who developed a novel statistical procedure that can be adjusted to detect both nonlinear and linear effects of treatments of biological samples [82,83]. In their experiments (exposure of male and female mice to 100 or 500  $\mu$ T at 60 Hz for 1 to 105 d or continuously for 175 d), the authors found statistically significant repression of lymphocytes that could, nevertheless, be scored only when the data were analyzed as if they were governed by nonlinear laws. When the relationship of the immune variables and the EMF was modeled as nonlinear, the results started to show a pattern, which suggested that lymphocytes respond to and are vulnerable to EMF transduction, but that it may be impossible to predict specific changes of immune-system variables, due to the innate nonlinearity of the regulatory mechanisms of the immune system. Moreover, the authors hypothesize that the inconsistencies in EMF research stem from the attempt to use linear methods and models to study inherently nonlinear input–output relationships and believe that the underlying behavior of the immune system may be extremely sensitive to its prior states.

In conclusion, the final pattern emerging from all of these studies suggests that while exposure to EMF and RF radiation has no direct effect on DNA, it does under some circumstances exert a biological effect reminiscent of heat shock and/or stress. This effect is mild and heavily dependent on the state of cell homeostasis prior to exposure. When experiments were done on animals, individual variation was often more pronounced than the results of the exposures. Moreover, most protracted exposure experiments on animals found that the dysbalance in the cell homeostasis introduced at the beginning of exposure was most often overcome before the end of the experiment.

Considering the variations in responses to EMF and RF, it is very difficult to either outrightly accept or reject any study that was controlled for “contamination” with thermal stress and included reasonable biological negative controls. In other words, the fact that the majority of studies failed to produce reproducible results depends on the innate complexity of the experimental design rather than any intrinsic “falseness” of the tests conducted. The immune system is one of the most important lines of defense of an organism against the hostile environment. The provocations coming from the environment can be met by the immune system with vehemence or almost passively, and two organisms of the same species do not always react the same way to the same stimuli. Furthermore, even a single individual does not respond the same way to the same stimulus at different times; as the immune system adjusts to the environment reactions to certain stimuli become intensified, while others gradually decrease.

Taking into consideration that many studies of the effects of EMF exposure showed opposite results for both EMF and RF exposures, we can either concern ourselves with trying to “prune” these data until they produce a coherent picture, or we can approach it in an all-encompassing manner. And while the first approach inevitably leads to the conclusion that the data “average” into a negative result, the second approach brings several tentative views on possible effects of EMF and RF exposure(s). If we consider the

totality of the data, we can claim that the effects of these exposures are mild in comparison to other known stressors, such as heat shock, ionizing radiation, nutrient deprivation, etc. and unpredictable based on our current modeling capabilities. Therefore, the most significant role of these exposures is likely going to be to intensify the effects of exposures to other physical or chemical stresses. Any future studies in this field would probably benefit the most from work *in vivo*, using protracted exposures and combining EMF and RF exposures with exposures to known stress factors. Needless to say, a very rigorous control of the exposure conditions and use of multiple biological “negative controls” would be a necessary condition of any such experimentation.

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# 2

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## *Evaluation of the Toxicity and Potential Oncogenicity of Extremely Low-Frequency Magnetic Fields in Experimental Animal Model Systems*

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David L. McCormick

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## 2.1 Introduction

Over the past two decades, the possible relationship between exposure to power frequency (50 and 60 Hz) electromagnetic fields (EMF) and adverse human health outcomes has received significant attention in both the scientific community and the general population. Based on widely circulated accounts in the popular press [1], and on mass media reports of the results of selected epidemiologic investigations [2,3], a public perception has developed that human exposure to EMF may be associated with a range of adverse health effects, including reproductive dysfunction, developmental abnormalities, and cancer. The results of a number of epidemiologic studies provide limited support for the hypothesis that EMF exposure may be associated with an increased risk of neoplasia in several organ sites in humans. Among these sites, the hematopoietic system, breast, and brain have been identified most commonly as possibly sensitive targets for EMF action (reviewed in [4]).

Although individual epidemiologic studies provide suggestive evidence of the potential oncogenicity of EMF, the total body of epidemiologic data linking EMF exposure and cancer risk is by no means conclusive. Since 1990, more than 50 epidemiologic studies designed to investigate the possible association between occupational or residential exposure to magnetic fields and cancer risk have been published (reviewed in [5]). The methods used in these more recent studies are often substantially improved in comparison to methods used in earlier investigations; specific areas of improvement include the use of larger sample sizes and better exposure assessment. However, despite these improvements in epidemiologic methods, EMF cancer epidemiology studies continue to generate both positive and negative results. When considered together, the results of these studies are insufficient to either support or refute the hypothesis that exposure to EMF is a significant risk factor for human cancer.

In situations where epidemiology does not support the conclusive identification and quantitation of the potential risks associated with exposure to an environmental agent, laboratory studies conducted in appropriate experimental model systems increase in importance. Well-designed and controlled animal studies permit evaluation of biological effects *in vivo* under tightly controlled exposure and environmental conditions, and in the absence of potential confounding variables. In consideration of the conflicting results of EMF epidemiologic studies, and difficulties associated with exposure assessment in such studies, animal studies may provide the best opportunity to identify effects of EMF exposure that could translate into human health hazards.

The body of published scientific literature addressing the potential toxicity and oncogenicity of EMF in animal model systems has expanded greatly in the past 20 y. This chapter provides a brief review and analysis of this literature. For the purposes of the chapter, published toxicologic and carcinogenic bioassays of EMF in animal model systems are divided into five sections.

- In [Section 2.2](#), studies designed to investigate the general and organ-specific toxicity of exposure to power frequency EMF are summarized. These studies include both general toxicity bioassays that have been conducted using standardized rodent model systems, and specialized toxicologic studies that are designed to identify possible specific toxic effects of EMF such as developmental toxicity (teratology), reproductive toxicity, or immunotoxicity.
- In [Section 2.3](#), investigations into the possible oncogenicity of EMF as a single agent are reviewed. The most comprehensive studies reviewed in this section are chronic oncogenicity bioassays that are performed in rodents using standardized toxicity testing protocols. The designs of these studies include evaluations of the risk of oncogenesis in essentially all major organ systems; the experimental designs have been developed to satisfy the safety assessment requirements of regulatory agencies such as the United States Environmental Protection Agency and the United States Food and Drug Administration. Over several decades, an extensive database has been developed that supports the utility of the chronic oncogenicity bioassay in rodents as a predictor of oncogenicity in humans.
- In [Section 2.4](#), studies designed to evaluate the activity of EMF exposure as a cocarcinogen or tumor promoter will be reviewed. These studies involve simultaneous or sequential exposures to EMF in combination with another agent in order to identify possible activity as a tumor initiator, tumor promoter, or cocarcinogen in specific organ sites. Most such studies have been conducted using animal models that were designed primarily for use as research tools to study site-specific carcinogenesis. As a result, the utility of these assays as

predictors of human oncogenicity is less completely understood than the results of the 2 y bioassay.

- **Section 2.5** encompasses a discussion of studies in which the potential oncogenicity of EMF exposure has been evaluated using genetically modified (transgenic or gene knockout) animals. Via insertion of cancer-associated genes (oncogenes) or deletion of tumor suppressor genes from the germ line, transgenic and knockout animal strains have been developed that demonstrate a genetic predisposition to neoplasia. The use of these models to evaluate the potential oncogenicity of EMF exposure may identify biological effects that occur in sensitive subpopulations, but which may not occur in the general population. Given the relative novelty of the genetically engineered model systems used in these studies, however, little information exists on which their utility as predictors of human oncogenicity can be evaluated.
- **Section 2.6** is a brief review of potential biological mechanisms through which EMF exposure has been proposed to induce or stimulate carcinogenesis. An exhaustive compilation and discussion of the literature related to all possibly relevant biological activities of EMF is well beyond the scope of this chapter. For this reason, this section is focused specifically on the effects of EMF exposure on biochemical and molecular markers that may be mechanistically linked to the process of neoplastic development, with specific emphasis on studies performed in whole animal model systems.

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## 2.2 Animal Toxicity Bioassays of Electromagnetic Fields

### 2.2.1 General Toxicologic Bioassays of Electromagnetic Fields

Although many thousands of humans have received occupational exposure to EMF for extended periods, no documented case reports of generalized human toxicity resulting from EMF exposure have been published in the peer-reviewed literature. On this basis, it is generally agreed that acute, subchronic, or chronic exposure to EMF is not a significant risk factor for systemic or generalized toxicity in humans. Because certain occupational environments contain high-field areas in which EMF flux densities often exceed residential EMF levels by factors of tens to hundreds, it is logical to expect that populations working in such high-field environments would be the primary groups in which EMF toxicities would be observed. Because no case reports of EMF-associated generalized toxicity or malaise in any exposed worker have been published, it is logical to conclude that EMF exposure is not a significant risk factor for the induction of such generalized toxicity.

The lack of systemic toxicity of EMF exposure in humans is clearly supported by the results of acute and subchronic bioassays conducted in animal model systems. In these studies, rats or mice have been exposed to EMF flux densities ranging from low milli-Gauss (mG) levels up to maximum flux densities as high as 20 G (Gauss; equivalent to 2000  $\mu$ T; bioassays conducted in different laboratories have studied the effects of both linearly polarized and circularly polarized magnetic fields.

Although the experimental end points evaluated in these bioassays vary by study, most include evaluations of the possible effects of EMF exposure on survival, overall health as monitored by clinical and physical observations, body weight, food consumption, hematology, clinical chemistry, and histopathologic evaluation of tissues. Although

occasional instances of statistically significant differences in EMF-exposed animals from sham-exposed controls have been observed in one or more end points, no reproducible pattern of EMF toxicity has been identified in any bioassay with known predictability for human responses (see for example [6–8]; other studies reviewed in [4]). The lack of hematologic toxicity of EMF identified in these animal studies is consistent with the data from an experimental exposure study conducted in humans [9].

Although long-term studies have generally been focused on the possible activity of EMF as a carcinogen or tumor promoter, many of the chronic EMF exposure studies (discussed in succeeding sections of this chapter) also included evaluations of toxicologic end points such as body weight, clinical and physical observations, and hematology. As was the case with the acute and subchronic exposure studies, studies involving chronic EMF exposures have failed to identify any evidence of systemic toxicity (see for example [10–12]).

Summarizing the available data, no evidence of generalized toxicity associated with EMF exposure has been identified in epidemiologic studies of human populations receiving occupational exposure, or in experimental exposure studies in either animals or humans. On this basis, it can be concluded that the risks of generalized or systemic toxicity resulting from acute, subchronic, or chronic exposure to EMF are very small.

### **2.2.2 Developmental and Reproductive Toxicity Studies of Electromagnetic Fields**

Investigations into the possible developmental and reproductive toxicity of EMF were stimulated by early reports of teratogenesis in chick embryos exposed to high-EMF flux densities (reviewed in [13]). Although the processes of prenatal development in chickens differ substantially from those in humans, the induction of terata in early chick embryos has been interpreted by some investigators to support the hypothesis that EMF is a teratogen. By contrast, however, teratologists who use animal models to develop human hazard assessments for chemical and physical agents have questioned the value of the chick embryo model for human hazard identification. In consideration of both the positive and negative results of studies designed to examine the effects of EMF exposure on chick embryo development, and the unknown ability of studies in chickens and other avian species to predict human developmental toxicity, data from developmental toxicity studies of EMF in chickens are considered to offer relatively little insight into human hazard assessment [13].

It should be noted, however, that the early teratologic data generated in chicken models were extended by reports of small increases in the incidence of birth defects and spontaneous abortions in women who used electric blankets and electric water bed heaters during pregnancy (reviewed in [4]). These early findings were hypothesis generating, but were limited in that they were retrospective studies that included no measurements of EMF exposure. Furthermore, the design of these studies essentially precluded the separation of possible EMF effects from thermal effects that could result from the use of an electric blanket or bed heater.

Since the publication of these early findings, a large number of epidemiologic studies have been performed to investigate the hypothesis that EMF exposure is a risk factor for teratogenesis. These studies have examined the incidence of birth defects, growth retardation, and premature delivery, among other end points, in the offspring of women who used electric blankets or electric bed heaters during pregnancy. Other studies have examined rates of these abnormalities in pregnant women with different levels of residential EMF exposure and in pregnant women with different measured or imputed levels of occupational EMF exposure. In general, although occasional EMF-associated differences have been reported, the results of these studies failed to confirm the hypothesis that

EMF exposure during pregnancy is a significant risk to the developing fetus (reviewed in [4]). In most studies, no statistically significant relationships have been found between EMF exposure and the risk of birth defects or other developmental abnormalities. The source of the EMF exposure (use of an electric blanket or bed heater, overall higher level of residential EMF, or occupational EMF exposure) was inconsequential with respect to possible effects on the risk of teratogenesis.

Supporting these epidemiologic data are the negative results of more than a dozen teratology studies in which pregnant rodents were exposed to EMF flux densities of up to 20 G (2000  $\mu$ T; cf. [14–18]; others reviewed in [13]). It is important to note that the rodent models, study designs, and periods of exposure used in several EMF developmental toxicity studies have been used for many years to evaluate the possible teratogenicity of pharmaceuticals and environmental chemicals; these models have been validated extensively, and have been shown to be generally predictive of human responses. The results of these EMF teratology studies, which have been conducted by several different teams of investigators in several different countries, have been uniformly negative.

When the negative results generated in these experimental evaluations of EMF teratogenesis are considered together with the negative results of most epidemiologic studies, the overwhelming majority of available teratology data do not support the hypothesis that exposure to power frequency EMF during pregnancy constitutes an important hazard to the developing fetus. Interestingly, although Mevissen et al. [19] found no significant adverse effects of fetal exposure to 50 Hz EMF in rats, they reported a significant reduction in fetal survival in rats receiving gestational exposure to 30 mT static magnetic fields.

As is the case with developmental toxicity (teratology) studies of EMF, no compelling body of evidence from animal studies implicates EMF as a significant risk for either male or female fertility. The total number of relevant studies is small, and includes assessments of the potential reproductive toxicity of both power frequency electric and magnetic fields ([20,21]; other studies reviewed in [22]). However, the results of these studies, which have commonly involved exposure of several generations of rodents to EMF, have failed to identify any decreases in fertility in either male or female animals receiving such exposures.

### **2.2.3 Immunotoxicity Studies of Electromagnetic Fields**

Evaluation of the possible effects of EMF exposure on immune function is important to support our understanding of the possible role of EMF in carcinogenesis and as a modifier of host resistance to infection. Any reproducible downregulation of immune function in EMF-exposed animals could have important consequences in terms of the host's ability to resist infectious disease challenge and to provide effective immune surveillance against neoplastic cells.

Over the past 20 y, an increasing database has developed in which immunotoxic effects observed in rodent models have been linked to similar effects in humans. On this basis, the results of immune function studies in established rodent models appear to provide the best experimental tool to predict possible effects in humans. Studies have been reported in which the effects of EMF on immune-related parameters have been evaluated in non-rodent species such as sheep [23]; however, the utility of these species as predictors of human responses is unknown. For this reason, the most relevant data set for the possible effects of EMF exposure on immune function is that which has been generated in rodents.

In the rodent studies, a battery of immune function assays (lymphoid organ weight, lymphoid organ cellularity, lymphoid organ histology, B- and T-cell-mediated immune responses, natural killer (NK) cell function, lymphocyte subset analysis, host resistance

assays) was evaluated in mice or rats exposed to magnetic fields flux densities of up to 20 G for period ranging from 4 weeks to 3 months. The results of these studies demonstrated that EMF-exposed animals demonstrated no deficits in lymphoid organ weights, lymphoid organ cellularity, or lymphoid organ histology; similarly, no deficits in T- or B-cell function were identified, and lymphocyte subset analysis did not reveal any effects of EMF exposure [24,25]. Interestingly, however, a statistically significant suppression of NK cell function was seen in two studies performed in female mice; this alteration was not seen in male mice of the same strain or in either sex of rat evaluated in a parallel study [24,26].

Although the authors conclude that this suppression of NK cell function is real [26], it appears to have little or no biological significance. The primary role of NK cells appears to be in immune surveillance against cancer cells. However, completely negative results were obtained in a 2-y carcinogenesis bioassay of EMF performed in the same strain of mice that demonstrated the reduction in NK cell function [12]. Because the deficit in NK cell function was not associated with an increase in cancer risk, it is concluded that either (a) the quantitative reduction (~30%) in NK cell function induced by EMF is too small to be biologically significant, or (b) other arms of the host immune system were able to perform required immune surveillance in these animals.

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## **2.3 Assessment of the Possible Oncogenic Activity of Electromagnetic Field Exposure Using Chronic Exposure Studies in Rodents**

### **2.3.1 Design of Chronic Exposure Studies**

Several teams of investigators have published the results of studies that are designed to investigate the possible carcinogenic activity of chronic exposure to EMF alone (as opposed to EMF exposure in combination with simultaneous or sequential exposure to other agents). These studies have generally been conducted using a standardized study design that is commonly referred to as the chronic oncogenicity bioassay in rodents. This study design has been used widely as the basis to evaluate the potential carcinogenicity of new drugs, agricultural chemicals, occupational chemicals, and a wide range of environmental agents, and is considered to be a useful predictor of human oncogenicity. Both the International Agency for Research on Cancer (IARC) and United States National Toxicology Program (NTP) have performed analyses demonstrating the utility of the rodent chronic bioassay as a predictor of predict human carcinogenicity. As a result, it is generally accepted among toxicologists that the chronic rodent bioassay currently provides the best available experimental approach to identify agents that may be carcinogenic in humans.

In the chronic rodent oncogenicity bioassay, groups of rats or mice are exposed to the test agent for a period that encompasses the majority of their normal life span (2 y in most studies; some studies in mice are conducted for 18 months). The bioassay includes a standard series of in-life toxicologic observations and evaluations; however, its key experimental end point is histopathology.

Histopathologic evaluations performed at the conclusion of chronic rodent bioassays generally include classification of all gross lesions identified at necropsy, and examination of 45 to 50 tissues from each animal in the high dose and control groups. In addition to the complete histopathologic evaluations of high dose and control animals, histopathology is generally performed on gross lesions and identified target tissues in animals in the middle and low dose groups.



A significant strength of the use of the chronic rodent bioassay to identify potentially carcinogenic agents is the ability to compare tumor incidence patterns of exposed groups to both contemporaneous controls, i.e., the control group from that specific study, as well as to tumor incidence patterns in historical control animals. Historical control databases for tumor incidence are commonly maintained both within conducting laboratories and by animal suppliers. The historical control database is particularly large for studies conducted for the NTP. Because all rats and mice used in NTP chronic oncogenicity studies are bred in the same animal colonies, the NTP database includes the results of tumor incidence data generated during histopathologic evaluations of many thousands of control animals.

### 2.3.2 Results of Chronic Rodent Oncogenicity Bioassays

#### 2.3.2.1 IIT Research Institute (United States): Chronic Oncogenicity Bioassays in Rats and Mice

The largest and most comprehensive evaluations of the potential carcinogenicity of EMF exposure in rodents were conducted at IIT Research Institute (IITRI), under the sponsorship of the NTP. The quality and comprehensive nature of these studies was specifically highlighted in a commentary written by the Director of the Food and Drug Administration's National Center for Toxicological Research; this commentary was published in *Toxicologic Pathology* [27].

In this evaluation program, parallel studies were conducted in F344 rats [11] and in B6C3F1 mice [12]. In each study, groups of 100 animals per sex were exposed for 18.5 h/d for 2 y to pure, linearly polarized 60 Hz magnetic fields. It is useful to note that this group size is twice that commonly used in chronic rodent bioassays; this increase in group size confers increased statistical power to the experimental design, and thereby increases its ability to identify weak effects. Experimental groups included animals receiving continuous exposure to field strengths of 20 mG (equivalent to 2  $\mu$ T), 2 G (200  $\mu$ T), or 10 G (1000  $\mu$ T), or intermittent exposures (1 h on/1 h off) to 10 G (1000  $\mu$ T). Parallel sham control groups were housed within an identical exposure array, but were exposed to ambient magnetic fields only.

The design of these studies included continuous monitoring of magnetic field strength and waveform throughout the 2 y exposure period. At termination, all animals received a complete necropsy, and complete histopathologic evaluations were performed on all gross lesions collected from all study animals, in addition to approximately 50 tissues per animal in all study groups.

The results from these studies provided no evidence of increased cancer incidence in any of the putative target tissues (hematopoietic system, brain, breast) for EMF oncogenicity, and do not support the hypothesis that EMF is a significant risk factor for human neoplasia in any site:

- The results of the study conducted in B6C3F1 mice demonstrated no increases from sham control in the incidence of neoplasia in any tissue. When compared to sham controls, the only statistically significant differences in tumor incidence in groups exposed to EMF were:
  - (1) A statistically significant *decrease* in the incidence of malignant lymphoma in female mice exposed intermittently to 10 G EMF
  - (2) Significant *decreases* in the incidence of lung tumors in both sexes of mice exposed to EMF at 20 mG or 2 G (but not at 10 G)

- The results of the oncogenicity study conducted in F344 rats also failed to provide evidence to support the hypothesis that EMF is a significant risk factor for human cancer. Similar to the results of the study in mice, no significant increases in the incidence of neoplasia in the hematopoietic system, breast, or brain were seen in any study group. Significant differences from control tumor incidences included:

- (1) A statistically significant *decrease* in the incidence of leukemia in male rats exposed intermittently to 10 G EMF
- (2) A statistically significant *decrease* in the incidence of preputial gland carcinomas in male rats exposed to 2 G (but not to 10 G) EMF
- (3) A statistically significant *decrease* in the incidence of trichoeplitheliomas of the skin in male rats exposed continuously to 10 G EMF
- (4) A statistically significant *decrease* in the incidence of adrenal cortical adenomas in female rats exposed intermittently to 10 G EMF
- (5) A statistically significant *increase* in incidence of thyroid C-cell tumors in male rats exposed to EMF at field strengths of 20 mG or 2 G (but not at 10 G)

When considered together, the results of these studies provide no evidence that EMF exposure is a significant risk factor for human cancer. No increases in the incidence of neoplasia in the brain, breast, or hematopoietic system were observed in either sex of either species in any group receiving chronic EMF exposure. Similarly, although a number of statistically significant differences from control tumor incidence were observed in the two studies, seven of the nine differences were *reductions*, rather than increases in tumor incidence.

### **2.3.2.2 Institut Armand Frappier (Canada): Chronic Oncogenicity Bioassay in Rats**

Scientists at the Institut Armand Frappier (IAF) in Laval, Quebec conducted a chronic bioassay in which F344 rats were exposed to pure, linearly polarized 60 Hz magnetic fields for 20 h/d for 2 y [25]. Although group sizes used in the IAF study were smaller than those used in the chronic rat oncogenicity study conducted at IITRI, the experimental designs of the two rat oncogenicity bioassays were generally comparable. Two factors did distinguish the experimental design of the IAF study:

- EMF exposures were initiated 2 d before animal birth, rather than in young adult animals
- Study participants were “blinded” to identify study groups until after study completion

The most common approach to the conduct of rodent oncogenicity bioassays is to begin exposure to the test agent when animals are young adults (6 to 8 weeks old), and to identify the exposure status of each experimental group so that all study participants are aware of group identities. Because the IAF study included exposures during the perinatal and juvenile periods, its design addresses the possibly enhanced sensitivity of younger animals to EMF bioeffects. Furthermore, the conduct of the bioassay using a blinded design precludes any possible influence of investigator bias on study results.

The design of this study included 50 rats per sex per group. Experimental groups included cage controls, sham controls, and groups exposed to EMF at field strengths of 20 mG (2  $\mu$ T), 200 mG (20  $\mu$ T), 2 G (200  $\mu$ T), and 20 G (2000  $\mu$ T). Toxicologic end points

included a standard battery of in-life evaluations and histopathologic evaluation of gross lesions and approximately 50 tissues per animal.

Similar to the data generated in the IITRI studies, the results of the IAF study failed to support an association between EMF exposure and increased risk of neoplasia in the brain, breast, hematopoietic system, or any other tissue examined. The study authors summarize their data by stating that “no statistically significant, consistent, positive dose-related trends with the number of tumor-bearing animals per study group could be attributed to EMF exposure.” Although not statistically significant at the 5% level of confidence, total tumor incidence in all groups exposed to magnetic fields was *decreased* in comparison to tumor incidences observed in the cage control and sham control groups.

In the IAF study, the incidences of brain tumors (astrocytomas, gliomas, etc.), malignant mammary tumors (adenocarcinomas), and leukemias were low in all groups, and demonstrated no pattern of differences between control groups and groups exposed to EMF. Benign mammary tumors (fibroadenomas) were common in all groups, and were present in similar incidences, regardless of EMF exposure. The most common tumor identified in the study was a benign pituitary tumor (adenoma); although differences did not always attain statistical significance, the incidences of pituitary adenomas in all groups exposed to EMF were *decreased* from those seen in both the sham control group and the cage control group. *In toto*, the results of the IAF study are comparable to those from the IITRI study, and extend these results to include EMF exposures that were initiated during the perinatal period.

### **2.3.2.3 Mitsubishi Kasei Institute (Japan): Chronic Oncogenicity Bioassay in Rats**

Scientists at the Mitsubishi Kasei Institute (MKI) of Toxicological and Environmental Sciences, Ibaraki, Japan conducted a 2 y bioassay in F344 rats to evaluate the potential oncogenicity of chronic exposure to linearly polarized, 50 Hz EMF [28]. The 50 Hz sine wave is the primary component of power frequency EMF associated with the generation and distribution of electricity in Europe and Asia. In this study, groups of 48 rats per sex were exposed for >21 h/d for 2 y to 50 Hz EMF at field strengths of 5 G (500  $\mu$ T) or 50 G (5000  $\mu$ T); an identically sized control group received sham exposure for the same period. At study termination, all animals received a complete necropsy. Complete histopathologic evaluations were performed on all gross lesions, and approximately 45 additional tissues per animal from all animals in all study groups.

Survival was comparable in all study groups, and differential white blood cell counts performed after 52, 78, and 104 weeks of exposures failed to identify any effects of EMF exposure. Consistent with the results of studies conducted at IITRI and IAF, the MKI study found no increased risk of neoplasia in animals receiving chronic exposure to EMF. The MKI team reported that EMF exposure had no effect on animal survival in either sex, and had no effect on the total incidence or number of neoplasms. Within a sex, incidences of leukemia, brain tumors, and mammary tumors in groups exposed to EMF were comparable to those of sex-matched sham controls.

The only statistically significant histopathologic findings from the study were an *increase* in the incidence of a benign lesion (fibroma) in the subcutis of male rats exposed to 50 G EMF, and a *decrease* in the total incidence of invasive neoplasms in male rats in the 5 G EMF group. Although the increased incidence of fibromas in male rats exposed to 50 G EMF was statistically significant when compared to concurrent male sham controls, lesion incidence was comparable to that observed in historical controls in the laboratory. On this basis, the authors concluded that this increase in fibroma incidence in rats exposed to 50 G EMF was not biologically significant. Similarly, because no differences in the incidence of metastatic neoplasms were seen in the study, the observed decrease in the incidence of invasive malignancy appears to be without biological significance.

#### **2.3.2.4 Overview of Chronic Rodent Oncogenicity Studies**

Although minor differences can be identified, the overall experimental designs used in the bioassays performed at IITRI (United States), IAF (Canada), and MKI (Japan) were very similar; all study designs conform with harmonized regulatory requirements for chronic oncogenicity testing in rodents. The results of the four studies conducted in these three laboratories are also in close agreement. In all studies, chronic exposure to power frequency EMF had no effect on the incidence of neoplasia in any of the three putative targets for EMF action (brain, breast, and hematopoietic system). Furthermore, chronic exposure to EMF did not induce any pattern of biologically significant increases in cancer incidence in other tissues. The very close comparability of the results of four chronic rodent oncogenicity bioassays conducted independently in three laboratories strengthens the conclusion that exposure to magnetic fields does not induce cancer in rodents. On the basis of the demonstrated predictive nature of the rodent 2 y bioassay, these findings do not support the hypothesis that EMF exposure is a significant risk factor in the etiology of human neoplasia.

### **2.3.3 Results of Site-Specific Oncogenicity Bioassays in Rodents**

#### **2.3.3.1 University of California at Los Angeles (United States): Lymphoma and Brain Tumor Bioassay in Mice**

A chronic murine bioassay was conducted at the University of California at Los Angeles (UCLA) and Veterans Administration Hospital to evaluate the potential interaction between EMF and ionizing radiation in the induction of hematopoietic neoplasia in mice [29]. Although the primary focus of this study was the interaction between EMF and ionizing radiation, the study design did include several experimental groups that were exposed to EMF alone. Neoplastic responses in EMF-exposed and sham control groups that received no exposure to ionizing radiation are suitable for use in assessing the potential oncogenicity of exposure to EMF.

In this study, one group of 380 female C57BL/6 mice was exposed to circularly polarized 60 Hz EMF at a field strength of 14.2 G (1.42 mT) for up to 852 d. The incidence of hematopoietic neoplasia in these EMF-exposed mice was compared with that observed in a negative (untreated) control group consisting of 380 female C57BL/6 mice, and in a sham control group consisting of 190 female C57BL/6 mice. In addition to the investigation of EMF effects on hematopoietic neoplasia, a *post hoc* histopathologic analysis of brain tissues from this study was performed to investigate the possible activity of EMF as a causative agent for primary brain tumors [30].

Chronic exposure to EMF had no statistically significant effects on animal survival or on the incidence or latency of hematopoietic malignancy in this study [29]. At study termination, the final incidence of lymphoma in mice exposed to circularly polarized EMF was 36.8%, as compared to a lymphoma incidence of 34.7% in sham controls. The incidence of histiocytic sarcomas was 23.7% in mice receiving EMF exposure versus 22.1% in sham controls, yielding a total incidence of hematopoietic neoplasia of 56.3% in sham controls versus a total incidence of 59.2% in the group exposed to circularly polarized EMF. Relative risks for various types of hematopoietic neoplasia in the EMF group ranged from 0.94 to 1.03; all 95% confidence intervals included 1.00, and none differed from sham control at the 5% level of significance.

Consistent with the results for hematopoietic neoplasia, histopathologic evaluation of brains from this study provided no support for the hypothesis that EMF exposure is a significant risk factor for brain tumor induction. In this study, brains from a total of 950 nonirradiated mice were evaluated (total of control and EMF-exposed groups that

received no exposure to ionizing radiation); only two benign brain tumors were identified [30]. Because the incidence of brain cancer in the negative control group, sham control group, and the EMF-exposed group were all below 0.25%, these data provide no evidence that exposure to circularly polarized EMF is oncogenic in the brain of C57BL/6 mice.

Although lack of proximity to power transmission and distribution lines ensures that most humans are not exposed to circularly polarized EMF, the possible carcinogenicity of circularly polarized fields has received only very limited study. The primary strengths of this study are that it evaluated the potential oncogenicity of a previously unstudied exposure metric (circularly polarized rather than linearly polarized EMF), and used very large experimental groups. The large group sizes used in the study greatly increase its statistical power, and therefore improve its ability to identify effects of modest magnitude.

It must be noted, however, that the study design was quite limited in that it included histopathologic evaluations of only the hematopoietic system, lymphoid system, and brain. As such, while the study data provide no support for the hypothesis that EMF exposure is causally associated with the induction of neoplasia in the hematopoietic system and brain, the lack of systematic evaluation of other tissues precludes any conclusions about possible influences on neoplasia in other sites.

### **2.3.3.2 Technical University of Nova Scotia (Canada): Lymphoma Bioassay in Mice**

Using a unique study design in which three consecutive generations of CFW mice were exposed to extremely high flux densities (250 G [25 mT] of 60 Hz EMF [31]), data were presented that suggested an increased incidence of malignant lymphoma in the second and third generations of exposed animals. The strength of this study lies in its innovative design involving exposure of multiple generations, and its examination of flux densities that greatly exceed those that have been used in other studies. In consideration of the exposure regimen used in this study, it can be argued that this investigation provided the maximum likelihood of identifying a positive oncogenic activity of EMF. Indeed, this study is the only report in the peer-reviewed literature in which long-term EMF exposure in otherwise untreated animals has been associated with an increased incidence of malignancy.

Although the results of this study were clearly a novel finding, it must be noted that the experimental design and interpretation of this study has been strongly criticized for several reasons [32,33]. These reasons include:

- Group sizes are small and variable, and the number of observed malignant lesions is very small.
- Exposure assessment, environmental control, and control of possible confounding of study results by the heat and vibration generated by the EMF exposure system present serious questions as to the validity of results. Indeed noise and vibration associated with ventilation equipment needed to dissipate the heat generated by the EMF generation and exposure system as well as temperature control for exposed animals were very different than control animals that were housed in a different room, and were not exposed to these environmental factors.
- The onset of lymphoma in mice is age related, yet the study design included large discrepancies between the ages of EMF-exposed mice and the control groups to which they were compared.
- Peer review of photomicrographs indicates that some lesions identified by the investigators as neoplastic may, in fact, represent hyperplastic rather than malignant lesions.

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## 2.4 Assessment of the Possible Cocarcinogenic or Tumor-Promoting Activity of Electromagnetic Field Exposure Using Multistage Rodent Models

It is generally accepted that exposure to power frequency EMF does not induce mutations or other genetic damage that is identifiable using standard genetic toxicology model systems (reviewed by McCann et al. [34]). Because EMF is apparently not genotoxic, it is logical to conclude that any oncogenic effects of exposure are mediated through cocarcinogenic or tumor promotion mechanisms. To address this possibility, a relatively large number of *in vivo* studies have been conducted to identify possible cocarcinogenic or tumor-promoting effects of EMF in various target tissues. The design of these studies involves simultaneous or sequential exposure to EMF in combination with another agent, and generally focuses on neoplastic development in a specific organ. It should be noted, however, that most multistage tumorigenesis studies of EMF have been conducted using animal models that were designed as research tools to study site-specific carcinogenesis. As a result, the utility of these assays as predictors of human oncogenicity is less completely understood than are the results of the chronic oncogenicity bioassay.

The design of most multistage tumorigenesis studies is based on the initiation–promotion paradigm that was originally developed in mouse skin. In the initiation–promotion model in mouse skin, mice are exposed to a single subthreshold dose of a genotoxic agent (e.g., benzo[*a*]pyrene or 7,12-dimethylbenz[*a*]anthracene [DMBA]), followed by repetitive administration of a nongenotoxic agent (e.g., 12-*O*-tetradecanoylphorbol-13-acetate). Over the past 50 y, this paradigm has been extended to include tumor induction models in more than a dozen organ sites, several of which have been used in EMF studies. Most multistage evaluations of the potential oncogenicity of EMF have been performed in animal models for neoplastic development in the breast, brain, skin, liver, and hematopoietic system.

In view of the relative large literature related to possible tumor promotion by EMF, analysis and discussion of all possibly relevant multistage oncogenicity evaluations is beyond the scope of this chapter. For this reason, discussion in this section is limited to a brief review of the studies that have been conducted in organ systems that have been suggested by epidemiologic data as likely targets for EMF oncogenicity (brain, breast, and hematopoietic system).

### 2.4.1 Design of Multistage Oncogenicity Studies

The design of multistage oncogenicity studies is site specific: the selection of animal species and strain, carcinogen exposure regimen, observation period, and end point analyses vary by animal model system. As a general rule, animals are exposed to one or a few doses of a genotoxic agent (the initiator), which is then followed by subchronic or chronic exposure to EMF (the putative promoter). Many, but not all, multistage studies that are designed to evaluate the activity of EMF as a promoter include a positive control group that is exposed to the same dose of initiating agent, followed by exposure to an agent with known promoting activity.

In consideration of their site specificity, multistage tumorigenesis studies are often conducted in a single sex, and employ group sizes that are smaller than those used in chronic rodent bioassays. The species and strain of animals used in these organ-specific tumorigenesis studies are selected on the basis of previous literature demonstrating appropriate sensitivity to neoplastic development in the organ site of interest. Under

optimal conditions, the carcinogen-dosing regimen (initiating regimen) is selected to be a threshold dose that will induce a low incidence (<10%) of neoplasia in otherwise untreated animals; unfortunately, the value of a several multistage tumorigenicity evaluations of EMF is undermined by inappropriate selection of initiator doses. The period of observation and type of end point observations are also model specific; optimal study design in these areas is based on previous studies using the same model system.

#### **2.4.2 Multistage Oncogenicity Studies in the Brain**

The peer-reviewed literature contains two reports of experimental studies in which the activity of 60 Hz EMF as a promoter of brain tumor induction has been evaluated. These reports include an evaluation of the activity of EMF as a promoter of neurogenic tumors induced in F344 rats by transplacental exposure to *N*-ethyl-*N*-nitrosourea (ENU) [35], and a study to evaluate the activity of EMF as a promoter of brain tumors induced in C57BL/6 mice by ionizing radiation [30]. Neither study demonstrated any significant activity of EMF as a promoter of brain tumor induction.

##### **2.4.2.1 Institut Armand Frappier (Canada): Brain Tumor Promotion Study in Rats**

Mandeville et al. [35] have reported a study to evaluate the influence of EMF exposure on the induction of brain and spinal tumors by transplacental exposure to the nitrosamide, ENU. The transplacental ENU model in rats has been used widely in studies of neurogenic carcinogenesis, and is considered to be an appropriate model system for such evaluations.

In this study, groups of 50 pregnant female F344 rats received a single intraperitoneal dose of 5 mg ENU/kg body weight on day 18 of gestation. The F1 generation was exposed to EMF beginning on day 20 of gestation and continuing until study termination; flux densities (20 mG [2  $\mu$ T], 200 mG [20  $\mu$ T], 2 G [200  $\mu$ T], and 20 G [2000  $\mu$ T]) and environmental conditions in the laboratory were identical to those used in the chronic oncogenicity bioassay conducted at IAF.

EMF exposure had no effect on animal survival in the study, and had no influence on the incidence of neurogenic tumors. The results of this study do not support the hypothesis that EMF is a potent promoter of neural tumorigenesis.

##### **2.4.2.2 University of California at Los Angeles (United States): Brain Tumor Promotion Study in Mice**

A chronic murine bioassay was conducted at the UCLA and Veterans Administration Hospital to evaluate the potential interaction between EMF and ionizing radiation in the induction of hematopoietic neoplasia in mice [29]. As an adjunct to this study, the possible activity of EMF as a promoter of brain tumor induction was evaluated via histopathologic evaluation of brains from mice exposed to graded doses of ionizing radiation followed by exposure to either ambient magnetic fields (sham controls) or circularly polarized EMF (14.2 G [1.4 mT]) for up to 852 d [30].

When compared to matched sham controls, EMF exposure had no statistically significant effect on animal survival in any group. Total brain tumor incidence in the study was <0.5% (a total of seven brain tumors in 1707 irradiated mice), and no evidence of increased brain tumor incidence was observed in any group exposed to EMF. These data provide no evidence that exposure to circularly polarized EMF has tumor-promoting activity in the brain of C57BL/6 mice.

In spite of its large group sizes, one issue in the interpretation of the results of this study is its sensitivity and ability to identify any neurocarcinogenic effects of EMF exposure. The small number of brain cancers seen in all experimental groups could be interpreted as evidence that the mouse brain is relatively insensitive to neural tumor induction. Alternatively, however, the low incidence of brain cancers observed in otherwise unexposed animals could suggest that brain cancer incidence in study animals is at a response threshold and that exposure to any agent that stimulates cancer induction should increase brain cancer response. Regardless of the strength of the finding, the data from the present study provide no support for the hypothesis that EMF exposure is a significant risk factor for cancer induction in the brain.

### 2.4.3 Multistage Oncogenicity Studies in the Mammary Gland

The carcinogen-induced mammary adenocarcinoma in female rats is an organ-specific cancer model that is highly suitable for use as a test system in which to identify and quantitate EMF bioeffects. Epidemiologic evidence suggests that the breast may be a sensitive target tissue for the biological activity of EMF, and a well-defined and extensively studied multistage model system that is suitable for the identification of possible mammary gland carcinogens and tumor promoters has been established [36]. Numerous studies have been conducted to investigate the possible activity of EMF as a promoter of carcinogenesis in the rat mammary gland, and considerable controversy has developed concerning whether EMF exposure does indeed stimulate neoplastic development in this model system.

The general design of studies to evaluate the possible activity of EMF as a promoter of tumor induction in the rat mammary gland involves administration of one to four doses of a chemical carcinogen (DMBA or *N*-methyl-*N*-nitrosourea [MNU]) to young adult rats, followed by whole body exposure to either EMF or sham fields. Tumor development is monitored by palpation at regular intervals throughout the in-life period; at study termination, tumors are excised and evaluated histopathologically to confirm their malignant phenotype.

Several critical factors must be considered in evaluating the quality of studies reporting evaluations of the potential oncogenicity of EMF in the rat mammary gland.

- It is essential that study designs include histopathological evaluation of induced tumors. In the absence of such histologic confirmation, it is impossible to demonstrate that masses palpated during in-life observations are indeed malignant, and do not represent either benign tumors (adenomas or fibroadenomas) or nonmalignant lesions (e.g., mammary cysts).
- Dose selection for the chemical carcinogen is also a critical factor. To demonstrate promotional activity, the dose of carcinogen that is administered to test animals must induce a relatively low incidence of lesions, thereby providing a baseline cancer incidence against which a statistically significant increase in incidence can be achieved. If the cancer incidence induced by the carcinogen dose is too high, demonstration of promotional activity will be impossible.
- The mammary tumor response to the carcinogen must fall within accepted incidence and multiplicity ranges; these ranges have been established over more than 30 y through the work of many independent researchers. It is critical to note that this model has been used for several decades in studies of hormonal carcinogenesis and in efficacy evaluations of cancer chemopreventive agents. As such, dose-response parameters for mammary tumor induction are well established.



In instances, where reported dose–response relationships fall outside of the established dose–response parameters, it becomes impossible to exclude technical deficiencies in study conduct.

#### **2.4.3.1 *Oncology Research Center (Georgia): Mammary Tumor Promotion Studies in Rats***

In 1991, Beniashvili et al. [37] published the first report of the enhancement of rat mammary carcinogenesis by EMF exposure and have subsequently published a confirming study [38]. In this work, exposure of rats to either static or 50 Hz magnetic fields alone did not induce mammary cancer. However, exposure of rats to static or 50 Hz EMF following administration of a chemical carcinogen (MNU) increased the incidence and reduced the latency of mammary tumors in comparison to controls exposed to MNU only. Tumor incidence was higher in animals exposed to MNU + 50 Hz EMF than to either MNU + static fields or MNU only. The authors interpret these findings to suggest that EMF is not oncogenic by itself, but may present a cancer hazard for both humans and animals.

The value of the data from these papers is compromised by the lack of information regarding the exposure system used and limited detail regarding experimental methods. However, these data were clearly suggestive and stimulated the conduct of a number of similar studies in other laboratories.

#### **2.4.3.2 *Hannover School of Veterinary Medicine (Germany): Mammary Tumor Promotion Studies in Rats***

The largest number of studies investigating the influence of EMF on rat mammary carcinogenesis have been published by Loscher and colleagues [39–46] from the School of Veterinary Medicine at Hannover in the Federal Republic of Germany. In these studies, rats were exposed to DMBA followed by exposure to 50 Hz EMF or sham fields for periods ranging from 13 to 27 weeks.

The initial study from this group [45] reported an increase in mammary tumor incidence in rats exposed to 50 Hz EMF at a flux density of 1 G (100  $\mu$ T). However, no histopathologic evaluations were performed in this study, and tumor size measurements were based on estimates performed via palpation. In a follow-up study conducted at lower doses that are more relevant to residential environments, Loscher et al. [39] reported that exposure to a 50 Hz EMF gradient of field strengths ranging from 3 to 10 mG (0.3 to 1.0  $\mu$ T) reduced nocturnal melatonin levels, but had no effect on mammary carcinogenesis. In this study, EMF exposure for 13 weeks had no effect on mammary cancer incidence, mammary tumor size, or the incidence of preneoplastic lesions. The data from these studies were interpreted to suggest that exposure to EMF at high flux densities stimulates mammary tumor induction in rats, but that such effects do not occur as a result of exposure to EMF at low field strengths, such as those encountered in residential environments.

In a subsequent study, Loscher and Mevissen [40] reported a linear increase in mammary tumor incidence at 13 weeks in rats receiving DMBA and exposed to 50 Hz EMF at field strengths of 100 mG, 500 mG, and 1 G (10, 50, and 100  $\mu$ T). Confirming these data, the same group of investigators reported that exposure to 500 mG (50  $\mu$ T) EMF increased both the number and growth rate of mammary tumors [41] and the total incidence of mammary tumors [46]. By contrast, however, another confirming study [42] published the same year as the initial investigation of this series reported no increase in mammary tumor incidence, number, or size in rats exposed to 50 Hz EMF at 100 mG (10  $\mu$ T). A fourth confirming study conducted for 26 weeks [43] found a much smaller increase from sham control in the incidence of mammary tumors in groups exposed to the 500 mG

magnetic field; although the authors state statistical significance for the finding, the reported difference appears to be within the intrinsic variability of the DMBA mammary cancer system.

#### **2.4.3.3 *Battelle-Pacific Northwest Laboratories (United States): Mammary Tumor Promotion Studies in Rats***

A series of studies supported by the NTP and conducted at Battelle-Pacific Northwest Laboratories (PNL) was designed to replicate and extend the mammary carcinogenesis studies reported by Loscher and colleagues. In these studies, groups of 100 female rats received sham EMF exposure or were exposed to either 50 Hz or 60 Hz EMF at field strengths of 1 G or 5 G (100 or 500  $\mu$ T).

The initial study conducted was a 13 week bioassay that was designed to replicate the findings of Loscher and Mevissen [40] and Loscher et al. [45]. The results of this replication study demonstrated no differences from DMBA-treated sham controls in mammary cancer incidence, mammary cancer number, or mammary cancer size in DMBA-treated rats that were exposed to either 50 Hz or 60 Hz EMF [47]. Therefore, the replication study conducted at PNL failed to confirm the findings of the Hannover group.

The second study conducted at PNL used the same exposure metrics, and was designed to replicate the results of the 26 week mammary cancer bioassays reported by Loscher et al. [45]. Similar to the results of the 13 week study conducted at PNL, the results of the 26 week study demonstrated no significant increases in mammary cancer incidence in any group exposed to EMF. Furthermore, statistically significant decreases in the total number of mammary cancers was seen in groups exposed to 50 Hz EMF or 60 Hz EMF field strengths of 1 G [10].

Investigators at Battelle and in Hannover have collaborated in the attempt to identify reasons underlying the inability of Battelle investigators to reproduce the results reported by Loscher and colleagues [48]. A recent paper published by the Hannover group has attributed the inability of the Battelle group to reproduce their findings to differences in EMF responsiveness of different substrains of Sprague-Dawley rats [44].

#### **2.4.3.4 *National Institute for Working Life (Sweden): Mammary Tumor Promotion Studies in Rats***

One study has been published by investigators from the National Institute for Working Life in Sweden in which the possible promoting activity of 50 Hz EMF was evaluated in the DMBA rat mammary carcinogenesis model system [49]. In this study, groups of female rats were exposed to DMBA followed by exposure to a transient-producing magnetic field at flux densities of 2.5 or 5 G (250 or 500  $\mu$ T) for 25 weeks. No statistically significant differences from sham control were observed in mammary tumor incidence, mammary tumor number, total tumor weight, or tumor volume in either group exposed to EMF. The results of this study do not support the hypothesis that EMF exposure is an important risk factor for breast cancer.

### **2.4.4 *Multistage Oncogenicity Studies in the Hematopoietic System***

Two studies have been reported in which the activity of EMF as an inducer of lymphoma in standard (nontransgenic) animals has been evaluated. Several additional studies using genetically modified animals (transgenic and knockout strains) have also been published; the results of these studies are summarized in [Section 2.5](#).

#### **2.4.4.1 Zhejiang Medical University: Lymphoma Promotion Study in Mice**

In 1997, Shen et al. [50] presented the results of a study designed to evaluate the possible activity of a 1 mT 50 Hz magnetic field in the promotion of lymphoma induction in newborn mice treated with DMBA. In this study, 320 newborn mice received a single injection of carcinogen within 24 h of birth; sham exposure (155 mice) or EMF exposure (165 mice) was initiated at 2 weeks of age, and was continued until study termination at 16 weeks. The incidence of lymphoma was histologically confirmed.

At the termination of the study, lymphoma incidence was 22% in sham controls, and 24% in the group receiving EMF exposure, demonstrating that EMF exposure had no promoting activity under the conditions of this study. However, the incidence of metastatic infiltration into the liver was higher in EMF-exposed mice than in controls; the significance of this finding, if any, is unclear.

#### **2.4.4.2 University of California at Los Angeles (United States): Lymphoma Promotion Study in Mice**

A chronic murine bioassay was conducted at the UCLA and Veterans Administration Hospital to evaluate the potential interaction between EMF and ionizing radiation in the induction of hematopoietic neoplasia in mice [29]. In this study, groups of mice were exposed to graded doses of ionizing radiation followed by exposure to either ambient magnetic fields (sham controls) or circularly polarized EMF (14.2 G [1.4 mT]) for up to 852 d. Details of the design of this study were provided in [Section 2.3](#).

When compared to matched sham controls, EMF exposure had no statistically significant effect on animal survival in any group, and did not increase the incidence of lymphoma in any group. In fact, the only statistically significant difference from matched sham controls was a reduction in lymphoma risk in mice exposed to EMF after exposure to an ionizing radiation dose of 5.1 Gy. Extending the results of the complete carcinogenesis component of this study, the results of the lymphoma promotion cohorts of the study provide no evidence that exposure to circularly polarized EMF promotes the induction of lymphoma in C57BL/6 mice.

#### **2.4.4.3 Overview of Multistage Oncogenicity Studies**

Overall, the peer-reviewed literature provides little compelling evidence that exposure to EMF has cocarcinogenic or tumor-promoting activity in any organ site in experimental animals. Although the results of epidemiologic studies have identified the brain, breast, and hematopoietic system as possible targets of EMF action, no consistent pattern of EMF effects has been seen in any of these sites in experimental carcinogenesis models. Similarly, carcinogenesis studies conducted in animal model systems have not identified any other organ as a potential target of EMF oncogenicity.

Studies conducted in animal models for lymphoma and brain cancer provide no evidence of tumor-promoting or cocarcinogenic activity associated with exposure to EMF. The observed lack of activity in the promotion of hematopoietic neoplasia in multistage model systems is supported by similar results from studies conducted in transgenic and knockout models of lymphoma induction.

Several reports of tumor-promoting activity in the rat mammary gland have been presented in the peer-reviewed literature. However, these reports come from only two laboratories, and tumor-promoting activity is identified on the basis of different end points in different studies. More importantly, however, independent efforts to replicate these studies in other laboratories have failed. On this basis, the body of evidence supporting tumor-promoting activity for EMF in the rat mammary gland must be considered to be weak, at best.

Although not reviewed in detail in this chapter, similar negative results have been obtained in experimental studies conducted in animal models for neoplasia of the skin and liver. Although one report of skin tumor promotion by EMF has been presented [51], subsequent studies conducted in several laboratories have failed to identify either any tumor-promoting effects of EMF in mouse skin [52–55] or any effects of EMF exposure on biomarkers of tumor promotion in the skin [56]. Studies designed to determine the effects of EMF exposure on the induction of altered liver foci (a preneoplastic lesion in rat liver) also fail to provide clear evidence of tumor promotion by EMF [57,58].

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## 2.5 Assessment of the Possible Oncogenic Activity of Electromagnetic Field Exposure Using Genetically Modified (Transgenic and Gene Knockout) Rodent Models

Recent insights into the molecular mechanisms of disease provide the scientific basis for the development and use of genetically modified animals to study disease processes, and the application of these models in hazard identification and toxicity evaluation. The use of genetically modified animals in safety assessments is an emerging technology that lacks the long historical precedent of the 2 y bioassay and other standardized approaches to toxicity testing. However, our understanding of the utility of these model systems in safety assessment is evolving rapidly, and their use in toxicology is expanding. In this regard, it should be noted that oncogenicity studies in one genetically modified model, the p53 knockout mouse, are routinely required by the FDA to support safety assessments of novel pharmaceuticals and natural products that induce chromosomal damage or other genetic effects in *in vitro* test systems.

The science supporting the use of genetically modified models in safety assessment stems from initial observations that the incidence of malignancy is increased and its latency is decreased in animal strains into whose germ line a tumor-associated gene (oncogene) has been inserted. Subsequent work demonstrated similar changes in patterns of neoplasia in animal strains from whose germ line a tumor suppressor gene has been deleted. On this basis, transgenic animals (into whose germ line a tumor-associated gene has been inserted) and knockout animals (from whose genome a tumor suppressor gene has been deleted) demonstrate a genetic predisposition to neoplasia; this predisposition may be exploited as an approach to the use of animal models whose sensitivity to oncogenesis is increased.

These models have several desirable attributes that support their use in the study of EMF bioeffects. First, oncogenicity studies in transgenic or knockout models involve evaluations of biological activity in animals in which exposure to a test agent is superimposed upon a genetic predisposition to disease. Such evaluations may identify oncogenic effects that occur in sensitive subpopulations, and rarely or not at all in the general population. As a result, genetically modified models may demonstrate sensitivity to EMF bioeffects that do not occur in standard animal strains. Secondly, transgenic or knockout animals provide experimental models that may be considered as analogous to cocarcinogenesis and initiation–promotion models; tumorigenesis in some transgenic and knockout model systems does not require exposure to a chemical carcinogen. Finally, the conduct of parallel assessments of the possible oncogenicity of EMF in animals with different genetic alterations can generate information regarding (a) the generality of any observed effects, and (b) possible molecular mechanisms underlying such effects.

Because genetically modified animals have a limited history of use in safety assessment, few data exist concerning their utility in the prediction of human oncogenicity. When considered alone, oncogenicity assessments in genetically modified animals may be of limited value in predicting human responses. However, when considered in combination with studies performed using standardized model systems, the increased sensitivity of transgenic and knockout models to disease processes may support the identification of low dose effects and effects in sensitive subpopulations. Given their rapidly expanding use in submissions to the FDA, studies in p53 knockout mice may become particularly valuable in assessing possible oncogenic hazards.

### **2.5.1 Design of Oncogenicity Studies in Genetically Modified Animals**

The design of oncogenicity studies in transgenic and knockout animals generally parallels the designs used for oncogenicity bioassays in standard strains of rodents. However, these bioassays differ from the design of chronic oncogenicity bioassays in duration, group size, and experimental end points. Primary differences from the 2 y bioassay are as follows:

- The duration of oncogenicity studies in genetically modified animals varies by animal strain. The primary determinant of study duration is the kinetics of spontaneous oncogenesis in the animal model used in the bioassay. Most commonly, oncogenicity assessments are designed for completion using an in-life period of 6 months; however, the in-life period in certain models can be extended to include much longer periods of exposure. For example, the protocol developed by the NTP for oncogenicity bioassays in heterozygous p53 knockout mice involves 26 weeks of agent exposure; this protocol is now used widely in safety assessments of novel drugs and natural products, being submitted for FDA approval. By contrast, studies in PIM transgenic mice (as cited below) have ranged in duration from 6 to 18 months.
- Group size in these studies rarely exceeds 30 per sex per group versus 50 to 100 in standard oncogenicity bioassays. A group size of 25 per sex per group is routinely required by the FDA for studies in the p53 knockout mouse.
- In most cases, studies in genetically modified animals are designed to examine effects of a test agent on neoplastic development in a specific site, and histopathologic evaluations are limited to that site. In EMF studies, the primary focus has been on the possible influence of EMF exposure on the incidence of lymphoma. As such, histopathologic evaluation of tissues in these studies has been limited to lymphoid organs.

### **2.5.2 Results of Oncogenicity Studies in Genetically Modified Animals**

#### ***2.5.2.1 IIT Research Institute (United States): Lymphoma Bioassays in PIM Transgenic and Heterozygous p53 Knockout Mice***

As part of a larger program supported by the NTP and the United States National Institute of Environmental Health Sciences (NIEHS), scientists at IITRI conducted 6 month studies to determine the influence of EMF exposure on lymphoma induction in PIM transgenic mice and heterozygous p53 knockout mice [59]. Both genetically modified mouse strains were used as models for lymphoma; the PIM mouse receives a single dose of carcinogen (ENU) prior to EMF exposure, and is considered to be a

high-incidence lymphoma model over a 6 month observation period. The heterozygous p53 knockout mouse receives no carcinogen exposure, and is a low incidence lymphoma model system over this same observation period.

#### 2.5.2.1.1 Results of IITRI (United States) Lymphoma Bioassay in PIM Transgenic Mice

The PIM transgenic mouse carries the *pim-1* oncogene [60] and is a rapid model for the study of lymphoma. We have previously demonstrated that this model system is useful for studies of the modulation of lymphoma promotion by cancer chemopreventive agents [61]. It should be noted, however, that the *pim-1* oncogene appears to be specific for the induction of murine lymphoma and has no known homology to any human oncogenes. Furthermore, there are few data to correlate neoplastic responses in other organs in the PIM mouse model with responses to chemicals in 2-y bioassays for oncogenicity. As such, the PIM mouse must be considered to be at an early stage of development for use in the identification of agents that may stimulate the induction of leukemia and lymphoma.

EMF exposure metrics used in the PIM mouse oncogenicity bioassay [59] were identical to those used in the chronic animal bioassays of EMF that were conducted at IITRI. Groups of 30 mice per sex received a single intraperitoneal dose of 25 mg ENU and were then exposed for 18.5 h/d for 23 weeks to pure, linearly polarized 60 Hz magnetic fields. Experimental groups included animals receiving continuous exposure to field strengths of 20 mG (2  $\mu$ T), 2 G (200  $\mu$ T), or 10 G (1000  $\mu$ T), or intermittent exposures (1 h on/1 h off) to 10 G (1000  $\mu$ T). Parallel sham control groups were housed within an identical exposure array, but were exposed only to ambient magnetic fields. At the conclusion of the exposures, animals underwent a limited gross necropsy focused on the lymphoid system, and histopathologic evaluations were performed on the spleen, thymus, mandibular lymph node, liver, kidney, and lung.

When compared to sham controls, exposure to 60 Hz EMF did not increase the incidence of lymphoma in any experimental group. In fact, the only statistically significant difference from sham control was a significant *decrease* in the incidence of lymphoma in male mice exposed continuously to EMF (23% incidence versus 49% incidence in sham controls;  $p = 0.041$  via Fisher's exact test). On this basis, the results of this study do not support the hypothesis that exposure to EMF is a significant risk factor for hematopoietic neoplasia.

#### 2.5.2.1.2 Results of IITRI (United States) Lymphoma Bioassay in p53 Knockout Mice

As a result of deletion of one allele of the p53 tumor suppressor gene, heterozygous p53 knockout mice are highly sensitive to spontaneous neoplasia in several sites, as well as to the induction of neoplasia by carcinogens. By 18 months of age, approximately 50% of otherwise untreated heterozygous p53 knockout mice will develop malignant lesions, with particular predisposition to tumors of the bone and hematopoietic system [62]. Carcinogen exposure greatly accelerates neoplastic development in this model; in this context, p53 knockout mice were used as a model system for the study of EMF effects on lymphoma induction.

In this study, groups of 30 mice per sex were exposed for 18.5 h/d for 23 weeks to either pure, linearly polarized 60 Hz EMF at 10 G (1000  $\mu$ T), or to sham fields. At the conclusion of the exposures, animals underwent a limited gross necropsy focused on the lymphoid system, and histopathologic evaluations were performed on the spleen, thymus, mandibular lymph node, liver, kidney, and lung.

Consistent with the results of the lymphoma bioassay in PIM mice, exposure to 60 Hz EMF at a flux density of 10 G had no effect on lymphoma induction in heterozygous p53 knockout mice. Lymphoma incidence in both sexes in the sham control group was 3% (1/30). By comparison, lymphoma incidence in male mice in the 10 G group was 0%

(0/30), while female mice exposed to 10G had a 7% incidence of lymphoma (2/30). Although this study was of limited statistical power, it provides no evidence to support the hypothesis that EMF exposure is a risk factor for lymphoma induction.

#### **2.5.2.2 *Walter and Eliza Hall Institute of Medical Research (Australia): Lymphoma Bioassay in PIM Transgenic Mice***

A very large lymphoma induction study using the PIM mouse model was conducted at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia [63]. In this study, groups of approximately 100 female PIM mice were continuously exposed for 18 months to linearly polarized 50 Hz EMF at flux densities of 10 mG (1  $\mu$ T), 1 G (100  $\mu$ T), or 10 G (1000  $\mu$ T), or intermittently exposed (15 min on/15 min off) to 10 G (1000  $\mu$ T) for the same period. In contrast to the design of the PIM mouse study conducted at IITRI, EMF and sham-exposed mice in the present study received no carcinogen.

Although the study designs were very different, the conclusions drawn from the results of this study were quite similar to those of the PIM mouse study conducted at IITRI. When compared to sham controls, no statistically significant increase in the incidence or latency of lymphoma was seen in any group of mice exposed to EMF; in comparison to a lymphoma incidence of 29% in the sham control group, the incidence of lymphoma in groups exposed to EMF ranged from 26% to 35% ( $p > 0.05$  for all comparisons). By contrast, mice exposed to a positive control material (ENU) demonstrated a lymphoma incidence of 60% at 9 months.

#### **2.5.2.3 *Overview of Oncogenicity Studies in Genetically Modified Animals***

Although the total number of completed oncogenicity evaluations of EMF in genetically modified animals is small, both the goals and results of these studies are very consistent. The peer-reviewed literature contains reports of three studies in which the influence of EMF exposure on lymphoma induction has been assessed using genetically modified animals. The three studies were conducted using vastly different experimental models and designs (carcinogen-treated PIM mouse, noncarcinogen-treated PIM mouse, and noncarcinogen-treated p53 knockout mouse). Furthermore, the three models used demonstrated large differences in the incidence of lymphoma in sham control groups. However, the results of all three studies were very comparable; in all studies, exposure to EMF was not associated with a significant increase in any experimental group.

The relatively recent development of genetically modified animals precludes a long history of their use in safety assessment. As a result, data are insufficient to support a broadly based assessment of the predictive nature of these models for human oncogenesis. However, the results of oncogenicity evaluations of EMF exposure that has been conducted in standard animal strains and in genetically modified animal models are quite comparable. On this basis, the results of EMF lymphoma studies in the PIM transgenic and p53 knockout model systems can be considered to confirm and extend the results obtained in 2 y bioassays of EMF.

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## **2.6 Proposed Mechanisms of Electromagnetic Field Action in Oncogenesis**

A number of mechanisms have been proposed through which exposure to EMF may induce or stimulate neoplastic development; these possible mechanisms include a wide

range of effects at the molecular, cellular, and organismal level. An exhaustive presentation and evaluation of the literature relevant to possible mechanisms of EMF action is beyond the scope of this chapter. However, a brief discussion of the more commonly proposed mechanisms for EMF action is relevant to an evaluation of exposure to EMF as a risk factor for cancer and other diseases. Detailed reviews of possible mechanisms of EMF action have been presented in the assessment of EMF health effects prepared by the U.S. NIEHS [5] and in the IARC evaluation of the potential carcinogenicity of EMF [6].

Review of the published scientific literature provides no clear support for any specific mechanism of EMF action in carcinogenesis. Furthermore, no reproducible body of scientific evidence exists to link EMF exposure to alterations in any biological end point that is mechanistically related to neoplastic development in any tissue.

Most hypothesized mechanisms of EMF action in carcinogenesis can be grouped into five types of effects:

- Genetic toxicity (DNA damage, clastogenesis, or mutagenesis)
- Alterations in gene expression
- Biochemical changes associated with regulation of cell proliferation
- Alterations in immune function
- Alterations in melatonin levels or action

These general classifications are necessarily arbitrary and were developed to simplify the purposes of this chapter. It is clear that several of these mechanisms overlap and that all possible mechanisms of EMF action are not included in these five groups. However, these groups encompass the vast majority of mechanisms proposed for EMF bioeffects, and include mechanisms that have received the most extensive study to identify possible linkages between EMF exposure and carcinogenesis.

### **2.6.1 Genetic Toxicology Studies of Electromagnetic Field Exposure**

The scientific literature includes nearly 100 reports of studies that were conducted to determine if exposure to EMF induces clastogenesis, mutagenesis, or other types of DNA damage. Many of these studies were conducted using standardized experimental model systems (Ames test, micronucleus test) that are widely used in safety assessments of new drugs, industrial and agricultural chemicals, environmental contaminants, and other agents. As such, the predictive nature of several of the assay systems used to study the genetic toxicology of EMF is well known.

The authors of a comprehensive review of the genetic toxicology literature related to EMF concluded “the preponderance of evidence suggests that extremely low-frequency (ELF) electric or magnetic fields do not have genotoxic potential” [34]. Several investigators have presented study data that indicate positive mutagenic or clastogenic activity of EMF exposure; however, an independent scientific review of these studies suggests that none of these results satisfy basic quality criteria for data reproducibility, consistency, and completeness. By contrast, a number of well-designed, complete, reproducible, and apparently well-conducted studies have failed to demonstrate any genotoxic effects of EMF [34].

It is generally agreed by investigators in the field that ELF magnetic fields do not possess sufficient energy to damage DNA via direct interaction (reviewed in [4]). Although it is possible that power frequency EMF could damage DNA through an indirect mechanism (i.e., via generation of free radicals), the vast majority of well-designed genetic toxicology studies conducted in models with demonstrated predictiveness



for DNA-damaging activity have failed to identify any significant mutagenic or clastogenic effects of EMF exposure. As such, it is the consensus among toxicologists that exposure to power frequency EMF presents little or no risk of genetic toxicity. On this basis, the induction of DNA damage by EMF exposure appears unlikely to provide a mechanism for cancer induction.

### 2.6.2 Alterations in Gene Expression

The possible influence of EMF exposure on the expression of cancer-related genes is one of the most controversial areas of research in bioelectromagnetics. Several investigators have reported increases in the expression of one or more protooncogenes (generally *c-myc* or *c-fos*) in cell cultures exposed to 60 Hz EMF [64–66]. However, replication studies conducted by numerous other investigators have not confirmed these findings [67–71], and other, more broadly based screening efforts to identify genes that are up- or down regulated by EMF exposure have generally yielded negative results [72,73]. On this basis, the robustness of the reports of altered gene expression, as well as the generality of those findings, must be questioned.

Given the huge universe of genetic targets in the mammalian cell, it is not possible to exclude the possibility that some condition of exposure to 60 Hz EMF may alter the expression of one or more genes. It is important to note, however, that a finding of enhanced expression of an oncogene or any other gene is not, by itself, sufficient evidence to suggest physiological or pathophysiological activity. Because many cellular pathways exhibit biological redundancy, alterations in one pathway may be compensated for by an antagonistic change in another pathway. For this reason, although findings of altered gene expression may be considered as generally suggestive of a biologically important effect of EMF exposure, demonstration of up- or downregulation of some effector function is necessary to establish the biological relevance of the observed alteration in gene expression. Lacking the demonstration of any physiological and pathophysiological effect, an isolated finding of altered gene expression cannot be construed to be biologically significant. Restated with emphasis on the process of carcinogenesis, a finding of altered expression of an oncogene or a tumor suppressor gene as a result of EMF exposure can by no means be equated with any activity of EMF as a carcinogen or tumor promoter.

### 2.6.3 Biochemical Changes Associated with Regulation of Cell Proliferation

Changes in the expression or activity of enzymes and protein products involved in cell proliferation, cell cycle regulation, and apoptosis would be expected if EMF exposure is significantly associated with either complete carcinogenesis or tumor promotion. For example, several reports of EMF-associated alterations in the activity of ornithine decarboxylase, the rate-limiting step in polyamine biosynthesis, have been presented in the literature [74,75]. However, other investigators have either failed to confirm these findings in replication studies [76] or have found no effects of EMF on ornithine decarboxylase activity in other *in vitro* or *in vivo* model systems [56,77,78]. A similar lack of consistency has been found in studies of biochemical end points, such as induction of heat shock proteins [79–82].

Clearly, a single finding of an alteration in a specific biochemical parameter is insufficient to support its alteration as a general mechanism for EMF activity. As discussed above with regard to possible alterations in gene expression, an isolated finding of changes in enzyme activity or other pathway in cells that are exposed to EMF can be considered to be no more than suggestive of a possible adverse outcome. Linkage of these

biochemical or cell-based effects to the physiological or pathophysiological effect is essential to demonstrate their biological significance.

#### **2.6.4 Alterations in Immune Function**

Downregulation of the host immune response by EMF is a tempting hypothesis through which EMF exposure could be associated with risk of carcinogenesis; however, this hypothesis is supported by very little experimental evidence. In fact, several studies conducted in standard rodent models for immunotoxicology testing have failed to demonstrate any significant adverse effects of EMF exposure on either cell-mediated or humoral immune responses [24,25,83].

The only exception to these negative studies is an apparently reproducible suppression of NK cell function that was seen in B6C3F1 female mice in two studies [24,26]. Interestingly, this suppression was not observed in male B6C3F1 mice, or male and female F344 rats used in these same studies. Furthermore, the reduction in NK cell function does not appear to result in a functional deficit, since negative results were obtained in a 2 y carcinogenesis bioassay of EMF in the same animal species and sex (female B6C3F1 mouse) in which the NK cell functional deficits were observed [12]. On this basis, although the deficit in NK cell function was statistically significant, it appears to be without biological significance in terms of host resistance to neoplasia.

As such, although the hypothesis that EMF exposure may stimulate tumorigenesis through an indirect mechanism involving suppression of host immunity may be intellectually appealing, it is unsupported by any credible body of experimental evidence.

#### **2.6.5 Alterations in Melatonin Levels or Action**

The melatonin hypothesis is perhaps the most widely accepted potential mechanism for the biological effects of EMF exposure. A simplified version of this hypothesis states that EMF bioeffects are mediated through alterations in the function of the pineal gland; these changes in pineal function are then responsible for alterations in patterns of melatonin synthesis, kinetics, or action [84]. Ultimately, reductions in melatonin synthesis may have a number of potential adverse effects on the host, including reduced activity as an endogenous free radical scavenger, and influences on the hypothalamic–pituitary–gonadal axis [85].

A considerable body of evidence to support the melatonin hypothesis was generated in bioelectromagnetics studies performed during the 1980s and early 1990s (reviewed in [86,87]). In these studies, reductions in circulating melatonin levels and time shifts in the circadian rhythm of melatonin biosynthesis were reported in both experimental animals and humans (reviewed in [4]). More recently, however, several investigators who had previously reported significant effects of EMF exposure on pineal function have been unable to replicate their own data [88–91]. This failure of replication was often associated with the acquisition of new, more sophisticated EMF exposure and monitoring equipment. Furthermore, the majority of recent papers from other laboratories have failed to demonstrate any consistent pattern of effects of EMF exposure on melatonin synthesis or kinetics in humans or in rodent model systems.

One notable exception to this trend is a series of studies that have been conducted using the Siberian hamster as a model system; EMF effects on melatonin secretion in this species appear to be a generally reproducible finding [92,93]. However, this animal demonstrates a seasonal circadian rhythm that is considerably different from that seen in either humans or rodent species that are commonly used for safety assessments; as such, a relationship between this finding and possible human health effects is difficult to establish.

Concomitant with the weakening of the data set relating EMF exposure to alterations in pineal function, the melatonin hypothesis has been revised to suggest that EMF influences on melatonin may be affected through alteration in melatonin effects at the level of the target cell, rather than through effects on its synthesis or secretion. The database supporting this hypothesis is very limited and is considered insufficient to provide strong support for this potential mechanism of EMF action.

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## 2.7 Conclusion

The results of acute, subchronic, and chronic toxicity, and oncogenicity studies conducted in experimental animal model systems provide little support for the hypothesis that exposure to power frequency magnetic fields is a significant risk factor for human disease. Although positive results have been reported in a small number of animal studies, essentially all of those results either (a) have not been replicated in subsequent studies conducted in other laboratories or (b) were generated using nontraditional experimental models whose value as predictors of human responses is largely or completely unknown.

Clearly, the safety of human exposure to EMF (or any other chemical or physical agent) cannot be conclusively determined on the basis of studies in animal models alone. Interspecies extrapolation of results, and requirements for extrapolation of high dose exposure data in animals to low dose exposures in humans both require the acceptance of mechanistic assumptions that may ultimately prove to be inaccurate. For this reason, the most effective hazard assessments are based on the integration of results from appropriate animal test systems with available data from studies of exposed human populations.

In the case of EMF hazard assessment, evaluation of data from specific toxicologic end points that have been examined both in experimental models and in epidemiologic studies demonstrates that the results of animal studies agree quite closely with data from studies in human populations. This relationship suggests that, where appropriate comparative data exist, the results of well-designed studies in appropriate animal model systems are largely predictive of human responses to EMF.

Ultimately, the key issue in EMF hazard assessment is whether animal data can be used to predict human risk for cancer and other disease processes for which the epidemiologic data are inconsistent or unavailable. On the basis of the close agreement between animal and human data in both general toxicology and organ site-specific toxicity studies, and the negative results of the overwhelming majority of oncogenicity evaluations of EMF in animal model systems, it is concluded that exposure to EMF is unlikely to be a significant risk factor for human oncogenesis.

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## *Interaction of Nonmodulated and Pulse-Modulated Radio Frequency Fields with Living Matter: Experimental Results*

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### 3.1 Introduction

Over the past several decades, research on electromagnetic fields (EMF) has been motivated primarily by public health considerations. Through the 1980s and early 1990s, investigations of extremely low-frequency (ELF) were preeminent in EMF research due to increased societal concerns in the area of power frequency. This work focused on biological effects of power transmission lines and appliances. Since the mid-1990s, however, as human-made sources of radio frequency (RF) energy continued to increase in the environment, so also did environmental and health concerns. Due to elevated levels of RF exposure, specifically the exponential increase in exposure from cellular telephony, the output from RF effects research has increased significantly since the publication of the last edition of the handbook, as judged from a survey of leading research journals from 1994 to 2004.

Other factors have also influenced the enthusiasm with which new investigators have ventured into EMF research and have affected the motivation of research administrators who influence public policy on resource allocation. Concerns over nonreproducibility of results and nonrobustness of effects have produced a voluble exchange of views on direction and magnitude of support for EMF research (Pickard and Foster 1987). Such problems are brought into focus by the fact that the database of this research typically enters directly into the public health policy forum and is used to establish permissible exposure limits, directly affecting diverse economic interests (IEEE 1991). EMF research is therefore subjected to a second, critical review, following scholarly peer review but in a more pragmatic context. The veracity of the work is again analyzed and, in addition, its

relevance for formulating safe limits for human exposure is scrutinized. This exercise requires extrapolation from conditions of experiments, often with animals, to human exposure. Inevitably the process requires the imposition of interpretations and judgments as to whether an effect might or might not constitute a hazard to humans and the database is seldom clear on that point. Utilization of the database in standards setting has been described by Petersen (1991). The role of culture and philosophy in the divergence of Eastern European and Western safety standards has been the subject of commentary by Sliney et al. (1985).

The fundamental interaction of RF radiation (3 kHz to 300 GHz (IEEE 1991)) with biological systems is set forth in detail in other chapters of this book. At sufficiently high RF intensities bulk thermal energy is imparted to the system, causing an increase in random kinetic energy and temperature. The thermal gradients differ from those of ambient heating, a fact that is marshaled occasionally to explain differences in effects between RF and ambient heating. Above certain intensities RF energy can quickly produce morbidity, and, after thermoregulatory mechanisms are overwhelmed, mortality. In recent years, work has focused on possible effects at nonthermal levels or under conditions in which physiologic temperature can be maintained in the presence of normally thermalizing specific absorption rates (SAR).

Studies of human perception indicate that the greatest cutaneous sensitivity to microwave (MW) heating is at frequencies with wavelengths comparable to or smaller than the thickness of skin, the millimeter wave range (EPA 1984). In this range, most of the energy is absorbed in the superficial dermis containing thermal sensors. At lower frequencies, 1 to 10 GHz, with wavelengths equal to or longer than the human body, much of the energy is absorbed below the superficial dermis. In this range, the threshold temperature for cellular injury about 42°C is below the threshold for pain (about 45°C). Consequently cutaneous perception of RF energy may not be a reliable response, which protects against potentially harmful levels of RF radiation at the lower microwave frequencies (Hardy, 1978).

### 3.1.1 Thermal versus Athermal or Nonthermal Effects

With exposure to an energy source such as EMF, there will always be some temperature change because of the finite heat capacity of any material system. From an experimental point of view, however, one can specify an effect as a thermal or nonthermal if during exposure of a system, using a nonperturbing temperature probe, the real-time temperature excursion is below an arbitrarily finite  $\Delta T$ , deemed nonthermal. A typical excursion could be  $\Delta T = 1^\circ\text{C}$ , but, whatever  $\Delta T$  is chosen as the nonthermal limit, it represents a judgment by an investigator carrying a set of presumptions into the experiment which he may or may not have made explicit. An effect can also be identified as nonthermal, even in the presence of a temperature excursion exceeding an arbitrary threshold if an RF-induced effect is found and the same effect is not elicited with ambient heating producing an identical  $\Delta T$ .

Theoretical definitions are also arbitrary. The introduction of RF energy into a system is inherently diffuse on a large scale compared with macromolecules or whole cells. In thermodynamic terms, one can posit a target structure, possessing a mode (e.g., vibration, rotational degree of freedom) in equilibrium with a surrounding heat bath (tissue). RF energy couples into the structure and heat bath simultaneously but unequally; perturbed from equilibrium, they proceed to interact with each other to achieve equilibrium. In this construct, a rigorously nonthermal interaction would be one resulting from direct electromagnetic coupling with the target structure independent of thermal interactions with the heat bath. In any experimental setting such a distinction would be difficult to make.

The displacive phase transition in a macromolecule produced by pumping RF energy into low-frequency vibrational modes of DNA, leading to unwinding and strand separation, was postulated by Girirajan et al. (1989). This process, if indeed it happens, could be considered nonthermal. One also finds in the literature the term “field” effect, suggesting again the concept of direct transduction of RF energy into a specific kinetic or metabolic change mediating the effect unrelated to a change in random kinetic energy.

A more empirical way of approaching the thermal versus nonthermal argument would be to identify an experimental effect that occurs or is observed at low RF energy and look for the presence or absence of the effect following the insertion of energy by other means such as conductive heating from a water bath. Changing the ambient temperature of a biological system, by whatever means, produces thermal gradients, which are different from those produced by RF energy. If such differences can be assumed not significant (an analysis is required for each experiment), then one may look for the presence or absence of a biological effect under each exposure condition and identify the effect as nonthermal if it occurs following RF exposure but not after ambient heating to the equivalent small  $\Delta T$ .

In evaluating the claims for thermal versus nonthermal effects, one must remember how elusive the distinction can be. Ease of application varies, depending on the experimental problem. Early claims that RF energy could produce a change in the intrinsic permeability of the blood–brain barrier (BBB) were later challenged, both as to the reality of the altered permeability and also as to whether generalized heating was the possible effector. “Window” effects with regard to calcium exchanges between compartments in the brain are viewed as inherently nonthermal effects by many investigators, but the validity of such effects has been challenged.

### 3.1.2 Vagaries and Pitfalls in the Literature

A critical analysis of the literature is a daunting task. A majority of interesting reports have not been replicated and often efforts to replicate studies have shown antithetical results. Discussions then arise over whether an attempt to reproduce a result was the same or, in some way, a significantly different experiment. The literature reports presented in this chapter will identify still unresolved issues and, where possible, describe a prevailing consensus. In addition, rather than presenting all publications available for specific subjects, this chapter will present a broad representative sample of the literature through 2004.

Table 3.1 and Table 3.2 identify factors which must be addressed in performing research in the field. In fact, since some of the parameters are not addressed in research reports appearing in journals known for meticulous peer review, perhaps because of practical limitations of space, the reader does not know if they were considered. It is even harder to establish whether a particular laboratory is imbued with a culture of rigorous science. Does it have a systematic approach to measurement assurance and quality control? The literature alone does not answer such questions. Because of the large number of potential sources of error and sources of variation from one laboratory to another, it is not surprising that the literature contains contradictions and produces frustration, especially in the risk assessment community. Conclusions that can be drawn therefore depend to a significant degree on the weight of the evidence instead of specific individual reports.

### 3.1.3 Physical and Physiologic Scaling

The cost–benefit analyses used to justify exposure of human beings to drugs of unknown hazard but potential benefit do not in general allow systematic RF experimentation on human beings for RF effects. A noteworthy exception has existed in medical oncology and

**TABLE 3.1**

Factors That Affect Microwave and RF Absorption

Physical Parameters	Biological Parameters	Artifacts	Environmental Factors
Frequency	Tissue dielectric properties	Ground or conductor plane	Temperature
Polarization	Size; geometry	Container (material, size)	Humidity
Modulation (AM, FM, Pulse, CW)	Animal orientation relative to polarization	Metal implants	
Power density (peak and average)	Spatial relations among animals	Shielding implants	
Field pattern (near or far)		Metal or nonmetallic objects in the field	
Field uniformity			
Type of transmitting and radiating equipment			
Chamber material			
Chamber dimensions			

more recently in the treatment of musculoskeletal, degenerative disorders where treatment efficacy has been established. Research in these areas focuses on maximizing benefit while minimizing adverse consequences.

Although exposure of humans is of primary importance and concern, the bulk of EMF research has been carried out using animal models. This requires an understanding and appreciation of biophysical principles and “comparative medicine.” Such studies require interspecies scaling (Guy et al. 1976; Gandhi et al. 1977; Massoudi et al. 1977; Gandhi 1980; Durney et al. 1986), the selection of biomedical parameters that reflect basic physiological functions, and differentiation between adaptational or compensatory changes and pathological effects. In comparing results of experiments performed in the same or different laboratories, standardization of conditions is important and, unfortunately, all too often not attained.

**TABLE 3.2**

Factors That Influence Biological Responses to the Same SAR

Subject Variables	Concomitant Variables	Environmental Variables	Experimental Variables
Species	Genetic predisposition	Temperature	Acclimation procedures
Sex	Baseline of the response	Humidity	Duration of exposure
Age	Functional and metabolic disorders	Air flow	Number and schedule of exposures
Weight		Lighting	Mode of exposure (partial or whole body)
Sensitivity		Noise	Time between exposure and sampling
Number of subjects		Odor	Time of day of exposure
Interventions (anesthetics, drugs, electrodes, lesions)			Restraint devices
Animal husbandry			Investigator—animal interaction

Even by using approaches where absorbed energy patterns in a test animal are set to approximate as closely as possible the patterns that may exist in man under certain exposure conditions, the intrinsic physical and physiological dissimilarities between species further confound the problem of extrapolating from animals to man. In addition to the obvious external geometric differences, the differences in internal vascular anatomy and mechanisms of heat dissipation in fur-bearing animals compared to man must be taken into consideration.

Because of the use of animals as a surrogate for humans in hazard analysis, one must create a set of experimental conditions, which are as relevant as possible for the purpose of the study. Many factors, such as methods of animal care, the role of seasonal and circadian rhythms, temperature and humidity, etc., as well as psychosocial interactions, must be considered in the experimental design as well as in the analysis of results. Additionally, one should not extrapolate results obtained in small laboratory animals to larger animals or man without consideration of size and energy distributions, as well as metabolic and physiological differences.

The possible difference between a biological response (effect) and a deleterious change in function is an important question in hazard assessment. Remarkable changes following exposure of cells or tissues *in vitro* do not necessarily have any significance *in vivo*. Most exposures are necessarily short term and do not provide information on possible long-term consequences. Even short-term exposures can produce interpretive problems. For example, could the human central nervous system (CNS) tolerate specific absorptions up to 30 kJ/kg delivered in periods from 360 ms to 1  $\mu$ s with no long-term sequelae, as do rats (Guy and Chou 1982). Although rats are stunned in such an exposure, they appear to make a complete recovery within minutes. No responsible investigator would be willing to assert that a human would make a similar recovery.

Johnson (1975) has described factors that affect absorption of electromagnetic energy in animals. This absorption is dependent on the size and geometry of the animal relative to wavelength and polarization. The wavelength-to-animal size relationship ( $\lambda$ -to- $a$ , where  $a$  is the longest axis dimension of the body and the electric field vector is parallel to the longest axis) is a critical factor in the relative absorption cross section, the ratio of the absorbed energy per second to the power incident on the geometrical cross-sectional area of the animal (Anne et al. 1962). This produces the immediate result that, at a given frequency and power density, the SAR is vastly different in animals depending on size. To produce an identical whole body average SAR, one must scale from one frequency to another. It is of practical importance to realize that experiments on biological effects at 2.45 GHz on small animals, such as mice and rats, do not scale to humans at 2.45 GHz, but approximately to effects on humans at 100 MHz. Much information on absorption of RF energy has been collected in the *Air Force RF Handbook* (Durney et al. 1986). Subsequently, considerable research has been done on the variation of localized absorption in animals and man, through both experimental measurements (see Adair 1995) and modeling (Gandhi 1990; see also Chapter 10 of *BBA*).

The process of scaling in RF bioeffects research is not restricted to extrapolation of the physical absorption of energy, but includes the problems of quantitatively comparing the relative efficiency of various biological processes in different animals to each other and to those processes in humans. The same biological processes in different animals are not necessarily affected by the same SAR. It is also possible that the absorbed energy in a particular area or tissue may not be the most useful indicator of disturbances of some biological functions, if those functions are perturbed by a systemic stimulus instead of a localized stimulus. The need for proper dosimetry in experimental procedures and the importance of using appropriate scaling factors for extrapolation of data obtained with small laboratory animals to man are thus clearly indicated.

### 3.1.4 Specific Absorption Rate

RF energy absorption is converted to thermal energy. This thermal energy is then rapidly redistributed by conduction, convection (blood flow) and to a lesser extent, radiation from the biological target. The tissue heat capacity and heat transfer processes influence the dose and dose distribution within the body. Present modeling techniques use the internal geometry and dielectric properties of different tissues to develop a profile of energy absorption and current flow as a function of position as described in [Chapter 10](#) of *BBA*. These calculations produce excellent agreement with phantoms (anatomically realistic models of the body with similar dielectric properties), but are not yet capable of simulating the complex redistribution of absorbed energy in living systems. Of course, they have only limited predictive value for actual physiologic or pathologic response. There is no substitute for animal experimentation.

There is a consensus that an important dosimetric measure of RF exposure is the SAR. This is the unit-mass, time-average rate of RF energy absorption specified in SI units of watts per kilogram (W/kg) (NCRP 1981). The amount of energy absorbed by a given mass of material, which is termed specific absorption (SA) is given in joule per kilogram (J/kg) and is the integral of the instantaneous SAR over the duration of exposure in seconds. Thus the SAR is the rate at which RF electromagnetic energy is imparted to unit mass of a biological body. The SAR is applicable to any tissue or organ of interest, or is expressed as a whole body average. As the science of dosimetry has evolved, localized SARs are more frequently reported, i.e., specified for a particular location or tissue, such as the eye.

Whole body SAR is maximal when the long axis of a body is parallel to the *E* field vector and is four tenths the wavelength of the incident field. At 2.45 GHz ( $\lambda = 12.5$  cm), for example, a standard human (long axis is 175 cm) will absorb about half of the incident energy. If the human whole body SAR is divided by the basal metabolic rate (BMR) for humans, a ratio is obtained that provides a measure of the thermal load incurred due to a known incident power density (Stuchly 1978). Table 3.3 illustrates the variation of this ratio with frequency at two incident power densities. In the region of human whole body

**TABLE 3.3**

Ratio of SAR to Basal Metabolic Rate for an Average Man Exposed to Far Field Incident Power Densities of 10 and 50 W/m<sup>2</sup>

Frequency (GHz)	Average SAR/BMR (%)	
	10 W/m <sup>2</sup>	50 W/m <sup>2</sup>
0.01	0.13	0.65
0.02	0.60	3.00
0.05	5.80	29.00
0.06	10.00	50.00
0.08	16.00	80.00
0.10	12.00	60.00
0.20	5.20	26.00
0.50	3.70	18.00
1.00	2.90	14.50
2.00	2.50	12.50
5.00	2.50	12.50
10.00	2.50	12.50
20.00	2.50	12.50

Source: From Stuchly, M.Q., *Health Aspects of Radio Frequency and Microwave Radiation Exposure*, Part 2, Department of National Health and Welfare, Ottawa, 1978.



resonance (60 to 80 MHz), this ratio reaches a maximum value (about 16 for an incident far field power density of  $10 \text{ W/m}^2$ ). The ratio drops off rapidly on either side of this peak.

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### 3.2 Response to Local Radio Frequency Exposure

Considerable attention has been paid in animal and human experiments to the temperature rise in various organs and tissues as functions of incident energy wavelength, thickness of the subcutaneous fatty layer, blood circulation rate, and other factors. The increase in temperature of tissues during local exposure is linear for short periods (1 to 3 min) and proportional to the magnitude of the MW energy absorbed. With exposures in excess of 3 min, the extent of the thermal effect and distribution of heat in tissues is determined by heat-regulating mechanisms. The thermal effect depends on exposure duration (Adair and Black 2003). Deep-lying muscles are heated to a greater extent only during the first 20 min of exposure. When the thigh region is exposed to microwaves, there is a greater temperature rise in the muscles than in the skin and subcutaneous (sc) fatty layer (Cook 1952; Herrick and Krusen 1953). For a full discussion and review of thermal interactions with animals and humans, including specific tissues (see Chapter 10 of *BBA* and Chapter 5 of this volume).

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### 3.3 Biochemical Changes

Biochemical alterations have been reported to result from exposure to RF energies. Such effects generally appear to be reversible and no well-defined characteristic response pattern has been determined, nor is it known whether the changes are direct or indirect effects of exposure. This is an area of research in which considerable work was conducted in the 1970s and 1980s. However, very few results have been reported in publications over the past decade.

Effects on mitochondria isolated from exposed animals have been reported (Dumansky and Rudichenko 1976) but there was no effect on rat liver mitochondria exposed *in vitro* to 2.4 GHz, 1 to 4 W/kg, or 10 to 12 GHz, at a maximum of  $20 \text{ W/m}^2$ . No effect of microwave exposure has been found on a number of enzymes and proteins irradiated *in vitro* (Belkhode et al. 1974; Allis 1975; Ward et al. 1975; Bini et al. 1978). Albert et al. (1974) exposed Chinese hamsters at 2.45 GHz,  $500 \text{ W/m}^2$  for 0.5 to 4.5 h over a period of 1 to 21 d and found no change in liver ATP.

The limited number of studies on oxidative enzyme systems has yielded mixed results. Exposure of suspension of the membrane-bound enzyme cytochrome oxidase to sinusoidally modulated 2.45 GHz MW energy at an SAR of  $26 \text{ W/kg}$  did not significantly affect its activity during exposure. On the other hand, Sanders et al. (1980), exposing the rat brain surface *in vivo* to 591 MHz continuous wave (CW) RF at 50 or  $138 \text{ W/m}^2$  under conditions of negligible systemic temperature change, reported that increases in NADH and decreases in ATP concentration occurred supporting an hypothesis of radio frequency radiation (RFR) inhibition of electron transport in brain mitochondria. These observations were later extended to effects of pulsed, and

sinusoidal amplitude-modulated microwaves on brain energy metabolism where similar changes were found (Sanders et al. 1985).

Dumansky et al. (1972) reported a decrease in liver glycogen content together with increased lactic acid levels and phosphorylase activity, which was interpreted as evidence of impairment of glycogen synthesis in the livers of rats chronically exposed to 2.45 or 10 GHz MWs at 500 to 2000 W/m<sup>2</sup>. Exposure to 2.45 or 10 GHz MWs at 0.25 to 10 W/m<sup>2</sup> decreased the proteolytic activity of the mucous membrane of the small intestines of experimental animals, where the invertase and adenosine triphosphatase activity increased. Altered proteolytic activity was suggested to be due to alteration in the structure and physicochemical characteristics of the mucous membrane. Reduced synthesis of macroglobulin and macroglobulin antibodies resulted from exposure of experimental animals to 0.5 W/m<sup>2</sup> (Malyshev and Tkachenko 1972).

Dose-dependent transient elevations in serum glucose, blood urea nitrogen, and uric acid were noted following far field exposure of rabbits to 2.45 GHz for 2 h at intensities of 50, 100, and 250 W/m<sup>2</sup> (Wangemann and Cleary 1976). There were detectable differences between CW and PW exposures of equivalent average power density. There was an increase in colonic temperature of 1.7 to 3.0°C at 100 and 250 W/m<sup>2</sup>. Exposure of rats for 15 min to pulsed 2.86 GHz at 50, 100, 500, or 1000 W/m<sup>2</sup> resulted in statistically significant changes in serum albumin and phosphorous levels only at 1000 W/m<sup>2</sup>, and there was no change in serum glucose levels in rats exposed to 2.86 and 0.43 GHz pulse-modulated fields at an average power density of 50 W/m<sup>2</sup>. Single or repeated exposures of rabbits to 3 or 10 GHz at 500 to 2500 W/m<sup>2</sup> resulted in alterations in serum albumin-globulin ratio, which was attributed to effects on the liver or adrenals (Swiecicki and Edelwejn 1963).

Baranski (1972a) found inconsistent changes in cholinesterase activity in both rabbit and guinea pig brains following 3 months of exposure, 1 h/d, with pulsed fields of 250 W/m<sup>2</sup>. He also found a decrease in cholinesterase activity in the brains of guinea pigs after a single 3-h exposure to 35 W/m<sup>2</sup> of pulsed 2.45 GHz MWs. Increase to 250 W/m<sup>2</sup> caused a further decrease in activity. Pulsed energy was found to produce a more severe effect than CW exposures of the same average power density, suggesting that these effects are due to peak fields. Nikogosyan (1962) found an increase in blood cholinesterase activity after a single 90-min exposure to 3 GHz waves at 400 W/m<sup>2</sup>. Revutsky and Edelman (1964) also reported an increase in specific cholinesterase activity in rabbit blood exposed *in vitro* to 2.45 GHz MWs. It should be noted that what is measured in blood is pseudocholinesterase, which has no neural-related activity. On the other hand, Olcerst and Rabinowitz (1978) found no effect on aqueous cholinesterase exposed to 2.45 GHz CW up to 1.25 kW/m<sup>2</sup> for half an hour or 250 W/m<sup>2</sup> for 3 h. No effect was found on cholinesterase activity in defibrinated rabbit blood exposed for 3 h to 210, 350, or 640 W/m<sup>2</sup>, 2.45 GHz, CW or pulsed. Under similar exposure conditions, there was no effect on release of bound calcium or magnesium from rabbit red blood cells (RBCs).

Mitchell et al. (1989) exposed or sham-exposed rats to 100 W/m<sup>2</sup> CW radiation at 2.45 GHz for a period of 7 h. Animals were subjected to behavioral, biochemical, and electrophysiological measures during and immediately after exposure. In this joint U.S. and former USSR study, the U.S. group found Na<sup>+</sup>, K<sup>+</sup>, and ATPase activity to be significantly lower in microwave exposed animals than in the shams. No significant differences were found by the former Soviet Union group. On review, the authors concluded that the U.S. result was spurious. This and other studies done emphasized the value of multilaboratory collaboration in promoting rigor in scientific design.

Ho and Edwards (1977, 1979) studied oxygen consumption in mice exposed to 2.45 GHz radiation at various SARs in a waveguide exposure system and found that the animals compensated homeostatically to SARs of 10.4 W/kg or greater by a decrease in

metabolic rate to compensate for thermal loading. Normal metabolic activity was resumed following cessation of exposure.

Oxidative stress has been proposed as a possible mechanism for the biological effects of MW exposure (Lai and Singh 1995–1997) and some experimental support for such a mechanism has been reported. Other experimental work, however, has not fully supported this proposal. Induction of stress proteins was investigated in HeLa and CHO cells following 2 h of RF exposure at 2450 or 2700 MHz, respectively (Cleary et al. 1997). The study was conducted under isothermal conditions at an SAR of 25 W/kg (HeLa cells) or 27 W/kg (CHO cells). Exposures had no detectable effect on stress protein induction with comparisons made to both sham and positive control cells. Reports of decreased camphor binding in rat olfactory tissue that potentially could indicate oxidative stress effects in exposed samples did not show correlation with SAR (Bruce-Wolfe and Adair 1985; Philippova et al. 1988, 1994). High levels of MW, which generated elevated temperatures in liposomes (*in vitro*), showed no effect on peroxidation (Logani and Ziskin 1996). However, in a series of studies in which the RF levels resulted in considerable temperature elevations, changes could be demonstrated in membrane fluidity, permeability, and protein shedding in a way that might be related to oxidative stress (Liburdy and Penn 1984; Liburdy and Magin 1985; Liburdy and Vanek 1985, 1987; Liburdy et al. 1988). Further work, suggesting that these observations were indeed due to temperature elevation, showed that even at high SAR RF exposure of sheep RBCs, no effect on NADH oxidase or glucose oxidase activity was observed when the temperature of the preparation was controlled (Kiel and Erwin 1986; Kiel et al. 1988). Using lower levels of RF, there was an apparent protection of temperature-induced oxidative hemolysis in RBCs (Kiel and Erwin 1984).

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### 3.4 Cellular and Molecular Biology

Studies to determine whether RF exposure can produce effects in biological systems can be approached on several levels. A primary goal in such experimental studies would be to establish reproducible effects resulting from exposure under well-controlled conditions. One such approach is to utilize cultures of either animal or plant cells under *in vitro* conditions. Cell culture experiments are relatively straightforward to reproduce; however, the behavior of cells in culture is never precisely the same as within an organism (*in vivo*) due to the isolation of cells from their usual environment, including contact with other cell types, missing intercellular factors, and lack of normal tissue architecture. *In vitro* experiments, therefore, can sometimes be more and sometimes be less prone to evidence responsiveness to an environmental exposure. The advantages of cellular experiments accrue to precise control of conditions, good dosimetry, and strong statistical power due to sample size and replicate designs. Cell culture studies, however, also have significant limitations in long-term exposure experiments and are often only remotely connected to potential human health issues.

Significant advances in our understanding of biological systems have occurred over the 10 y since the previous edition of this handbook. These advances have had demonstrable effects on the understanding of biology, which in turn have facilitated efforts to determine if and how RF could influence biological systems. The design of experiments and collection of data from culture studies has also improved from technical advances in modeling the inherent complexity of biological systems, thus providing a theoretical foundation for interpreting experimental data. Cell and tissue culture techniques in the last decade or so

have moved from simple biochemical assays and single-molecule detection methods to investigations using identified signal transduction pathways, polymerase chain reaction technology permitting DNA amplification from even single cells, and differential display techniques and cDNA microarray approaches that permit studies of gene expression of large numbers of genes at one time. Other new techniques include high performance liquid chromatography, mass spectroscopy of protein chips, and studies of protein distributions, protein dynamics, and protein-protein interactions. In addition, developments in microscopic techniques provide a basis for advanced studies of processes such as cell dynamics and inter- and intracellular interactions. These advanced techniques are not fully developed in MW research but are just recently beginning to be employed in the investigation of biological preparation response to MW exposure. Some aspects of MW effects on cellular biology have recently been reviewed by Heynick et al. (2003).

### 3.4.1 Chromosome and Genetic Effects

Early on in MW bioeffects research, investigators have focused on chromosome changes, including translocations and structural aberrations (Heller 1970; Chen et al. 1974; Yao 1978, 1982), polyploidy (Yao 1976), and stickiness (Manikowska et al. 1979). Exposures ranged from  $70 \text{ W/m}^2$  to more than  $2 \text{ kW/m}^2$ . In a study of meiotic cells removed from exposed male mice (Manikowska et al. 1979), translocations were observed at meiosis I at 10 and  $50 \text{ W/m}^2$ . Other investigators reported no exposure-related effects (Huang et al. 1977; Lloyd et al. 1984, 1986). An extensive study conducted by Kerbacher et al. (1990) examined Chinese hamster ovary (CHO) cells exposed to 2450 MHz at high SAR levels ( $33.8 \text{ W/kg}$ ). Even though the temperature of the cultures was increased to  $40^\circ\text{C}$  by the exposure, an assessment of a large number of indicators of chromosomal alteration showed no changes due to exposure. This study included many experiments and independent replication for each exposure condition. Following this work, a number of more recent studies have reported results in agreement with these findings that induction of chromosomal aberrations is not a result of RF exposure (Maes et al. 1997, 2000, 2001; Vijayalaxmi et al. 1997a,b, 1998, 2001a,b, 2003). Induction of micronuclei (MN) as an indicator of chromosomal damage has also been thoroughly investigated following MW exposure. Although there are reports of MN induction in mammalian cells exposed to RF *in vitro* (D'Ambrosio et al. 2002; Tice et al. 2002), there are a far greater number of reports suggesting the lack of MW involvement in MN formation (Vijayalaxmi et al. 2001a,b, 2003; Bisht et al. 2002; McNamee et al. 2002a,b). Even studies that are positive for MN production on exposure failed to show a corresponding evidence of DNA breakage (Tice et al. 2002). The problems inherent in the use of chromosomal aberrations as indicators of genetic damage have been noted by Savage (1977).

Ciaravino et al. (1991) exposed CHO cells simultaneously to adriamycin and pulsed 2.45 GHz microwaves at a SAR of  $33.8 \text{ W/kg}$  for 2 h. Temperatures did not exceed  $39.7^\circ\text{C}$ . RF radiation did not affect changes in cell progression produced by adriamycin and did not change the number of sister chromatid exchanges induced by adriamycin, an agent which is known to damage DNA. Similar results were obtained for mitomycin C (Ciaravino et al. 1987). Lloyd et al. (1986) examined human peripheral blood lymphocytes *in vitro* exposed up to  $200 \text{ W/kg}$  of 2.45 GHz radiation for 20 min with temperatures ranging from  $37$  to  $40^\circ\text{C}$ . Using standard techniques for evaluating unstable chromosome and chromatid-type breaks, exchange aberrations or sister chromatid exchanges, no significant variations from appropriate control cultures were found. In a series of studies, Maes et al. investigating sister chromatid exchange (SCE) in exposed cultures initially reported an increase in SCE (Maes et al. 1996), then inconsistent changes

(Maes et al. 1997), and finally two studies with no evidence of an effect (Maes et al. 2000, 2001). In another study, mice were exposed to 2.45 GHz RF at 200 W/m<sup>2</sup> (SAR 21 W/kg) for 8 h/d for 28 d. Incidences of SCE in bone marrow cells of irradiated mice, sham-irradiated control mice and standard control mice were compared. No statistically significant differences were detected (McRee et al. 1981).

More recent genetic work has focused on formation of single- or double-strand breaks in the DNA of exposed samples. As seems to be a pattern in much of this work, initial reports indicated an increase in DNA breakage in exposed cultures (Kurt and Milham 1988; Lai and Singh 1995, 1997). Subsequent attempts at replication, however, have failed to confirm the changes initially observed (Malyapa et al. 1998; Hook et al. 1999; Lagroye et al. 2004). Additional work at cell phone frequencies also showed no increased DNA single-strand breakage with exposure (Tice et al. 2002). The large majority of studies using mammalian cell lines as well as isolated human cells indicate a lack of strand breaks associated with MW exposure (Maes et al. 1997; Malyapa et al. 1997a,b; Vijayalaxmi et al. 2000, 2001b; Alekseev and Ziskin 2001; Li et al. 2001; McNamee et al. 2002a,b).

Hamrick (1973), studying the response of mammalian lymphocytes exposed to 2.45 GHz MW (CW), reported that power densities from 67 to 160 W/kg had an effect identical to that of heating on the secondary structure of DNA, as determined by comparison of thermal denaturation curves. In other work, circular DNA plasmids, exposed to 2.55 GHz microwaves at SARs ranging up to 85 W/kg for 20 min exhibited a decrease in supercoiled DNA with an increase of relaxed and linear DNA indicating single- and double-strand breaks (Sagripanti and Swicord 1986). The authors indicated that this could be a result of excitation of internal vibrational modes of the DNA but that a nonspecific or thermal mechanism could not be excluded. Furthermore, the presence of copper electrodes in the DNA solution led the investigators to conclude that DNA breakage was not due to RF exposure (Sagripanti et al. 1987).

Hamnerius et al. (1985) exposed *Salmonella typhimurium* in exponential phase growth to a number of different frequencies in pulse wave (PW) and continuous wave modes producing SARs up to 130 W/kg over exposure periods ranging from 2.5 to 6 h. Temperatures were closely controlled between exposed and control samples. Ames' assays on bacteria showed no elevated mutation frequency. The same group, utilizing similar exposure conditions, studied mutations in a gene controlling eye pigmentation in *Drosophila*. No elevated mutation frequency was found.

The mutagenic potential of microwave energy has been evaluated by various techniques including point mutations in bacterial assays (Anderstam et al. 1983), the dominant lethal test in mammalian systems (Varma et al. 1976), or genetic transmission in *Drosophila* (Pay et al. 1972; Mittler 1976) with inconsistent results.

Anderstam et al. (1983) found no change in mutation induction in *Escherichia coli* or *S. typhimurium* exposure to 0.027 and 2.45 GHz continuous wave (CW) and 3.07 GHz pulsed wave (PW) at SARs from 4 to 100 W/kg. Baranski et al. (1976) were not able to attribute mutagenic effects or metabolic changes in *Physarum polycephalum* or *Aspergillus nidulans* to 2.45 GHz, 100 W/m<sup>2</sup> CW or PW microwaves. Correlli et al. (1977) also reported no mutagenesis after exposure to RF at 2.6 to 4.0 GHz, 20 W/kg. They investigated the effects of RF on colony-forming ability (CFA) and molecular structure (determined by infrared (IR) spectroscopy) of *E. coli* B bacterial cells in aqueous suspension. Cells were exposed for 10 h at SARs of 20 W/kg (equivalent to 5 kW/m<sup>2</sup>). No RF-induced effects on either CFA or molecular structure were observed.

Other studies of mutagenic effects of RF in bacteria and yeasts have given negative results. A study by Blackman et al. (1976) involved exposure of *E. coli* WWU to 1.70 or 2.45 GHz RF at 20 to 500 W/m<sup>2</sup> for 3 to 4 h. Dutta et al. (1979) exposed *Saccharomyces*

*cerevisiae* D4 to 2.45 GHz CW RF at 400 W/m<sup>2</sup> or to 8.5 to 9.6 GHz pulsed RF at 10 to 450 W/m<sup>2</sup> for 120 min with negative results.

Saunders et al. (1988) exposed mice to 100 W/m<sup>2</sup> at 2.45 GHz CW for 6 h/d for an 8-week period. Exposed male mice were mated to an unexposed group of female mice and followed. There was no significant reduction in pregnancy rate and cytogenetic analysis at the end of the mating period showed no significant variation from the controls.

Meltz et al. (1987) investigated whether there were any RF-induced alterations in DNA repair in normal human fibroblasts maintained *in vitro* after the DNA was damaged by a selected dose of UV light. Power densities of 10 or 100 W/m<sup>2</sup> (0.350 and 1.2 GHz) caused no perturbation of the DNA repair process. Brown et al. (1981) treated mice with streptozocin, a mutagenic and carcinogenic agent known to damage DNA in the rodent liver, and exposed the mice to 400 MHz RF to determine if excision repair of the DNA would be inhibited. Power densities of 16 and 160 W/m<sup>2</sup> (SAR 0.29 and 2.9 W/kg) did not alter the level of excision repair.

Although Mickey (1963) reported increased mutagenesis in *Drosophila* exposed to a 0.02 GHz field, no effects on mutagenesis were observed by Pay et al. (1972) using 2.45 GHz or by Mittler (1976) with 0.003 and 0.150 GHz RF energy.

Varma and Traboulay (1976) reported increased mutagenesis, using the dominant lethal test with male mice exposed to 1.7 GHz continuous wave at 100 and 500 W/m<sup>2</sup> for 90 and 30 min, respectively. Mice exposed to 2.45 GHz CW at 1 kW/m<sup>2</sup> for 10 min and at 500 W/m<sup>2</sup> three times, 10 min each, within 1 d also showed increased mutagenesis. Mice subjected to four 10-min exposures at 500 W/m<sup>2</sup> over a period of 2 weeks, showed no increase in dominant lethality above control levels. Saunders et al. (1983) found no evidence of change in dominant lethality in mice exposed to 2.45 GHz CW, 43 W/kg.

Berman et al. (1980) exposed male rats daily to 2.45 GHz CW (day 12 of gestation to 90 d of age, 100 W/m<sup>2</sup>, 4 h/d) or 2.45 GHz CW at 50, 100, or 280 W/m<sup>2</sup> from day 6 of gestation to 90 d of age, 4 or 5 h/d. No significant evidence of germ cell mutagenesis or alteration in reproductive efficiency was detected. In a recent study, Roti Roti et al. (2001) found no evidence of neoplastic transformation in C3H10T1/2 cells after exposure to 835 MHz frequency division multiple access (FDMA) and 847 MHz code division multiple access (CDMA) signals.

It is known that the rate of induction of mutations will increase with increasing temperature. It is possible that artifacts or thermal stress could be factors in some of the reported studies. Baranski and Czernski (1976) noted that there is no satisfactory evidence of microwave-induced genetic effects at low to modest power densities. Additional experimental studies in rodents (Varma et al. 1976) have confirmed earlier findings that microwave exposure at power densities below 100 W/m<sup>2</sup> is not mutagenic in these organisms. Other studies (Liu et al. 1979) have failed to find effects that differ from those resulting from RF heating. Janiak and Szmigielski (1977) likewise reported no significant differences in the sequence and time course of cell membrane injury between cells treated in a water bath and those heated with 2.45 GHz microwaves.

Theories have been proposed that absorption of energy by the DNA from the MW field might result in chemical- or reactive species-mediated damage to the molecule (Swicord and Davis 1982; Edwards et al. 1984, 1985; Davis et al. 1986; Sagripanti et al. 1987). In the actual experimental work in several laboratories, definitive conclusions are less clear. In the interaction of millimeter waves with biological media, a number of investigators reported sharp, distinct resonances in the absorption of the RF energy by various biochemical and biological preparations (Webb and Booth 1969, 1971; Stamm et al. 1974; Dardanoni et al. 1976; Lee and Webb 1977) or that exposure to millimeter waves produces biological effects which exhibit a sharp-frequency dependence (Berteaud et al. 1975; Stamm et al. 1975; Grundler and Keilmann 1978, 1983). These studies suggest sharp

millimeter wave, frequency-dependent lethal and mutagenic effects on microorganisms, on metabolic control of growth of cells, on oncogenic viruses, viability properties of cells, protective effects on x-irradiated and cytotoxic drug-treated animals, etc. Because of poor documentation of a number of these studies and failure to replicate the observation of resonances in studies, which were adequately documented (Gandhi et al. 1980) there is little confidence in the validity of such resonances. Experiments in two other laboratories have failed to support either the theoretical propositions or the earlier observations (Foster et al. 1987; Gabriel et al. 1987, 1989).

Absorption resonances were also reported for DNA in solution for the range 8 to 12 GHz (Swicord and Davis 1982). These observations were later retracted by the authors, who identified artifacts of the experiment (Rhee et al. 1988). An attempt to identify such resonances in another laboratory was not successful (Gabriel et al. 1987).

In summary, there is no solid evidence that exposure to RF radiation induces mutations in bacteria, yeasts, or fruit flies. The results of some studies suggest that RF radiation induces mutations in mammals. Critical review has cast doubt on these findings. Other studies have shown no mutagenic effects of RF on mammals. Evidence for cytogenetic effects of RF is mixed. The lowest power density at which cytogenetic effects have been reported is  $200 \text{ W/m}^2$  (Stodolnik-Baranska 1974) but these results are contradicted by Chen et al. (1974), who failed to find cytogenetic effects at 2 to  $5 \text{ kW/m}^2$ .

### 3.4.2 Kinetic or Functional Changes and Membrane Effects

Many investigations were performed in the 1980s and early 1990s on the potential for MW irradiation to produce functional changes and membrane effects in exposed cell cultures. However, there is very little work reported with such a focus since the last edition of this handbook. The conclusions stated then have not changed; that RF effects were observed; however, consistent changes appeared generally to be associated with accompanying temperature increases and not due to a direct RF field influence. Kinetic or functional changes demonstrated in cells exposed to RF radiation have been reported, as reviewed below.

Dutta et al. (1992) exposed neuroblastoma cells in culture to varying powers of RF at carrier frequencies of 915 and 147 MHz, amplitude modulated at 16 Hz. Enhanced release of calcium ions from the cells was found at two different power densities, between which no enhancement was found. There was enhanced acetylcholinesterase activity under the same exposure conditions. Saffer and Profenno (1989, 1992) found an increase in beta-galactosidase expression exposed to RF between 2 and 4 GHz at  $10 \text{ W/kg}$  for 4.5 h with temperature changes not exceeding  $0.1^\circ\text{C}$ . Control cultures were adjusted to identical temperatures. The authors concluded that the effect was not thermal but possibly caused by thermal gradients due to RF radiation different from conductive heating produced by ambient conditions.

D'Inzeo et al. (1988) studied acetylcholine-activated single channel openings of cultured myotubes from chick embryos by patch clamp techniques. During exposure to RF at 10.75 GHz,  $0.5 \text{ mW/m}^2$  at the surface of a bathing solution, the frequency of channel openings decreased but channel-opening time and conductance were not affected. The authors speculated that exposure to microwave energy decreased channel-opening probability via an alteration of intracellular enzymatic processes through a nonthermal mechanism.

Rotkovska et al. (1987) studied stem cell colony-forming units (CFUs) derived from bone marrow cells of female mice utilizing a spleen exocolony method. At 2.45 GHz CW at a range of temperatures there was a significant enhancement of the number of CFUs in RF-exposed systems versus temperature-matched controls.

Rotkowska et al. (1993) also attempted to assess the role of a membrane receptor in the enhancement effect of microwave energy described above. Bone marrow preparations were incubated with a beta-receptor blocker, trimepranol (TMP), during microwave exposure and controlled with preparations without TMP; in addition, comparable samples were exposed only to ambient heat. The microwave stimulatory effect appeared to be blocked in the presence of TMP. The authors stated that activation of the beta-receptors results in enhancement of cAMP synthesis and an increase of  $\text{Ca}^{2+}$  ions in the cell caused by increased membrane permeability. This is followed by reactions of the adenylate cyclase system, resulting in the enhancement described above.

Cleary et al. (1990a) exposed cultured glioma cells to 0.027 and 2.45 GHz CW RF radiation for 2 h at temperatures maintained at 37°C. At SARs up to 50 W/kg, there was significantly more incorporation of tritiated thymidine and uridine into exposed versus control cultures. These changes persisted for up to 5-d postexposure. Kinetic changes of cell populations exposed to RF energy are also described in [Section 3.10](#).

However, Parker et al. (1988) exposed rodent cell lines to 2.45 GHz microwave radiation at SARs up to 103 W/kg for varying lengths of time at temperatures from 37 to 45°C, using DNA probes for oncogenes, heat protein and long terminal repeat sequences, hybridized to mRNA in exposed and control cells. No significant differences in mRNA expression were observed after microwave exposure.

Brown and Marshall (1986) reported that microwave energy produces no perturbation in cultures of murine erythroleukemic cells undergoing erythroid differentiation in response to hexamethylene bisacetamide (HMBA). Exposures were for 48 h at 1.18 GHz at SARs up to 73 W/kg with temperature maintained at 37.4°C.

Effects of RF on cell membrane dynamics have also been reported. Liburdy and Vanek (1985) exposed rabbit erythrocytes to 2.45 GHz microwaves under a number of conditions and observed a change in membrane permeability to sodium ions within the temperature range 17.7 to 19.5°C. This change was described as linear with the internal electric field strength inside the sample, saturating at 600 V/m. The same investigators studied the effects of microwave energy on liposomes carrying cytosine arabinofuranoside (ARA-C) (Liburdy and Magin 1985). Below the membrane phase transition temperature of 41°C, liposome membranes are normally not leaky. In buffered saline exposure to 2.45 GHz microwaves at a SAR of 60 W/kg ARA-C is released at 33°C. In plasma release occurs at 27°C. Release is enhanced by oxygen and attenuated by antioxidants.

Phelan et al. (1992) studied the effect of microwave energy on membrane fluidity using a B16 melanoma cell line exposed to 2.45 GHz pulsed waves 10  $\mu\text{s}$  wide at 100 pulse per second (pps) for 1 h at 100 W/m<sup>2</sup>. An increase in membrane ordering as measured by an electron paramagnetic resonance (EPR) technique, reflecting a shift from a more fluid-like phase to a more solid-like phase, was found. A similar result with artificially prepared liposomes constituted with melanin was discovered. Melanotic cells rendered amelanotic did not show the change. The ordering process was inhibited by superoxide dismutase, implicating oxygen radicals as a cause of the membrane changes. The authors proposed that microwaves stimulate melanin to free radical production and that reactive oxygen species may subsequently produce membrane alterations via lipid peroxidation resulting in the ordering effect.

Sandblom and Thenander (1991) studied the effects of microwaves on the kinetics of gramicidin A channels in lipid bilayer membranes. Artificial membranes into which the decapentapeptide, gramicidin A, was inserted were exposed to 10 GHz pulsed microwaves, pulse width 1  $\mu\text{s}$  at a pulse repetition rate of 1000 per second in waveguide for 1 min at an average output power of 300 W, peak power 300 kW. Temperature excursions were up to 5.1°C and estimated average SAR was 350 W/kg. Although microwave energy did not affect single channel conductance of channel lifetime compared to matched



temperature controls, the rate of channel formation was decreased during exposure. The authors stated that this is opposite of the effect expected for a purely thermal effect and offer theories for a nonthermal mechanism.

Philippova et al. (1988, 1994) observed that microwave radiation decreased specific  $^3\text{H}$ -camphor binding to rat olfactory membrane. Exposures were achieved in a waveguide using 0.9 GHz fields pulsed modulated at frequencies at 1–100 Hz. SAR was varied from 0.5 to 18 W/kg and steady-state temperatures of 4°C maintained. The inhibition of binding appeared to decrease with increasing SAR. Inhibition did not depend on pulse modulation rate. The shedding of protein during microwave exposure of erythrocytes and lymphocytes had been previously reported by Liburdy and Penn (1984).

Allis and Sinha-Robinson (1987) investigated the ATPase activity in human erythrocyte membranes *in vitro* exposed to 2.45 GHz CW microwave radiation for a period of 20 min at a SAR of 6 W/kg and stabilized temperatures from 17 to 31°C. At 25°C, but not at other temperatures, there was a 35% decrease in the activity of the enzyme in microwave-exposed samples. The authors proposed that a transition state of the protein–lipid interaction within the membrane is available at the transition temperature and not at other temperatures.

The activity of a specific enzyme, ornithine decarboxylase, is increased in certain cell lines following exposure to amplitude-modulated microwave fields (Byus et al. 1988). There are many more studies that have documented membrane fluidity and ion transport effects of exposure to thermal levels of RF (Portela et al. 1979; Olcerst et al. 1980; Webber et al. 1980; Friend et al. 1981; Pickard and Barsoum 1981; Barsoum and Pickard 1982a,b; Arber and Lin 1983; Shnyrov et al. 1984; Kim et al. 1985; Liu and Cleary 1988; Sandweiss 1990; Saalman et al. 1991; Bergquist et al. 1994; Neshev and Kirilova 1994; Orlando et al. 1994; Fesenko and Gluvstein 1995; Weaver 1995; Eibert et al. 1999).

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### 3.5 Reproduction, Growth, and Development

Developing fetuses, as well as perinatal organisms, are acknowledged to be particularly sensitive to changes in environmental conditions. On this basis and because of general concern for potential effects on reproduction and development from MW exposures, a large number of research efforts were focused in this area from the early 1970s through the early 1990s. The results from that body of research indicate that little evidence exists for MW exposure effects on either reproduction or physiological processes in the developing organism. Primarily due to the generally observed lack of effects, little new information is available other than a few studies specifically targeting cell phone frequency RF exposure.

#### 3.5.1 Reproduction

MWs have been investigated extensively for possible effects on the testes (Ely et al. 1964; Goud et al. 1982; Kowalczyk et al. 1983). Exposure of the scrotal area at high power densities ( $>500\text{ W/m}^2$ ) results in varying degrees of testicular damage, such as edema, enlargement of the testis, atrophy, fibrosis, and coagulation necrosis of seminiferous tubules in rats and rabbits, exposed to 2.45, 3, and 10 GHz radiation. Saunders and Kowalczyk (1981) exposed the caudal area of anesthetized mature male mice to 2.45 GHz RF in a waveguide system for 30 min. Half-body SARs ranging from 18 to 75 W/kg

were estimated from measurements of forward, reflected, and transmitted powers. The corresponding colonic temperatures at the end of exposure ranged from 35.3 to 42.2°C. Other anesthetized mice were sham-exposed. Their mean colonic temperature was 32.6°C, 4 to 5°C lower than unanesthetized mice. For comparison, the caudal areas of other anesthetized mice were inserted for 30 min in a copper well heated by a water bath to 37, 41, 43, or 45°C, which resulted in colonic temperature from 36.4 to 40.7°C. Six days after treatment, sections of testes were scored for cell damage and enumeration of sperm. Extensive degeneration of the spermatogenic epithelium was evident for RF exposure at 75 W/kg and for direct heating to 45°C. At SARs of 57 and 46 W/kg (43 and 41°C), marked depletion of spermatids and spermatocytes, but not spermatogonia, was observed. At the lower SARs (37, 30, and 0 W/kg) or a temperature of 37°C, no effects were seen.

Temperature-sensing probes were also implanted in the testes of other groups of mice, and testicular temperatures were related to SAR values (Berman et al. 1980; Lebovitz and Johnson 1987a). Such measurements of testicular temperature indicated the existence of a threshold of about 39°C for depletion of spermatocytes and of about 41°C for 50% cell death after 6 d of RF exposure or direct heating. The corresponding SARs for these two thresholds were 20 and 30 W/kg, respectively.

Lebovitz and Johnson (1983, 1987a) exposed rats for 8 h to 1.3 GHz CW radiation at a whole body averaged SAR of 9 W/kg. This produced a deep rectal temperature elevation of 4.5°C. Subsequent sequential histologic examination, measurements of daily sperm production, circulating follicle-stimulating hormone (FSH), and leutinizing hormone (LH) were all normal, suggesting that the level of acute hyperthermia was not sufficient to produce disruption for any of the indicated measures of testicular function.

Lebovitz et al. (1987b) compared pulse-modulated 1.3 GHz MW, 1  $\mu$ s pulse width, 600 pps, to conventional heating in rats. Anesthetized animals were sacrificed over a 13 d cycle of seminiferous epithelium after exposure. At 7.7 W/kg, whole body average SAR for 90 min, there was a modest decline in daily sperm production, primarily from effects on primary spermatocytes. Exposure at 4.2 W/kg produced no change. Temperatures in the first exposure reached 40°C and in the second 38°C. Above 7.7 W/kg, all germ cell types were destroyed. Conventional heating in excess of 39°C for 60 min produced a significant decrease in daily sperm production. The authors stated all damage could be explained by heating.

Dasdag and colleagues exposed rats to cell phone RF and observed a reduction in seminiferous tubule diameter (Akdag et al. 1999; Dasdag et al. 1999). However, further study in the same laboratory was unable to replicate the earlier findings and found no effects on measures of testicular structure and function (Dasdag et al. 2003). With temperature controlled to 37°C, *in vitro* experiments, where RF exposure was to relatively high power (>50 W/kg), resulted in a reduction in sperm fertility, although a mechanism was not presented (Cleary et al. 1989). Additionally, an *in vivo* exposure study suggested a reduction in fertility in rats exposed to 27 MHz without an increase in body temperature (Brown-Woodman et al. 1989). This study has not been independently confirmed and appears to be inconsistent with the larger body of evidence that indicates reproductive effects from RF exposure occur only when temperature in the tissues is elevated.

Exposure to 3 GHz radiation, 80 W/m<sup>2</sup> did not affect mating of mice or rats (Miro et al. 1985). Pituitary gonadotropic function was preserved in female mice exposed to 3 GHz radiation, 100 W/m<sup>2</sup>, twice daily for 5 months (Bereznitskaya and Kazbekov 1974).

Reports on reproductive effects of MW exposure in humans include workers using radars, video display units (VDUs), RF heat sealers, and devices used for magnetic resonance imaging (MRI) and medical diathermy. These reports show principally negative results although some positive results were also identified. Whereas a slightly increased risk of infertility (Smith et al. 1997) and a small elevation in risk of miscarriage

(Goldhaber et al. 1988; McDonald et al. 1988) have been reported, no association between exposure to VDUs and pregnancy outcomes was observed in the large majority of studies (Michaelson 1983; Nurminen and Kurpa 1988; Bryant and Love 1989; Taskinen et al. 1990; Larsen 1991; Schnorr et al. 1991). No elevation in risk for adverse reproductive effects was observed in female MRI workers (Evans et al. 1993; Kanal et al. 1993).

Miscarriage in physical therapists exposed to medical diathermy units at 915 and 2450 MHz was reported to be slightly elevated (Ouellet-Hellstrom and Stewart 1993). Unlike many studies showing positive results, a weak dose–response relationship of effects was observed in this study; however, significant questions have arisen concerning the validity of the association between the reported effect and the absorbed RF energy (Hocking and Joyner 1995). Although low birth weight of offspring was reported to be associated with exposure of pregnant physiotherapists to shortwave equipment (Lerman et al. 2001), in another study of a comparable group of female workers, no evidence of malformations or spontaneous abortions was found in their offspring (Taskinen et al. 1990).

Lower sperm concentration, motility and number of normal sperm were observed in RF workers (Lancranjan et al. 1975), although others could not replicate the finding of reduced sperm numbers (Weydant et al. 1996; Schrader et al. 1998). Fertility, measured as semen quality and hormone levels in male heat-sealer workers, was not adversely affected with exposure (Grajewski et al. 2000).

### 3.5.2 Embryonic Development and Teratology

A recent review of MW exposures and embryonic development has been published by Heynick and Merritt (2003). There are a few reports that suggest particular combinations of frequency, duration, and power density produce effects on embryonic development and postnatal growth. Alterations in development have been reported in insects (Carpenter and Livstone 1971), chick embryos (Van Ummersen 1961), and rodents (Rugh et al. 1975).

Green et al. (1979) and Olsen (1982) have provided a thermal basis for an explanation of the MW-induced teratogenic effects in the mealworm, *Tenebrio molitor*, reported by Carpenter and Livstone (1971), Lindauer et al. (1974), and Liu et al. (1975). Olsen (1977) noted the threshold of teratogenesis in *Tenebrio* to be associated with a rise of pupal temperature in excess of 10°C.

Van Ummersen (1961) reported inhibition of growth and development of chick embryos exposed to 2.45 GHz (CW), 200 to 400 W/m<sup>2</sup>, 4.5 min to 5 h. Since all embryos in which effects were produced experienced a significant temperature increase, the observed deleterious effects were ascribed to heating. In other studies in chick eggs, 2450 MHz exposures at 2.9 W/kg showed no effect on hatchability (Braitewaite et al. 1991) and more recently Talau et al. (2003) investigated temperature changes in chicken eggs exposed to 1250 MHz fields of 1.45–10.44 W/kg.

An extensive series of MW exposure studies at 2450 MHz have been completed to examine embryo development in Japanese quail eggs (Hamrick and McRee 1975, 1980; McRee et al. 1975, 1983; Hamrick and Fox 1977; Galvin et al. 1980, 1981; Inouye et al. 1982; Clark et al. 1987; Gildersleeve et al. 1987a,b, 1988a,b).

Quail embryos, exposed during the first 12 d of development to 2.45 GHz MWs at an incident power density of 50 W/m<sup>2</sup> and SAR of 4.03 W/kg, showed no gross deformities in the exposed quail when examined and sacrificed at 24 to 36 h after hatch. No significant changes in the total body weight or weights of the heart, liver, gizzard, adrenals, and pancreas were found in the treated birds. Hematological parameters were also measured in the study. The results showed a statistically significant increase in hemoglobin and

decrease in monocytes in birds exposed to MWs. No statistically significant changes in hematocrit, RBCs, total white blood cells (WBCs), lymphocytes, neutrophils, basophils, or eosinophils were detected (McRee and Hamrick 1977).

Although effects were observed in isolated parameters when exposures were of sufficient intensity to produce elevation in temperature, in exposures under 4 W/kg no changes were reported in development of the heart (Hamrick and McRee 1975; Galvin et al. 1980), immune response (Galvin et al. 1981; Gildersleeve et al. 1987a), mortality, egg production, fertility, hatchability of eggs, and reproductive performance (Gildersleeve et al. 1987b).

In additional quail egg studies, Spiers and Baummer (1991) exposed eggs from day 1 through 15 of incubation to sham or 2.45 GHz microwave radiation, 8 h/d at SARs from 3.3 to 15.2 W/kg at a number of ambient temperatures. It was concluded that microwave radiation can be used to increase egg temperature and embryonic growth rate at ambient temperatures below the normal incubation level without altering basic metabolic processes of the embryo.

A number of studies, at various MW frequencies, have been concerned with effects of exposure on mammalian embryonic and fetal development. In many of these studies, a single acute exposure was administered at a power density that causes an increase in body temperature. Some investigators, however, have reported studies in which protracted exposures have been given at power density levels, which apparently do not cause a significant increase in colonic temperature. These and other studies and results are described below.

Much of the work examining development in MW-exposed animals has been performed at 2450 MHz. Rugh et al. (1975) reported abnormalities in mouse fetuses exposed in an environmentally controlled waveguide on gestation days (GDs) 7 to 13 to 2450 MHz (CW) in the range of 84 to 112 W/kg. Hemorrhages, resorptions, exencephaly, stunting, and fetal death were observed. In a further study, Rugh and McManaway (1976) demonstrated that lowering the body temperature of the dam with pentobarbital anesthesia could prevent the teratogenic effects of thermal loading with MWs, thus demonstrating the thermal influence in the earlier studies.

Employing a multimodal cavity at 2.45 GHz, Chernovetz et al. (1975) irradiated pregnant mice with a single intense dose of 38 W/kg for 10 min (22.8 J/g) on GD 11, 12, 13, or 14. This dose resulted in 10% maternal lethality. The exposure during late organogenesis or early fetal stage caused no change in fetal mortality or morbidity when compared to shams. In another study, Chernovetz et al. (1977) noted an increased rate of resorptions in rats after an absorbed dose of 30 W/kg for 20 min once on GDs 10 to 16.

Berman et al. (1978) exposed CD-1 pregnant mice to 2.45 GHz at 34, 136, or 280 W/m<sup>2</sup>, GD 6 through 17 for 100 min daily. Estimates of mean dose rate ranged from 2.0 to 22.2 W/kg. No significant increase in colonic temperature was reported. Another group was similarly sham-exposed. The mice in half of each group were examined on GD 18. The incidence of pregnancy, the number of live, dead, and resorbed fetuses, and the total number of fetuses were found to be similar for the exposed and sham-exposed mice. The mean body weight of the live fetuses in the RF-exposed group, however, was significantly smaller (by 10%) than those in the sham-exposed group, a finding consonant with their previous results. In addition, ossification of sternal centers was significantly delayed in the RF-exposed mice. The mice in the other half of each group were permitted to come to term. Significant fetal growth retardation occurred at the highest power density level. A significant increase in CNS anomalies occurred, but only if the data from three irradiated groups were combined. At 7 d of age, the mean body weight of the suckling mice of the RF-exposed group was also significantly smaller (by 10%) than that for the sham-exposed

group. The survival rate was not affected. The authors concluded that MW radiation at a frequency of 2.45 GHz was embryopathic at a power density of 280 W/m<sup>2</sup> (22.2 W/kg).

The embryofetal toxicity and teratogenicity of 2.45 GHz at different intensities were investigated in the CD-1 mouse by Nawrot et al. (1981). Mice were exposed on GD 1 to 15 at an incident power density of 50 W/m<sup>2</sup> (SAR of 5 W/kg), and either on GDs 1 to 6 or 6 to 15 to 210 W/m<sup>2</sup> (SAR of 28.14 W/kg) or to 300 W/m<sup>2</sup> (SAR of 40.2 W/kg) for 8 h daily. Exposure either on GD 1 to 6 or 6 to 15 to a power density of 210 or 300 W/m<sup>2</sup> caused an increase in colonic temperature of exposed dams of 1 and 2.3°C, respectively. To distinguish between thermal and nonthermal effects of 210 or 300 W/m<sup>2</sup>, groups of mice were also exposed to elevated ambient temperature to raise their body temperature to the level of the animals exposed to MW. Ambient temperatures of 30 and 31°C increased the deep colonic temperature to that obtained with the 210 and 300 W/m<sup>2</sup> MW exposure, respectively. The mice exposed to higher ambient temperature were handled in exactly the same manner as the MW-exposed mice. A significant decrease in implantation sites per litter and reduction in fetal weight was noted in the group exposed at 300 W/m<sup>2</sup> during GDs 1 to 6 compared to that of mice exposed to elevated ambient temperature. Exposure of mice at 300 W/m<sup>2</sup> (GDs 6 to 15) resulted in a slight but significant increase in the percentage of malformed fetuses, predominantly with cleft palate, when compared to all other groups.

In studies of effects of *in utero* exposure of Long-Evans rats to 2.45 GHz CW microwaves, Michaelson et al. (1976) found no adverse effects on the dam or offspring when gestation length or litter size was examined. The rats were exposed at 100 or 400 W/m<sup>2</sup> for 1 h on GD 9 (organogenesis) or day 16 of gestation. Enhanced maturation, as indicated by adrenocortical response, was suggested.

Laskey et al. (1970) observed an increase in resorption rate and a decrease in term fetal weight in offspring of rats exposed to 2.45 GHz for 8 to 13 min on GD 2, 2 and 5, 8, or 15 at 1 kW/m<sup>2</sup>. Maternal body temperature increased from 3 to 4°C during exposure. Smialowicz et al. (1979a) exposed rats to 2.45 GHz MWs daily for 4 h at a power density of 40 W/m<sup>2</sup> *in utero* from GD 6 d through 40 d of age. The SAR was determined by twin well calorimetry for several ages of the animals. Pregnant dams weighing 300 to 350 g had a mean SAR of 0.7 W/kg; animals that were 1 to 5 d of age and weighed 6 to 10 g absorbed approximately 4.7 W/kg. There was no significant difference between the mean body weights of the males (female offspring were not used in this experiment) in the 12 sham-irradiated litters when compared to the mean body weights of the males in the 12 MW-irradiated litters.

Jensh et al. (1983a,b) reported on the effects of protracted prenatal exposure of Wistar-derived rats to 2.45 GHz for 8 h daily throughout gestation. The mean exposure time to incident energy at 200 W/m<sup>2</sup> was 115 h. No statistically significant differences were observed between control and exposed animals for maternal mass, embryonic and fetal resorption rate, abnormality rate, and the term fetal and placental masses. These authors (Jensh et al. 1982a,b) also reported similar experiments in which 915 MHz MW were employed. They exposed Wistar rats at 100 W/m<sup>2</sup> for 8 h daily throughout the period of gestation for an average of 110 h of exposure. Postexposure colonic temperatures of dams showed no increase over baseline rectal temperatures. No differences were observed in embryonic or fetal death, abnormalities, fetal mass, litter size, placental mass, fetal sex ratio, maternal mass, or maternal gain of mass.

In additional experiments, rats exposed during gestation to high intensity 2450 MHz fields (16.6 to 22 W/kg) showed lower brain weight (Shore et al. 1977; Berman et al. 1984). However, long-term continuous exposure of rats during gestation at lower SAR (0.4 W/kg) produced no effects in development or fetal and brain weight (Merritt et al. 1984). Furthermore, in a study of brain development at 15, 20, 30 and 40 d of age following prenatal and postnatal 2.45 GHz exposure, no effects on brain development were detected

histologically (Inouye et al. 1983). In these studies, the brain SAR was 9.5 W/kg or more. In an additional study, Galvin et al. (1986) exposed rats prenatally between GD 5 and 20 and postnatally between days 2 and 20. Exposed animals exhibited larger body mass and less endurance at 30, but not at 100 d of age. SARs in the fetal rats were 4 W/kg and in the postnatal exposures were 5.5–16.5 W/kg. Jensh and Ludlow (1980) reported that exposure at 2.45 GHz to 3.6–5.2 W/kg did not alter growth or development and no changes were observed in five of six behaviors when the animals were tested as adults.

RF fields at SARs of 0.2, 1.0, and 5.0 W/kg demonstrated no developmental effects on rat embryos grown in culture (Klug et al. 1997). Studies have also been conducted at frequencies other than 2.45 GHz and the results are essentially the same. Lin et al. (1979b) studied the effects of repeated exposure of C3H mice to 48 MHz. The animals were exposed to 5 W/m<sup>2</sup> in a transmission electron microscopy (TEM) exposure chamber for 1 h/d, 5 d/week, beginning on the 4th to 7th day of postpartum, for 10 weeks. The formed elements in the blood were not affected by the exposure. The means of body mass of the irradiated and control animals were comparable. No significant differences in lesion onset, incidence, prevalence, extent, or type were observed when animals repeatedly exposed to RF were compared with sham control groups. During the period of the study, no cataracts were noted. Fertility differences among irradiated and control animals were not detected. Body growth patterns did not differ among sham- and RF-exposed animals.

Bereznitskaya and Kazbekov (1974) exposed mice to 3 GHz, 100 W/m<sup>2</sup>, which resulted in increased fetal wastage. No definite abnormalities or inborn genetic defects were found. Neonatal mice exposed to 19.27, 26.6, or 105 MHz pulsed waves in a magnetic field of 55 A/m and an electric field of 8000 V/m, 40 min/d for 5 d did not show any evidence of alteration in growth and development.

Lin et al. (1979b) exposed C3H mice in a rectangular coaxial exposure system at 148 MHz at 5 W/m<sup>2</sup> for 1 h daily from GD 2 through day 19, corresponding to a SAR of 0.013 W/kg. The experiment was conducted as three separate replications. In each of the experiments, some of the dams were allowed to come to term and the fetuses were assessed for weight at birth and again at 60 d of age, whereas in others the uterus was extirpated on GD 19 of gestation. No differences in percentage of resorbed, stillborn, or abnormal fetuses were observed in any of the three experiments.

Dietzel and coworkers exposed pregnant rats using a 27.12 MHz diathermy unit at 55, 70, or 100 W, which was sufficient to raise the animals' colonic temperatures to 39, 40, or 42°C (Dietzel et al. 1972; Dietzel 1975). The rats were exposed once between GDs 1 and 16. The fetuses were examined near term. The peak incidence of anomalies was found to occur on GDs 13 to 14, when 16% of the fetuses were abnormal. This fetal wastage, as in the studies of Rugh et al. (1975), was clearly associated with a general body temperature increase in the dam.

Brown-Woodman and Hadley (1988) exposed pregnant rats at GD 9 to thermalizing levels of RF at 27.12 MHz and found increasing developmental abnormalities with increasing core temperature. Maximum temperature elevation was 5°C. Only a few seconds at this temperature produced abnormalities. Rats heated to comparable levels and for comparable durations in a water bath demonstrated similar levels of abnormalities, suggesting that the teratogenicity of RF radiation is related to hyperthermia.

A series of experiments was conducted on rats to assess the teratologic effects of 27 MHz because of the prevalence of this frequency in industries using RF heat sealers (Conover et al. 1979; Lacy et al. 1980a,b). At various days of gestation, the investigators used energy levels sufficient to cause and sustain high body temperatures (up to 43°C, 20 to 40 min). These experiments showed that GD 9 was the most sensitive day for the rat. A variety of malformations, including decreased body weight, was found. Thermal (maternal) threshold was 41.5°C. Gross microscopic, teratogenic effects were equivalent when

increased maternal temperature was caused by MW or hot-water immersion. The threshold was approximately 12 W/kg for 30 min.

Conover et al. (1978) reported on single 20- and 30-min exposures of fetal rats on GD 10, 12, or 14 to 27.12 MHz at SARs of 17 to 35 W/kg. Preliminary results suggested that fetal wastage occurred after exposure on the 10th or 14th day of gestation. Grossly observable malformations occurred in animals exposed during the 10th day for 30 min, while visceral abnormalities occurred after exposure on the 10th and 14th days. Fetal mass as well as crown-rump length was smaller numerically in the fetuses exposed to MWs. The same investigators reported on eight groups of 16 to 28 gravid rats exposed to 27 MHz. The facility for exposure was an RF near field synthesizer operating in the dominant magnetic field mode at a field strength of 55 A/m. The dams were exposed on GDs 2, 4, 8, 10, 14, or 16 for 20 to 40 min at an average dose rate of 125 W/kg until their colonic temperature reached 43°C. Eight groups were sham-irradiated for 30 min and one group of 29 rats served as cage controls. Rats irradiated on GDs 8 to 16 (organogenesis) had a significant increase in gross malformations and a significant decrease in fetal weight and fetal crown-rump length.

Boak et al. (1932) administered shortwave radiation (1 MHz) to rabbits from the 29th day of life through several matings and pregnancies. The total exposure time ranged between 30 and 75 h, during which the temperatures of the animals were raised to 41 to 42°C. There was no interference with mating, fertilization, or development of the young *in utero*. Litter sizes were not significantly different from those of the control animals.

Roux et al. (1986) reported a study of rats exposed to 434 MHz radiation and ionizing radiation, indicating that the two forms of radiation potentiate teratogenic effects in the rat. In a study designed to examine possible effects of chronic RF exposure on mother-offspring behavioral patterns and the electroencephalogram (EEG), Kaplan et al. (1982) exposed 33 female squirrel monkeys near the beginning of the second trimester of pregnancy to 2.45 GHz RF in multimode, mode-stirred MW cavities at whole body SARs of 0.034, 0.34, or 3.4 W/kg, the last value equivalent to about 100 W/m<sup>2</sup> of plane wave RF, for 3 h/d, 5 d/week, until parturition. Eight pregnant monkeys were sham-exposed for the same periods. After parturition, 18 of the RF-exposed dams and their offspring were exposed to RF for an additional 6 months; then the offspring were exposed without the dams for another 6 months. No differences were found between RF- and sham-exposed dams in the numbers of live births or in the growth rates of the offspring. The major difference between RF- and sham-exposed offspring was that four of the five exposed at 3.4 W/kg both prenatally and after birth unexpectedly died before 6 months of age. They apparently had developed a form of pneumonia. Although the numbers of animals used in the behavioral and EEG studies were adequate, the mortality values were too small to place much confidence in statistical inferences.

O'Connor (1980) noted that with respect to basic design, procedure, and variables assessed, the teratogenic studies reported were more diverse than decisive. Wide variation in exposure parameters makes it difficult to compare the results; additional difficulty is generated because many of the reports do not contain information on critical variables such as the manner in which the day of gestation was timed. The day on which the animal is sperm or sperm-plug positive can be timed as day 0, although a more common procedure is to consider this day 1. The manner in which control animals were treated is often not given. Many kinds of controls have been employed including passive cage controls, sham-exposed controls, heat (IR-irradiated) controls, and historic controls. In some reports, probability statements have been substituted for data from concurrently studied control animals. Multiple control procedures in single experiments have not been used extensively. In addition, many of the investigators who have reported

defects have employed acute, intense irradiation, which has placed a thermal burden on the exposed subject. Some studies attempt to control heat by including controls heated by means of IR. Of importance in this regard are the comparisons with the literature on heat stress. Many of the teratogenic studies of RF exposure have been performed without a sufficient number of animals, and others lack sophisticated design and thus do not allow for observation of low-probability events. Statistical analyses are usually not given in enough detail to permit evaluation (O'Connor 1980).

However, if attention is focused, not on procedural questions, but only on similarities in the results of the studies, several trends are apparent. The most common result from fetal exposure to MWs suggests that below thermal levels of exposure few changes are observed. An occasional nonspecific, general response of reduced or retarded gain of body mass may occur. However, further study would be required to know if such an effect is maintained after birth.

A more general deleterious response seen in the mammal is increased rate of fetal resorption. The increase may be indicative of malformed fetuses, but the resorbed nature of the fetal material precludes a more fine-grained analysis and thus identification of which, if any, specific structure was damaged. The increased rate of resorption and the range of exposures within which it occurs is remarkably similar to the effects of heat stress. Particularly in the rat, the resorption rate appears to increase within a rather narrow thermal window, the other side of which is fetal death. The majority of defects have been observed following high level, acute exposures with obvious thermal effect (O'Connor 1980).

It does appear that abnormalities in small animal fetuses can be produced with MWs in conjunction with systemic hyperthermia of 2.5 to 5°C above the normal temperature for the species during a time period of specific critical development. From a survey of the literature, it appears that it is the temperature rise in the fetus, irrespective of the manner in which it is produced, that causes damage. It is important to realize that in all species there is a dynamic pattern of maturation during gestation, and in the rat this continues during the first 3 weeks of postnatal life. In interpreting the few teratological effects of MWs that have been reported, as with any agent, it is important to realize that many fetal defects such as hemorrhage, resorption, stillbirth, and exencephaly also occur spontaneously in mice (O'Connor 1980).

Thermal stress, therefore, appears to be the primary mechanism by which RF energy absorption exerts a teratogenic action. Chernovetz et al. (1975) and others have pointed out evidence that indicates increases in mortality and resorption are probably related to peak body temperature and its duration, regardless of the method by which the temperature elevation is elicited. The teratogenic effects of hyperthermia, regardless of the source, have been well-documented (e.g., Edwards 1968, 1969; Pleet et al. 1981). Rugh and McManaway (1976) were able to prevent the increase in incidence of teratogenic activity, which they had previously reported, by lowering the maternal body temperature through controlled use of pentobarbital anesthesia.

Concepts of thermoregulation and thermal stress have been discussed by Michaelson (1982) and Way et al. (1981). The absence of a core temperature change does not necessarily indicate that physiologic adjustments induced by exposure to RF energy are not heat-related. The organism has numerous thermoregulatory mechanisms by which it can maintain homeostasis such as modification of skin blood flow, alterations in peripheral conductance, changes in blood temperature, and alterations in evaporative loss mechanisms. This does not imply that the organism is not stressed. The stress induced by maintaining thermal homeostasis may result in abnormal growth and development in the offspring.



In humans, infections such as rubella, influenza, and smallpox, occurring during early pregnancy, are known to cause abortions and fetal malformations (Wilson 1959). In general, it appears that any infection, giving rise to fever in early stages of pregnancy in humans or animals, is capable of producing fetal malformation or abortion. It is well known that induction of fever can lead to the early termination of pregnancy (Cameron 1943). There are numerous reports of abnormalities from the induction of systemic hyperthermia of 2.5 to 5°C above the normal temperature for the species for 1 h or longer during specific critical developmental stages of the fetus by exposure of the pregnant animals to elevated temperatures and humidity in environmental chambers. Fetal resorption, growth retardation, microphthalmia, and malformations affecting the CNS, musculoskeletal system, and other organs have been observed in mammalian species. In experiments on rats, hyperthermia of 4 to 4.5°C for 40 to 60 min during specific developmental stages produces increased fetal resorptions, retardation of growth, microphthalmia, anencephaly, and defects of tails, limbs, toes, palate, and body, depending upon the gestational stage at which hyperthermia occurred (Edwards 1968). These results indicate that the occurrence of fetal malformations in mammals in early pregnancy is probably related, not as much to the viral or bacterial toxemia, but to the fever, hyperthermia occurring at a particular critical stage of organogenesis. The threshold appears to be an elevation of 2.5 to 5.0°C above the normal temperature of the species, sustained for an hour or more.

As noted by Marston and Voronina (1976) from the standpoint of public health, one must consider the difficulty of extrapolating data from experimental teratology to the human fetus. Such an extrapolation becomes feasible only after detailed analysis of the fine mechanisms of teratogenesis. Also, of great importance is the need for appropriate scaling factors to permit extrapolation of experimental data obtained on small animals, to the human.

The reports of effects of MW exposure on early development have been reviewed by Baranski and Czerski (1976), who concluded that no serious effects are to be expected at power densities below 100 W/m<sup>2</sup> under usual exposure conditions. They further note that defects, when observed, are the result of hyperthermia. The more recent review by Heynick and Merritt (2003) offers no evidence to refute this conclusion. In fact, additional work performed supports the conclusion that no effects on early development are due to MW exposures that do not produce temperature increases in the biological target. There are numerous reports of abnormalities from the induction of systemic hyperthermia of 2.5 to 5°C above the normal temperature for the species, by exposure of the pregnant animal to elevated temperatures at specific critical developmental stages of the fetus (Edwards 1968, 1969). It would thus appear that in the reports of MW-induced developmental abnormalities, it is the temperature rise in the fetus, irrespective of the manner in which it was produced, that caused the damage.

RF energies must be applied at high SAR (>15 W/kg) to rodents, approaching lethal levels, for teratogenesis to result. High maternal body temperatures are known to be associated with birth defects. There appears to be a threshold for the induction of experimental birth defects when a maternal colonic temperature of 41 to 42°C is reached. Any agent capable of producing elevated internal temperatures in this range is a potential teratogen.

Most studies involving prenatal exposures have not shown effects on growth and development. Temperature in the testes of >45°C induced by any modality can cause permanent sterility; from 37 to 42°C, mature sperm may be killed with a temporary loss of spermatogenic epithelium. Changes in reproductive efficiency have not been directly associated with RF exposure.

### 3.5.3 Postnatal Development and Behavior

Few studies have been reported in which postnatal psychophysiological parameters have been examined in animals exposed prenatally to MWs. Johnson et al. (1977) observed behavioral alterations in rats subsequent to protracted (20 h daily for 19 d during gestation) prenatal exposure to 0.918 GHz at a power density level of 50 W/m<sup>2</sup>. Such exposure resulted in a significantly lower level of achievement in the conditioned avoidance response test. Jensch et al. (1982a) were not able to detect significant behavioral changes associated with protracted 915 MHz MWs at 100 W/m<sup>2</sup> in Wistar rats, approaching the power density level required to increase maternal colonic temperature. Chernovetz et al. (1975) subjected mice to a single 10-min exposure to 2.45 GHz radiation at an SAR of 38 W/kg, without observing any postnatal functional alterations.

In another study, gravid Wistar rats were exposed 8 h daily throughout the pregnancy to 915 MHz MWs at 100 W/m<sup>2</sup> (Jensch and Ludlow 1980). The average exposure time was 109 h. In this study, dams were allowed to deliver their offspring. Within 3 d after birth, the pups were given three tests for reflexes. After the pups were weaned, a series of performance tests was given. The postweaning tests began on postpartum day 60 and were completed by the 90th day. No detrimental effects were observed on performance in a water T-maze, avoidance behavior, open-field behavior, forelimb hanging, or performance in a 24-h activity wheel. Jensch (1984a,b) also reported no observable teratology, growth, eye opening, or postexposure behaviors (open-field tests and water-maze performance) in rats exposed throughout gestation to 350 W/m<sup>2</sup> MWs at 6 GHz. The SARs in pre- and perinatal exposures that are associated with behavioral effects are consistent with the generally accepted threshold for such effects as about 4 W/kg. Prenatal exposure of rats to cell phone RF signals had no effects in behavioral tests on the adult animals (Bornhausen and Scheingraber 2000).

Japanese quail, irradiated continuously in ovo during the first 12 d of embryogenesis at 50 W/m<sup>2</sup>, SAR 4.03 W/kg, core temperature 37.5°C for exposed and controls, were followed to reproductive maturity. The reproductive histories of the exposed hatchlings were not different from the controls (Gildersleeve et al. 1987a).

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## 3.6 Effects on the Nervous System

Signal transmission occurs in biological organisms through complex electrical and chemical events. The very nature of the electrical communication within animals and humans suggests a potential interactive target for influences from external EMFs. Thus the intense interest in the early investigations of possible MW exposure effects on the nervous system (see review by Elder and Cahill, 1984). More recent concerns on cellular telephone safety, particularly from exposure of the CNS to such devices have revived wide-ranging research efforts investigating MW and RF exposures to the nervous system. In this section, results will be presented from studies in the 1960s through 1980s at a variety of frequencies, but also discussed are more recent studies using RF exposures principally at cell phone frequencies.

Transient changes in CNS function have been reported following MW exposure. Although some reports describe the thermal nature of MW energy absorption, others implicate nonthermal or specific MW effects at the molecular and cellular level. The first report of the effect of MW energy in the centimeter range on the conditional response activity of experimental animals was made by Gordon et al. (1955). In subsequent years,

study of nonthermal effects of MWs gradually occupied the central role in electrophysiological studies in the former Soviet Union (Novitskiy et al. 1971).

Yakovleva et al. (1968) reported that single and repeated exposures of rats to MWs, 50 to 150 W/m<sup>2</sup>, weakened the excitation process and decreased the functional mobility of cells in the cerebral cortex. Edematous changes were most often noted throughout the cortex. The greatest number of altered cells was noted with repeated exposures at 150 W/m<sup>2</sup>.

Seizure response to noise was transiently suppressed in audiogenic seizure-susceptible mice and rats after exposure to 3 GHz pulsed MWs at an average power density of 100 W/m<sup>2</sup> (Kitsovskaya 1960, 1968). Tolgskaya and Gordon (1960) studied the effects of both CW and PW MWs in rats at 3 and 10 GHz at various intensities. More pronounced morphological changes in the CNS were reported following 3 than 10 GHz at 10 to 100 W/m<sup>2</sup>. Pulsed waves were initially considered to be more effective than CW, a phenomenon also reported by Marha (1963). While it is clear that the amount of energy absorbed from MW exposures depends on factors such as wavelength body size and shape, and orientation in the fields (Gandhi 1974; Durney et al. 1986), the overall evidence for differential nervous system or behavioral responses to continuous or pulsed-wave MWs is somewhat tenuous (Frey and Feld 1975). Additional studies comparing pulsed and CW MWs can be found in the monograph by Tolgskaya and Gordon (1973), who noted that exposure to MWs can cause conditioned reflex activity and functional changes in CNS activity were reversible. The functional changes disappeared at the same time that the conditional reflex activity of the animals was restored upon cessation of exposure to MWs. A proliferative reaction of glial cells was also described. Even at high peak powers and ultra-wideband exposures, no evidence was observed of differences between CW and PW (Sherry et al. 1995). A more detailed review of PW investigations is presented by Lu and deLorge (2000) and Pakhomov and Murphy (2000).

Some investigators suggested that MW energy absorption may affect hypothalamic and midbrain function and also affect cerebral, cortical, and reticular system function (Yermakov 1969; Thompson and Bourgeois 1975). According to Gvozdikova et al. (1964), the greatest cortical sensitivity occurs in the meter range, less in the decimeter, and least in the centimeter MW band.

Guy and Chou (1982) and D'Andrea et al. (1994) studied response thresholds in rats for short, high-intensity microwave pulses. The energies were substantial in excess of those producing auditory responses (500  $\mu$ s wide or less, 5 to 180 mJ/kg in brain; see [Section 3.13.1](#)). Rats were subjected to local exposures to the head and especially the brain of a single 915 MHz pulsed field sufficient to produce a local SAR as high as  $4 \times 10^5$  W/kg and with pulse widths from 1  $\mu$ s to 360 ms. Animals displayed no reaction other than that due to the hearing effect until the specific absorption exceeded 28 kJ/kg. This correlated with a temperature rise in the brain of 8°C. Seizures lasted for 1 min after exposure followed by a 4- to 5-min unconscious state. Rats then recovered without apparent effect from the exposure. Brain histology revealed some demyelination of neurons 1 d after exposure and focal gliosis 1 month after exposure. Similar studies were performed by Petin et al. (2000).

By contrast Brown et al. (1994), also looking at high power pulses in intervals from 25 ms to 3.2 s, found effects at lower energies. Utilizing a 1.25 GHz source, comparing pulses at a repetition rate of 80 per second to equivalent CW for each episode of exposure, varying peak powers and total duration for different episodes of exposure, a threshold was found for an evoked body movement, an involuntary movement measured by a motion sensor attached to the tail of the rat. No differences were observed between PW and CW (or wide pulses) at any given SAR and specific absorption (SA, total energy absorbed per unit mass in J/kg). When the SAR was held constant at 7.3 kW/kg

under either mode and the SA was allowed to increase by varying the total exposure time from 0 to 3.2 s, the percentage of animals displaying the body movement increased proportionally up to 0.9 kJ/kg and then leveled off at 84% of the animals, reflecting a dependency on SA. On the other hand, holding SA constant at 0.9 kJ/kg and varying SAR revealed that above 900 W/kg most animals responded. However, lowering SAR (by lengthening the interpulse interval at constant SA) produced a proportionate decrease in percentage of responding animals out to 3.2 s, reflecting the ability of the thermoregulatory mechanisms to adjust to these lower SARs. At the highest energies, brain temperatures rose by 0.4°C. Skin and subcutaneous temperatures facing the energy source at the higher energies were measured and found to be at levels known to produce a nonpainful thermal sensation. The authors were uncertain as to the mechanism underlying the evoked movement. In further work of Brown et al. (unpublished), skin and subcutaneous regions of the snout and surrounding areas were anesthetized and animals subjected to pulsed energy as before. Thresholds and nature of responses were identical to those of the previous exposures, suggesting that cutaneous mechanisms did not mediate the evoked movement. Wachtel, reflecting on his earlier work with *Aplysia ganglia* (Wachtel et al. 1982), has proposed that the evoked movement reported by Brown et al. (personal communication) may result from the rate of temperature rise rather than the temperature change *per se* in a direct coupling with nervous tissue. The issue is, however, unresolved. Akyel et al. (1991) demonstrated that the evoked body movement produced by PW microwaves is differently affected by a number of neuropharmacologic drugs from that of the classic startle response. Additional work on high peak, low average power acute, and short-term effects is summarized in [Section 3.7](#).

### 3.6.1 Electroencephalographic Changes

Several investigators have reported that MW exposure produces alterations in the EEG (Baldwin et al. 1960; Livanov et al. 1960; Kolodov 1966; Baranski and Edelwejn 1967; Bawin et al. 1973; Servantie et al. 1975; Suvorov and Kukhtina 1984). Chizhenkova (1988) exposed unanesthetized rabbits to 2.4 GHz for 1 min at 400 W/m<sup>2</sup> and observed an increase in the number of slow waves in the EEG, and a change in the discharge frequency of neurons in the visual cortex. Evoked response of visual cortex neurons to light was enhanced and was regarded as the most sensitive indicator of an MW effect. Baldwin et al. (1960) exposed the heads of rhesus monkeys to 225 to 400 MHz CW in a resonant cavity and noted a progressively generalized slowing and some increase in amplitude of EEG patterns accompanied by signs of agitation, drowsiness, akinesia, and nystagmus, as well as autonomic sensory and motor abnormalities. There were signs of diencephalic and mesencephalic disturbances: alternation of arousal and drowsiness, together with confirming EEG signs. The response depended on orientation of the head in the field and reflections from the surrounding enclosure. In this report from 1960, the animals were described as exposed in a copper mesh resonant cavity into which a quarter wave antenna was inserted at the top. No direct dosimetry is described, but instead temperature measurements were performed in a vessel filled with water the size of an average monkey head. Temperature changes were merely described as trivial. Monkeys were fitted with metallic electrodes driven into the outer table of the skull for EEG measurements. It is a reasonable speculation that greater amounts of energy than suspected produced localized heating around electrodes affecting the results. Additional studies where effects of MW on EEGs were observed include rabbits exposed at 30 MHz, 0.5 to 2 kV/m (Takishima et al. 1979); cats at 147 MHz, unspecified SAR (Bawin et al. 1973); rats at 945 MHz, unspecified SAR (Vorobyov et al. 1997); rats at 900 MHz, 1.3 W/kg (Thuroczy et al. 1994). In the

range of studies that have been performed the types of changes in EEG are not consistent across studies.

In a joint U.S. and former USSR study, Mitchell et al. (1989) studied rats exposed for 7 h at 2.45 GHz and  $100 \text{ W/m}^2$ ; in comparing results between U.S. and former USSR investigators, he found statistically significant effects in the power spectral analysis of EEG frequency, but the two groups found such effects at different frequencies. EEG tracings in rabbits exposed to 3 GHz (PW)  $50 \text{ W/m}^2$  showed slight desynchronization from the motor region; at  $200 \text{ W/m}^2$  variations in the amplitude were observed; 300 MHz had a greater effect than 3 GHz. It was suggested that PW MWs produced a greater effect than CW MWs.

Baranski and Edelwejn (1975) reported that rabbits exposed to 10 GHz (pulsed),  $40 \text{ W/m}^2$  (single exposure) showed no changes in EEG tracings, but exposure to 3 GHz,  $70 \text{ W/m}^2$ , 3 h/d for 60 d, produced functional and morphological changes. Changes in conditional reflexes were also reported in rats exposed to 70 MHz, 150 V/m, 60 min/d for 4 months.

A review of the literature on EEG effects requires awareness of certain deficiencies in methodology and interpretation. The EEG is difficult to quantify due to its time-varying waveform. The use of metallic electrodes either implanted in the brain or attached to the scalp also makes many of the reports on EEG or evoked responses (ER) questionable. Johnson and Guy (1972) have pointed out that such metallic electrodes grossly perturb the field and produce greatly enhanced absorption of the energy in the vicinity of the electrodes. Such enhancement produces artifacts in the biological preparation under investigation. Recording artifacts also result from pickup of fields by the electrodes and leads during the recording of EEGs while the animal is exposed. For example, Tyazhelov et al. (1977) have pointed out that even for the coaxial electrode described by Frey (1968), diffraction of EM waves is still a major source of error because of the metallic nature and large dimensions of the electrode.

Bawin and Adey (1977) reported that electromagnetic energy of 0.147 MHz, amplitude modulated at brain wave frequencies, influenced spontaneous and conditioned EEG patterns in the cat at  $10 \text{ W/m}^2$ . These amplitude modulated 147 MHz fields induced changes only when the amplitude modulation frequency approached that of physiological bioelectric function rhythms; no effects were seen at modulation frequencies either below 8 Hz or above 16 Hz.

Human studies of RF fields have sometimes reported increases in parts of the EEG spectra (Reiser et al. 1995; von Klitzing 1995) often around the 10 Hz band (Borbély et al. 1999; Huber et al. 2000; Lebedeva et al. 2000, 2001). Additional reports failed to show the increases observed in above studies and reported either decreases (Croft et al. 2002) or no effects of exposure on spontaneous, awake EEG assessments (Kim 1998; de Seze 2000; Heitanen et al. 2000).

In studies examining RF mobile phone signals a shortening of sleep onset time and changes in EEG during sleep were observed (Mann and Roschke 1996; Mann et al. 1998a). However, in follow-up studies, the changes reported earlier were not statistically altered, even at greatly increased (100 to 250 times) exposure levels (Roschke and Mann 1997; Wagner et al. 1998, 2000; Mann and Roschke 2004). Huber et al. (2000) examined the spectral power of the EEG in humans for 900 MHz exposures. They found a short increase in the spectral power of nonrapid eye movement (NREM) sleep during the first 30 min but no effects on sleep latency, sleep stages, or rapid eye movement (REM) sleep spectrum. In a subsequent study, the same group observed that EMF alters waking regional cerebral flow and pulse modulation of the MW exposure is necessary to induce waking and sleep EEG changes (Huber et al. 2002).

A few studies of human event-related brain potentials have reported varied effects following RF exposure (Eulitz et al. 1998; Freude et al. 1998, 2000; Kellenyi et al. 1999).

Other studies looking at similar issues have reported no effects (Kim 1998; Urban et al. 1998; Hladký et al. 1999). Early findings by Krause et al. (2000a,b) of event-related potential changes during working memory tasks were not repeatable in replication efforts.

### 3.6.2 Calcium Efflux

In 1975, Bawin et al. (1975) reported changes in the calcium released from chick brain tissue following *in vitro* exposure to amplitude-modulated RF. The levels used appeared to preclude RF heating as a mechanism for the effect. Furthermore, effective calcium changes were dependent on amplitude modulation (AM), with significant effects reported for 6, 9, 11, 16, and 20 Hz. The effect also appeared to be power-dependent (Sheppard et al. 1979; Blackman et al. 1980a), suggesting the occurrence of power and frequency windows of effectiveness. Following these initial reports, a variety of studies were performed to investigate the calcium efflux effects of RF exposure (Bawin and Adey 1977; Bawin et al. 1978; Blackman et al. 1979, 1980a,b, 1982, 1985a,b, 1988, 1989, 1991; Joines and Blackman 1980; Albert et al. 1987).

In addition to chick brain, other neurological tissues have been examined following exposure to RF fields. These studies include cats showing irregular calcium efflux (Adey et al. 1982); increased efflux in rats exposed *in vivo* (Paulraj et al. 1999); no effect on rat brain tissue exposed *in vitro* (Shelton and Merritt 1981; Merritt et al. 1982); increased calcium efflux in rat brain synaptosomes exposed *in vitro* (Lin-Liu and Adey 1982); and human and rodent neuroblastoma cell lines, which showed effects similar to chick brain when exposed (Dutta et al. 1984, 1989). An overall evaluation indicates generally inconsistent results in calcium efflux from both *in vitro* and *in vivo* experiments. A detailed review of this literature is available (NRPB 2001).

### 3.6.3 Histopathology

According to some authors, cellular changes have been found in the nervous system of small animals following MW exposure at  $100 \text{ W/m}^2$  (Tolgskaya 1959; Tolgskaya and Gordon 1960, 1964). Albert and Sherif (1988) exposed 1 and 6 d old rats in the far field to 2.45 GHz, CW microwaves at  $100 \text{ W/m}^2$  for 5 consecutive days, 7 h/d (SAR  $2 \text{ W/kg}$ ). Animals were sacrificed 1 d after exposure cessation, and histology revealed evidence of widespread cell death with associated mononuclear cell infiltration.

Degeneration of neurons in the cerebral cortex and tissue changes in the kidney and myocardium of rabbits have been produced by exposure to 200 MHz. Accinni et al. (1988) found evidence for a condition resembling immune complex glomerulonephritis in rabbits exposed to 27.12 MHz with a diathermy apparatus sufficient to elevate rectal temperatures 2 to  $3^\circ\text{C}$  per episode of exposure. The exposures occurred daily for 3 weeks up to 20 min/d.

Exposure of cats for 1 h to 10 GHz,  $4 \text{ kW/m}^2$  resulted in injury to cerebral and spinal cord nerve cells; changes occurred in tigroid substance (Nissl bodies) and other components of nerve cells (Bilokrynytsk'ky 1966). On the other hand, rabbits exposed to 10 GHz (pulsed),  $40 \text{ W/m}^2$  showed no evidence of morphological damage to the brain, but comparable exposure to 3 GHz did produce such changes (Baranski and Edelwejn 1967).

Tolgskaya and Gordon (1960) have investigated the influence of PW and CW 3 and 10 GHz on the morphology of nervous tissue in rats and rabbits. With exposure to 3 GHz ( $1.1 \text{ kW/m}^2$  and  $400 \text{ W/m}^2$ ), severe symptoms of overheating were observed, often leading to death. Severe vascular disorders such as edema and hemorrhages in the

brain and internal organs were prominent. In repeated but less prolonged exposure, vascular disorders and degenerative changes in internal organs and the nervous system were less severe. With repeated exposures, the animals were better able to withstand successive exposures; they continued to gain weight; body temperature after irradiation quickly recovered; and temperature increase was not evident. Such a response resembles thermal adaptation on a physiological level.

At high field intensities, when death is a result of hyperthermia, the vascular changes are those of hyperemia, hemorrhage, and acute dystrophic manifestations (Dolina 1961; Minecki and Bilski 1961; Baranski et al. 1966). At low field intensities, the changes are of a more general dystrophic character, and proliferation of the glia and vascular changes are not as prominent.

Albert and DeSantis (1975) reported morphological changes in the brains of Chinese hamsters following exposure to 2.45 GHz CW MWs at power densities of 250 and 500 W/m<sup>2</sup>. Exposure durations varied from 30 min to 14 h/d for 22 d. Both light and electron microscopic findings revealed alterations in the hypothalamus and subthalamic structures of exposed animals, whereas other regions of the brain appeared unaltered. It should be noted that extremely high local SAR could be present under these conditions. Peak SAR could reach 40 to 200 W/kg in selected brain regions.

In subsequent studies at 1.76 and 2.45 GHz, 100 and 200 W/m<sup>2</sup>, Albert (1977, 1978) described cytoplasmic vacuolization of neurons, irregular swelling of axons, and decrease in dendritic spines of cortical neurons. The axonal swelling and spine changes were seen only in chronic exposures, whereas neuronal changes were observed in acute exposure. In all studies, no signs of permanent degenerative changes were recorded and reversibility was noted 2 h after exposure (Albert 1977). The author concluded that while it was possible that higher exposure levels (250 and 500 W/m<sup>2</sup>) could have resulted in thermal effects, it was unlikely that 100 W/m<sup>2</sup> would result in significant thermalization of the whole brain. He did not, however, rule out the possibility of hot spots. Exposure of rats to 100 W/m<sup>2</sup> of 2.45 or 2.8 GHz RF resulted in average hypothalamic temperature increases of 0.4°C or less. This increase is lower than hypothalamic temperature increases observed during the normal activity of an animal (Albert et al. 1977, 1978).

Albert et al. (1981b) looked at the effects of RF exposure pre- and postnatally on the Purkinje cells of the rat cerebellum. In one experiment, Sprague–Dawley rats were exposed *in utero* to 100 MHz CW RF at 460 W/m<sup>2</sup> (SAR 2.81 W/kg) for 6 h/d on GDs 16 to 21, and then for 4 h/d for 97 d after birth. Four exposed and four sham-exposed animals were sacrificed 14 months after cessation of irradiation. Quantitative assessment of the cerebella showed that the relative number of Purkinje cells was significantly smaller (12.7%) in experimental animals than in control animals. In another experiment, Sprague–Dawley rats were exposed *in utero* to 2.45 GHz CW RF at 100 W/m<sup>2</sup> (SAR 2 W/kg) for 21 h/d on GD 17 to 21. Power density measured was variable from 40 to 300 W/m<sup>2</sup> because of group exposure conditions. Half of the litters were used shortly after delivery and the other half 40 d after cessation of irradiation to assess effects of the exposure on cerebellar Purkinje cells. Because of the immaturity of the neonates, the Purkinje cell layer was not clearly displayed and quantitative results could not be obtained. Experimental animals sacrificed 40 d postexposure showed significantly fewer (25.8%) cells than did the controls. In a final experiment, rat pups were exposed to 2.45 GHz RF at 100 W/m<sup>2</sup> for 7 h/d on postnatal days 6 to 10. Half the pups were sacrificed at the end of exposure and half at 40 d postexposure. Only those sacrificed immediately showed a significant decrease in the relative number of Purkinje cells as compared with sham-exposed controls. Thus, exposure to two frequencies of RF at similar SAR values (2.8 and 2 W/kg) yielded a reduction in the relative number of Purkinje cells for fetuses and newborn rats. Although the change appeared permanent for rats exposed *in utero*, it appeared to be reversible for those exposed postnatally.

In a related study, Albert et al. (1981a) examined the effects of RF exposure on the Purkinje cells of squirrel monkey cerebella. Pregnant squirrel monkeys were exposed to 2.45 GHz pulsed RF at an equivalent power density of  $100 \text{ W/m}^2$  (SAR  $3.4 \text{ W/kg}$ ) 3 h/d starting in the first trimester of pregnancy. The offspring were exposed similarly for the first 9.5 months after birth. At the end of the irradiation period, seven exposed and seven sham-exposed animals were sacrificed and their cerebella examined. There were no statistically significant differences between control and exposed animals in any of the Purkinje cell parameters examined. Several factors were suggested by Albert et al. (1981b) to explain the discrepancy between these results and those with the rat. Factors that might have contributed were differences in geometrical configurations of the head, exposure methods, and daily exposure durations, as well as variations in gestational periods and species differences. It is quite possible that high local SAR values in the rat brain, but not in the squirrel monkey brain, at comparable whole body SARs, may be the important factor in explaining these differences.

### 3.6.4 Effects on the Blood–Brain Barrier

The existence of a BBB in most regions of the brain normally provides resistance to movements of large molecular weight, fat-insoluble substances (e.g., proteins or polypeptides) from the blood vessels into the surrounding cerebral extracellular fluid, presumably to protect the brain from invasion by various blood-borne pathogens and toxic substances. Several investigators have reported that RF can increase the permeability of the BBB to certain substances of large molecular weight, as described below. However, a majority of studies have been unable to confirm such effects.

The concept of the BBB is replete with definitional problems even preceding the interpretation of experiments. It is known that normal physiologic mechanisms of substance transport can occur by simple diffusion, facilitated diffusion, active transport or pinocytosis, and vesicular transport. Different substances are subjected to different mechanisms, making comparisons of differing substances as markers an uncertain process. The concept of an intrinsic permeability, which can be separated from other factors such as local cerebral blood flow (LCBF), has proved to be especially difficult to characterize. Changes in temperature, which are inevitable concomitants of RF experiments, alter several processes simultaneously making it difficult to identify changes in an intrinsic permeability.

The initial report in Western literature of possible impacts of MW exposure on the BBB was of a study of 2.45 GHz at an SAR of approximately  $1 \text{ W/kg}$  (Frey et al. 1975). Their report of increased BBB permeability was followed up by reports from Oscar and Hawkins (1977) that supported the original findings. They reported that a single exposure of rats to 1.3 GHz pulsed MWs for 20 min ( $1$  to  $20 \text{ W/m}^2$ ) induces a temporary change in the permeability of small inert polar molecules across the BBB of rats. Increases in permeability were observed for mannitol and inulin, but not for dextran, both immediately and 4 h after exposure, but not 24 h after exposure. They found statistically significant changes in the brain uptake index (BUI), also known as the Oldendorf (1970) technique, at average power densities less than  $30 \text{ W/m}^2$ . They also found that, depending on the specific pulse characteristics used, pulsed RF could be more or less effective in altering BBB permeability than CW RF of the same average power density. For pulses of long duration and high pulse power density, but only a few pulses per second, mannitol permeation could be induced at average power densities as low as  $0.3 \text{ W/m}^2$ . The authors considered the possibility of local heating due to hot spots since the greatest BBB alteration occurs in the cerebellum and medulla or close to the neck region of rats.



The Oldendorf technique involves injecting a measured amount of a  $^{14}\text{C}$ -labeled test substance and  $^3\text{H}$  water arterially, allowing one pass of the bolus through the brain, and following by decapitation and measurement of radioactivities in specific parts of the brain. A measurement of the ratio  $^{14}\text{C}$ - $^3\text{H}$  in brain tissue is normalized to the identical ratio in the injectate. The technique assumes that tritiated water freely diffuses between the brain and its vascular system. In fact, the passage of tritiated water is a function of LCBF, which itself varies, and therefore distorts the interpretation of the overall ratio.

Preston et al. (1979) and Preston and Prefontaine (1980), using methods similar to those of Oscar and Hawkins (1977), attempted to determine whether exposure to 2.45 GHz CW RF increased BBB permeability to  $^{14}\text{C}$ -labeled D-mannitol. They exposed rats to 1, 5, 10, or 100  $\text{W}/\text{m}^2$  and compared with sham-exposed rats for controls, they found no evidence that RFR exposure increased the permeability of the BBB for mannitol. In a second series, rats were exposed to 3, 10, 30, 100, and 300  $\text{W}/\text{m}^2$ . Again, there were no differences between results from exposed and sham-exposed animals. Like Oscar et al. (1981), Preston et al. (1979) believed changes in LCBF confounded the results of earlier studies.

Following up on earlier studies and in light of Preston's suggestion, Oscar et al. (1981) subsequently used a technique employing  $^{14}\text{C}$  iodoantipyrine to measure LCBF in rats. Male rats weighing 250 to 300 g were individually sham-exposed for 5, 30, and 60 min, or individually exposed for 5, 15, 30, 45 or 60 min to pulsed RF at 150  $\text{W}/\text{m}^2$  average power density. Carrier frequency was 2.8 GHz, pulse rate was 500 pps, and pulse width was 2  $\mu\text{s}$ . Within 5 min after sham or RF exposure, the previously venous-catheterized, but conscious, animals were infused with isotonic saline containing 5  $\mu\text{Ci}/\text{mL}$  of  $^{14}\text{C}$ -iodoantipyrine. The rats were decapitated 50 s after the start of the infusion. Brain regions were dissected out and assayed for radioactivity by routine liquid scintillation counting. LCBF was then calculated by established procedures. The results show that MW exposure caused a significant increase in LCBF (minimum 39% to >100%) in all 17 brain regions sampled. Because of these findings, the authors indicated that their earlier reported ratio measurements (Oscar and Hawkins 1977) may be an overestimate (Gruenau et al. 1982).

Attempts to duplicate the findings of Oscar and Hawkins (1977) have yielded equivocal results, as Chang et al. (1982) found, or failure as reported in a series of studies by Williams et al. (1984a–d). They (Williams et al. 1984e) concluded that studies reporting increased permeability of the BBB in many cases were subjected to technically derived artifact invalidating the conclusion. The authors found decreased entry of horseradish peroxidase (HRP) and  $^{14}\text{C}$  sucrose into the microvessel endothelium of hyperthermic rats at cerebral temperatures up to 43°C. They stated that it is possible that temperatures in excess of 43°C might disrupt the barrier by overt thermal injury as has been reported in clinical studies. They concluded that hyperthermia produces disruption of membrane functions including pinocytosis, but pointed out that other modes of transport, such as active and facilitated transport mechanisms, have not been evaluated. Others have also been unsuccessful in attempts to replicate Oscar and Hawkins (Ward et al. 1982; Ward and Ali 1985). Studies in mice exposed for either 1 h or after a lifetime of exposure at 4  $\text{W}/\text{kg}$  also demonstrated no permeability changes in the BBB (Finnie et al. 2001, 2002).

Merritt et al. (1978) exposed rats for 30 min to 1.2 GHz pulsed RF at peak power densities in the range of 20 to 750  $\text{W}/\text{m}^2$  and 0.5 duty cycle, corresponding to average power densities of 10 to 380  $\text{W}/\text{m}^2$ , or for 35 min to 1.3 GHz pulsed or CW RF at average power densities in the range of 1 to 200  $\text{W}/\text{m}^2$ . They examined brain slices under UV light for transfer of fluorescein and under white light for transfer of Evans blue dye (a visual tracer) across the BBB, and chemically analyzed various brain regions for fluorescein content. They also measured the brain uptake of  $^{14}\text{C}$ -labeled D-mannitol and determined the BUI values. To validate these detection methods, they used hypertonic urea, known to alter the BBB, as an alternative agent to RF. Finally, sham-exposed rats

were heated for 30 min in a 43°C oven to approximate the hyperthermia obtained at 380 W/m<sup>2</sup>. In their examination of brain slices, Merritt et al. (1978) found no evidence of enhanced fluorescein or Evans blue dye transfer across the BBB of RF-exposed rats, whereas penetration of the BBB was apparent for rats treated with urea instead of RF. The analyses of fluorescein content corroborated these findings. However, fluorescein uptake was high for the sham-exposed rats that were heated in the oven, an indication that hyperthermia of the brain is necessary to alter BBB permeability. In the <sup>14</sup>C-mannitol study of the various brain regions, there were no significant differences in BUI between RF and sham-exposed rats, whereas BUI changes were evident for rats treated with urea. Also, the results showed no evidence of the power density window reported by Oscar and Hawkins (1977). No change in the BBB occurred unless brain temperature was increased by 4°C.

Early on work relevant to MW exposure at high levels was conducted in which the effects of heating were examined on the integrity of the BBB. Sutton et al. (1973) used 2.45 GHz to produce selective hyperthermia of the brain in rats. He then studied the integrity of the BBB with HRP, a protein tracer that can be detected both morphologically and quantitatively. Brains were heated to 40, 42, and 45°C. Barrier integrity was disrupted after heating for more than 45 min at 40°C. Animals with brains heated to 45°C survived for only 8 to 15 min. The most common site of vascular leakage was the white matter adjacent to the granular cell layer of the cerebellum. Sutton concluded that to prevent BBB disruption, brain temperatures must not exceed 40°C in the absence of body core hypothermia.

Albert et al. (1978) also used HRP as a tracer and reported regions of leakage in the microvasculature of the brains of Chinese hamsters exposed to 2.45 GHz MWs at 100 W/m<sup>2</sup> for 2 to 8 h. In control animals, extravascular reaction product was found only in brain regions normally lacking a BBB. In a later paper, Albert (1979) reported that continuation of these earlier studies indicated that a partial restoration of the BBB's impermeability may have occurred within 1 h after exposure ceased, and that restoration was virtually complete within 2 h. Albert believed that the transient changes may be clinically subacute and probably cause no lasting ill effects. It is important to note, however, that such leakage of the microvasculature of the brain occurs irregularly; this was observed in approximately 50% of exposed and 20% of control animals studied by Albert and DeSantis (1975).

In another study, Albert (1979) exposed 52 animals (34 Chinese hamsters and 18 rats) to 2.8 GHz RF for 2 h at 100 W/m<sup>2</sup>. Of these, 30 were euthanized immediately, 11 at 1 h after exposure, and 11 at 2 h after exposure. Another 20 animals (12 hamsters and 8 rats) were sham-exposed. Leakage of HRP in some brain regions was reported for 17 of the 30 animals euthanized immediately after RF exposure and for 4 of the 20 sham-exposed animals. Fewer areas of increased BBB permeability were evident for animals euthanized 1 h after RF exposure, and except for one rat, virtually no leakage of HRP was seen for the animals euthanized 2 h after RF exposure. These results indicate that increased BBB permeability due to RF exposure at levels insufficient to denature brain tissue is a reversible effect. Albert suggested that such BBB changes may be clinically subacute and would probably cause no lasting ill effects. However, the increased BBB permeability seen in 4 of 20 sham-exposed animals may indicate that factors other than RFR in the experimental procedure could alter the BBB. One possible confounding point in the use of injected HRP as a tracer is the existence of endogenous peroxidase, the detection of which could yield false positive results. Also, no positive (BBB-altering) control agent was used in these studies for comparative purposes.

Comparable results were reported by Albert and Kerns (1981) on 51 Chinese hamsters exposed to 2.45 GHz CW RF for 2 h at 100 W/m<sup>2</sup>. There were 39 original sham-irradiated controls. Of the RF-exposed animals, 12 were allowed to recover for either 1 or 2 h prior to

HRP injection and subsequent fixation. This study appears to be an extension (for Chinese hamsters) of the work reported by Albert (1979) for 2.8 GHz RF exposure, with the same conclusions as that study.

Neubauer et al. (1990) reported that 2.45 GHz PW microwave radiation at  $100 \text{ W/m}^2$ , SAR approximately  $2 \text{ W/kg}$ , pulse width  $10 \mu\text{s}$  at 100 pps, resulted in enhanced uptake of rhodamine–ferritin complex by capillary endothelial cells of the cerebral cortex in rats, and was related to exposure duration and power density. Brain temperatures were not reported.

By using a small, dielectrically loaded coaxial applicator, Lin and Lin (1980, 1982) were able to irradiate only the heads of anesthetized adult male Wistar rats with pulsed 2.45 GHz RF ( $10 \mu\text{s}$ , 500 pps) for 20 min at average power densities of  $5 \text{ W/m}^2$  to  $30 \text{ kW/m}^2$ . The distribution of absorbed MW energy inside the head was determined by thermographic procedures, and average SARs were found to range from 0.4 to  $240 \text{ W/kg}$ . Evans blue dye was injected into a catheterized femoral vein following sham or RFR exposure, and 5 min later the animal was perfused via the left ventricle with normal saline. The brain was removed, examined, and scored for degree of tissue staining by the tracer. For average power densities up to and including  $26 \text{ kW/m}^2$  ( $200 \text{ W/kg}$ ), staining was not significantly different between exposed and control animals. For exposures at  $30 \text{ kW/m}^2$  ( $240 \text{ W/kg}$ ), extravasation of Evans blue dye could be seen in the cortex, hippocampus, and midbrain. The degree of staining decreased with increasing time to euthanasia postexposure, indicating that the effect was reversible. No alteration in the BBB was evident unless brain temperature was increased by  $4^\circ\text{C}$ . Neilly and Lin (1986) found that ethanol inhibits microwave-induced permeation of the BBB through reduced heating of the brain. Other groups have obtained results consistent with thermal effects (Goldman et al. 1984; Moriyama et al. 1991; Ohmoto et al. 1996; Fritze et al. 1997).

Chang et al. (1982) used a technique involving  $^{131}\text{I}$ -labeled albumin to investigate alterations of the BBB in dogs. The heads of dogs were exposed at various average power densities between 20 and  $2000 \text{ W/m}^2$ . In general, no statistically significant differences were found between exposed and sham-exposed animals, but the number of animals used in this study was too small to ascribe a high level of statistical confidence.

One group has reported increases in BBB permeability in rats exposed to CW 915 MHz fields independent of SAR, from 0.02 to  $8.3 \text{ W/kg}$ . With modulated RF effects were also not SAR-dependent with the confusing result of significant effects at low SARs of 0.0004 to  $0.008 \text{ W/kg}$  but no effect at the highest SARs ( $1.7$  to  $8.3 \text{ W/kg}$ ) (Persson et al. 1992, 1997; Salford et al. 1993a, 1994, 2003).

It is important to realize that the methods used to investigate BBB permeability are still somewhat controversial. Permeability changes in cerebral blood vessels occur under various conditions, including those that produce heat necrosis (Rozdilsky and Olszewski 1957). Most techniques used to measure BBB permeability in fact measure the net influence of several variables on brain uptake and do not differentiate among the effects of changes in the vascular space, alterations of blood flow, and membrane permeability.

The uncertainty in earlier research on the influence of RF on the BBB is no doubt related to significant artifacts introduced by the kinds of biological techniques used. Several investigators have indicated that exposure to RF may alter the size of vascular and extravascular volumes and cerebral blood flow rate, thereby yielding changes in the BUI that are not necessarily related to BBB permeability alterations. Blasberg (1979) reviewed many of the methods previously used for investigating BBB changes and the problems associated with these methods. Rapoport et al. (1979) developed a method for measuring cerebrovascular permeability to  $^{14}\text{C}$ -labeled sucrose that yields results independent of cerebral blood flow rate. As already noted, Oscar et al. (1981) confirmed

experimentally that LCBF is increased in the rat brain by exposure to pulsed RFR at 150 W/m<sup>2</sup> average power density.

There is still not a complete consensus among students of the BBB question. There is a widespread view that little confidence can be placed in the results of early experiments on RF-induced BBB alterations. The consensus is that hyperthermic levels of RFR, producing temperatures >43°C, can alter the permeability of the BBB, but in the majority of BBB studies at levels of MW exposure under 4 W/kg, exposure did not affect permeability of the BBB in animals.

### 3.6.5 Combined Effect of Radio Frequency and Drugs, and Radio Frequency Effects on Neurotransmitters

A pharmacodynamic approach has been taken by some investigators in the study of MW-exposure effects on the CNS and behavior (Baranski and Edelwejn 1968; Edelwejn 1968; Thomas and Maitland 1979). Following exposure to 3 GHz PW MWs, altered sensitivity to neurotropic drugs was noted. Decreased tolerance of rabbits to pentylentetrazole and increased tolerance to strychnine were observed after a single exposure to 200 W/m<sup>2</sup>. Repeated exposures at 70 W/m<sup>2</sup> produced a decreased tolerance to pentylentetrazole, strychnine, and acetophenetidin (Lin and Lin 1982). Servantie et al. (1976) reported that exposure of rats to 3 GHz at 50 W/m<sup>2</sup> for several days resulted in an altered reaction to pentylentetrazole. Using curare-like compounds, a neuromuscular site of action for this MW effect was implicated. Edelwejn (1968) observed alterations in the effects of chlorpromazine and D-tubocurarine on EEG recordings in rabbits repeatedly exposed at 70 W/m<sup>2</sup>. The author concluded that synaptic structures at the level of the brain stem are affected by MWs. It should be noted that drugs such as pentylentetrazole and chlorpromazine influence physiologic thermoregulation.

In some cases, drugs and microwave irradiation might have parallel effects, suggesting effects via a common mechanism. Lai, for example, has suggested that the parallel effects of MW and endogenous opioids on choline metabolism may be due to activation of endogenous opioids by MWs. Lai et al. (1987a), studying the effect of RF on central cholinergic activity, measured sodium-dependent, high-affinity choline uptake in different parts of the rat brain *in vivo* exposed to 2.45 GHz microwave radiation, 100 W/m<sup>2</sup>, CW and PW (100 W/m<sup>2</sup>, 2 μs pulses, 500 pps), whole body average SAR 0.6 W/kg. PW was found to decrease choline uptake in the hippocampus and frontal cortex. There was no effect on the hypothalamus, striatum, and inferior colliculus. Pretreatment with a narcotic antagonist blocked the effect of PW on hippocampal choline uptake but did not alter the effect on the frontal cortex. CW decreased uptake in the frontal cortex but not in the hippocampus, striatum, or hypothalamus. These effects have been further characterized by the same group of investigators (Lai et al. 1987b, 1988, 1989, 1991, 1992a,b; Lai 1992). The authors' conclusion from this series of experiments is a suggestion that the effect of RF on central acetylcholine metabolism is probably a secondary effect of endogenous opioids, which may result from the character of RF as a stressor. The auditory effect of pulsed RF could act as such a stressor. According to Lai, microwave-induced changes in central high-affinity choline uptake may also be mediated by corticotropin-releasing factor (Lai et al. 1990).

Microwave energy can affect the action of psychoactive drugs. Modulation of the action of barbiturates and related effects on the EEG has been reported. It has been reported that warming an animal with microwaves during anesthesia can attenuate the effects of pentobarbital (Bruce-Wolfe and Justesen 1985). Microwave energy can prolong the narcolepsy and hypothermia induced by pentobarbital. The role of local absorption patterns was found to be important.

A number of drugs can modify the thermal responses to microwaves. For example, chlorpromazine can attenuate the thermal effect of microwaves in ketamine-anesthetized rats (Jauchem et al. 1985a). On the other hand, chlorpromazine was reported to cause an increase in susceptibility to microwave heating in the dog (Michaelson et al. 1961).

Anticonvulsant drugs such as chloral hydrate can decrease susceptibility of rats to MW-induced convulsions (Lobanova 1985). The subject of drug modifications of thermal responses to microwaves has been reviewed by Jauchem (1985).

Pulsed microwaves have been reported to enhance the hypothermic effects of acetylcholinesterase inhibitors in the CNS (Ashani et al. 1980). Quock et al. (1987) reported that microwave exposure facilitates the antagonism of domperidone- to apomorphine-induced stereotypic climbing in mice. Quock et al. (1986) reported that microwave exposure facilitates the antagonism of methylatropine to central cholinomimetic drug effects.

Lai et al. (1984) found that microwave radiation enhances ethanol consumption but attenuates ethanol hypothermia at low, nonthermalizing levels in the rat. Hjeresen et al. (1988, 1989) further characterized the ethanol effect. These investigators found, utilizing low-intensity exposures (0.3 W/kg, 45 min exposures, 2.45 GHz), that attenuation of ethanol-induced hypothermia by microwaves is more effective at lower doses of ethanol than at higher doses. They argued for a nonthermal effect of microwaves, possibly an effect on neurotransmitter systems based on similarities to pentobarbital-induced hypothermia.

Neilly and Lin (1986) studied the possible impact of ethanol on the BBB in rats. Varying amounts of ethanol were administered followed by high intensity (30 kW/m<sup>2</sup> for 15 min) microwaves. Rectal temperature remained constant at 37°C and brain temperatures were monitored. After radiation, Evans blue dye in saline was injected and degree of staining in brain parenchyma was examined. The steady-state brain temperatures were found to be the highest (48°C) in animals receiving saline or the lowest doses of alcohol. The degree of staining decreased with increase in the amount of alcohol administered. The authors suggested that ethanol inhibits microwave-induced permeation of the BBB through reduced heating of the brain.

The direct effect of microwave energy on neurotransmitters has also been studied (Lai 1992). In one investigation, a decrease in brain concentration of acetylcholine was seen in mice exposed to pulsed microwaves, which produced a brain temperature increase of 2 to 4°C. This was believed to be due to an increased release of the transmitter (Modak et al. 1981).

Gandhi and Ross (1987) investigated effects of nonionizing radiation on neurotransmitters. Rats were subjected to 0.700 GHz radiation at 150 W/m<sup>2</sup>, raising core temperature by 2.5°C. Six brain regions were investigated, but only the hypothalamus showed significant changes in receptor states. The study concluded that both norepinephrine and acetylcholine are released in response to heat. A case was made that norepinephrine suppresses efferent impulses for heat production, whereas the cholinergic system initiates the heat loss or heat dissipation mechanism.

Inaba et al. (1992) investigated the effect on biogenic amines in the rat brain exposed to 2.450 MHz microwaves at 50 and 100 W/m<sup>2</sup> for 1 h at ambient temperatures of 21 to 23°C. At the lower intensity, rectal temperatures stabilized after rising 2.3°C and at the upper intensity 3.4°C. Under these conditions, norepinephrine content was reported to be significantly reduced. 5-Hydroxyindoleacetic acid was increased and serotonin was reported to be unchanged. These and other results suggested that microwaves can affect the function of some neurotransmitters. Browning and Haycock investigated the effect of low level MWs on a neuron-specific phosphoprotein, synapsin I, and found no effect on neurotransmitter levels or brain function at the intensity of microwaves studied (Browning and Haycock 1988). Gandhi and Ross (1989) investigated the effect of low level MWs

on rat brain synaptosomes and found that MWs alter the metabolism of inositol phospholipids by enhancing their turnover. It was suggested that this may affect transmembrane signaling in the nerve endings. Further information on combined nonionizing radiation and drugs effects can be found in the review of Lai et al. (1987c).

### 3.6.6 Neurophysiologic Effects *In Vitro*

Although this section is concerned with effects on the CNS, it seems appropriate to summarize work on the direct effect of RF on transmission of the nerve impulse, even though the bulk of this work has been done on peripheral nerves and ganglia *in vitro*.

Chou and Guy (1978) studied the effects of CW and PW 2.45 GHz MW on frog sciatic, cat saphenous, and rabbit superior cervical ganglia and vagus nerves. These investigations revealed that there is no direct production of an action potential by CW or pulsed microwaves (up to pulsed RF at 220 kW/kg peak or CW RF at 1500 W/kg). Compound action potentials were excited electrically in the presence and absence of RF. No changes in conduction velocity or amplitude were detected except with temperature elevations of at least 1°C in the solution. Identical changes could be produced by ambient changes in temperature.

Wachtel et al. (1975) and Seaman and Wachtel (1978), studying the effect of 1.5 or 2.45 GHz CW RF on abdominal ganglia from *Aplysia*, reported that patterns of pacemaker activity could be altered by MW and that comparable temperature elevations by ambient heating did not produce such changes. Studies by McRee and Wachtel (1982) on frog sciatic nerves suggested that MWs could produce diminished vitality of nerve function independent of temperature changes.

Khramov et al. (1991) investigated the effects of 34 to 78 GHz MWs on the spontaneous firing rate of the stretch-receptor neurons of the crayfish under thermally controlled conditions. The firing rates slowed in the presence of RF and temperature increases from 0.1 to 0.2°C, but not in the absence of temperature elevations. An analysis of the data suggested a thermal rather than direct RF effect.

On the other hand, Bolshakov and Alekseev (1992), studying neurons of *Lymnea stagnalis*, compared the effects of CW and PW at 900 MHz in cells exhibiting a burst-like irregularity in firing. MW above 0.5 W/kg PW (0.5 to 110 pps), but not CW, enhanced the probability of the burst-like irregularity. This was identified as possibly a MW pulse-specific effect, which could have arisen from mechanical oscillations in the exposure system.

Pakhomov et al. (1997) studied the effect of CW RF exposure (40 to 52 GHz, 0.24 to 3.0 mW/cm<sup>2</sup>, 10 to 60 min) on isolated frog spinal cord preparations. In the presence of RF-induced heating, effects were identical to conventional heating. Pakhomov et al. (1992) earlier looked at the effects of 915 MHz PWs (peak SAR of 5 to 70 kW/kg) on isolated frog nerves under a variety of conditions. In the exposed groups compound action potential amplitudes decreased significantly over the controls. At the highest SARs microwave heating produced temperature increases of 2.7°C, but comparable conventional heating did not produce the effect. Nerve velocities and refractory characteristics did not change. It was concluded that a particular type of exposure can produce MW-specific changes.

A group has investigated the effects of MW (CW 2.45 GHz, SAR from 12.5 to 125 W/kg on input resistance and action potential in snail neurons (Arber and Lin 1983, 1984, 1985a; Arber et al. 1987; Ginsburg et al. 1992). Although changes occurred, they were not consistent. On the other hand, conventional temperature changes caused consistent changes in input resistance and action potentials. The authors suggested that CW MWs may enhance degenerative effects in neuron metabolism but do not indicate a specific mechanism for MW interaction with neurons (Arber and Lin 1985b).

The hippocampal slice preparation was used by Tattersall et al. (2001) for studying *in vitro* effects of 700 MHz RF exposure. An examination of population spike amplitude showed at low field intensities a 20% potentiation of spike amplitude. At higher intensities, the population spike could change (either increase or decrease) by up to 120 or 80%, respectively. The most significant result was the variability and not the consistency of responses. There were a number of methodological difficulties in this study including use of halothane anesthesia, use of metal-stimulating electrodes, and *post hoc* calculations of dosimetry for the exposures.

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### 3.7 Behavioral Effects

Animal behavioral response to MW exposure has been used by essentially all standard-setting bodies to provide RF-exposure guidelines (NCRP 1986; ICNIRP 1998; D'Andrea 1999; IEEE 1991). Almost all the existing evidence links behavioral responses to MW exposures to the generation of temperature increases in tissues and reinforces the general conclusion that behavioral effects are thermally mediated. These effects have been demonstrated under a variety of conditions and in a range of animals. Parallel studies in humans assessing thermal sensation of MW exposures support the conclusion that changes in behavior of animals exposed to RF are likely to be thermally induced (Vendrik and Vos 1958; Hendler and Hardy 1960; Hendler 1968; Michaelson 1972; Justesen et al. 1982; Justesen 1983, 1988; Brown et al. 1994; Schwan et al. 1996; Blick et al. 1997; Riu et al. 1997; Walters et al. 2000; Adair et al. 2001a; D'Andrea et al. 2003; [see also Chapter 4](#) of this volume). Thermally influenced responses in animals may include perception, aversion, work stoppage or perturbation, reduction in endurance, or even convulsions (Phillips et al. 1975; Justesen 1979; Modak et al. 1981; Guy and Chou 1982; Frei et al. 1995).

Based on thermal responses in animals to MW exposures, behavioral changes show animals to be most sensitive at frequencies close to the resonant frequency (~2500 MHz for mice, ~6 to 700 MHz for rats, and ~70 MHz for humans). Exposures close to the resonant frequency, particularly at levels sufficient to cause thermal responses, can have significant effects on behavior (D'Andrea et al. 1977; de Lorge and Ezell 1980; Gordon 1983, 1987; Gordon and Ferguson 1984; Durney et al. 1986; Mitchell et al. 1988).

#### 3.7.1 Experimental Behavioral Studies

A large body of data exists and has been extensively reviewed for MW behavioral studies (D'Andrea et al. 2003). A portion of that database is briefly reviewed below ([see also Chapter 4](#) of this volume). Justesen and King (1970) utilized a 2.45 GHz (CW) multimodal resonating cavity to investigate conditioned operant behavior in rats. They used a recurrent cycle of exposure, 5 min on and 5 min off, over a 60 min period at average absorbed energy rates of 3.0, 6.2, and 9.2 W/kg. The performance of the animal usually stopped near the end of the 60 min test period during exposure with an energy absorption rate of 6.2 W/kg; at 9.2 W/kg, this effect occurred much earlier in the test period. Hunt et al. (1975) also, using a multimodal resonating cavity to expose rats to 2.45 GHz PW, found effects on exploratory activity, swimming, and discrimination performance of vigilance task, after a 30 min exposure at about 6 W/kg. Lobanova (1960) exposed rats to 3 GHz pulsed MWs, after which the rats were tested for swimming time. A decrease in endurance was noted after exposure to power–time combinations ranging from 1 kW/m<sup>2</sup> for

5 min to 100 W/m<sup>2</sup> for 90 min. Wang and Lai (2000) also used the swimming test for rats exposed to 2.45 GHz pulsed MW fields. It took exposed rats significantly longer times to locate the submerged platform, suggesting that exposure may have caused a deficit in spatial memory. Variations of the swimming test to assess spatial learning are performed in radial arm mazes (dry land spatial navigation). Testing rats with 900 MHz GSM fields showed no differences between exposed and control animals (Dubreuil et al. 2002). A similar study in mice produced the same results, that is, no evidence of effects on radial arm maze performance indicating the lack of an effect on spatial learning and working memory (Sienkiewicz et al. 2000). In earlier studies, however, Lai et al. (1989, 1994) observed deficits in learning in rats tested in the radial arm memory task following exposure to 2.45 GHz pulsed MWs (average whole body SAR of 0.6 W/kg).

The threshold for observed memory disruption in rats from exposure was demonstrated to be about 10 W/kg in animals even when brain hyperthermia occurred at SARs slightly lower (5, 8.5 W/kg) (Mickley et al. 1994; Mickley and Cobb 1998). In a completely opposite result, Luttgies (1980) examined the effects of 3 GHz MW exposure on learning and memory in mice and actually reported an enhancement of performance with exposure at whole body SAR of 13 W/kg. The study, with its unusual result, was repeated by Beel (1983) with the same finding but also with evidence of decreased performance with continued irradiation.

Lin et al. (1977) exposed rats to 0.918 GHz CW at levels of 100, 200, or 400 W/m<sup>2</sup> for 30 min. No effects on response rates were noted at the two lower levels, but at 400 W/m<sup>2</sup> the performance of the animal decreased after 5 min of exposure and ceased after about 15 min of exposure. The average energy absorption rate measured thermographically was 2.1 W/kg incident or 8.4 W/kg absorbed at 400 W/m<sup>2</sup>.

Diachenko and Milroy (1975) studied the effects of pulsed and CW MWs on an operant behavior in rats trained to perform a lever-pressing response on a differential reinforcement of low rate (DRL) schedule and tested immediately after 1 h daily exposure to 10, 50, 100, and 150 W/m<sup>2</sup>, 2.45 GHz MWs. No behavioral effects were found at these levels. The subjects exposed to 100 W/m<sup>2</sup>, while showing no significant decrement in performance, did show signs of heat stress. Thomas et al. (1975, 1982) reported response rate changes in rats exposed between 50 and 200 W/m<sup>2</sup> to 2.86 GHz (CW) and 9.6 GHz PW MWs. Response rates increased in five of ten tests. Decrements in timing of responses were suggested as indicating that low level (estimated at 0.2 to 3.6 W/kg) MWs produce effects when pulsed but not continuous fields were used.

Galloway (1975) studied the performance of four monkeys when using a 2.45 GHz (CW) waveguide applicator (total absorbed power of 10, 15, or 25 W) applied to the head. The duration of the irradiation was 2 min or until convulsions began (20 and 25 W produced convulsions). Because of skin burns, only two subjects completed this series of experiments. There were no performance decrements in a discrimination task that the subjects performed immediately after exposure. Acquisition of a new task during the first ten trials of training was impaired at 25 W, which resulted in convulsions.

Roberti et al. (1975) measured the running time of rats in an electrifiable runway in which each subject was trained to peak performance. Exposure for 185 h at 10.7 GHz (CW), 3 GHz (CW), and 3 GHz pulsed, to 10 W/m<sup>2</sup>, caused no performance decrements. No change in baseline performance was noted when rats were irradiated with 3 GHz pulsed for 17 d at a power density of 250 W/m<sup>2</sup>.

D'Andrea et al. (1986b) observed significant differences in shuttle box performances and schedule-controlled lever pressing for food pellets in rats exposed to 2.450 GHz CW microwaves at an average power density of 0.05 W/m<sup>2</sup>, 7 h/d, 7 d/week, up to 630 h. Similar findings occurred at 2.5 W/m<sup>2</sup> (D'Andrea et al. 1986a). DeWitt et al. (1987) exposed rats 7 h/d for 90 d to CW 2.45 GHz microwaves at an average power density



of  $0.05 \text{ W/m}^2$  followed by operant testing. In general, no behavioral alterations were found except for a variable effect on a time-related operant task. Galvin et al. (1986) studied long-term exposures of rats prenatally and perinatally (4.45 GHz,  $100 \text{ W/m}^2$ , 3 h/d, GDs 5 to 20 and days 2 to 20 postnatally) and found altered endurance and changes in gross motor activity in exposed versus control animals.

Mitchell et al. (1988) exposed rats to 2.45 GHz (CW) microwave radiation at  $100 \text{ W/m}^2$  for 7 h and then performed a number of behavioral tests. Exposed mice exhibited reduced locomotor activity and diminished responsiveness to acoustic stimuli. Average SAR of the freely moving animals was estimated to be  $2.7 \text{ W/kg}$ .

Lebovitz (1983) compared CW and PW radiation at levels known to affect behavior. The pulsed source utilized could produce  $1 \mu\text{s}$  wide pulses at 600 pps with a peak pulse power of 750 kW versus the CW source, which could produce a maximum power of 100 W. Rats, trained in an operant task, were exposed during the behavioral sessions for 3 h/d to CW or PW (1.3 GHz) at 3.7, 5.8, and  $6.7 \text{ W/kg}$ . At the two higher SARs, core temperature was elevated from  $0.5$  to  $1.0^\circ\text{C}$ . The two modes of energy delivery were equally effective in reducing response rates of the operant behavior. From the dosimetry, the auditory effect was probably present.

In some studies, MW exposure was coupled with the administration of various behavior-altering drugs. Thomas and Maitland (1979) investigated the effects of pulsed 2.45 GHz MWs,  $1 \text{ W/m}^2$  in combination with dextroamphetamine on behavior in rats. Both acute and repeated exposures modified the normal dose–effect function. The maximum drug effect was obtained at lower MW exposures as the drug dose was increased. Galloway and Waxler (1977) employed a serotonin-depleting drug (fenfluramine) to investigate the effect of 2.45 GHz CW RF applied to the head of 7 to 9 kg rhesus monkeys at dose rates of 2 to 15 W. Combinations of the drug and RF at a dose rate of 15 W resulted in behavioral deficits, whereas the drug or RF alone up to 15 W failed to produce this effect. In respect to drugs such as dextroamphetamine and fenfluramine, their influence on thermal regulation may be significant in these results.

In contrast to the previous studies in which postexposure behavior was measured, de Lorge (1983, 1984) exposed rats and monkeys to 2.45 GHz MWs under far field conditions, while the animals were performing on operant schedules for food reinforcement. Exposure sessions lasted 60 min and were repeated on a daily basis. Stable performance on the operant schedules was disrupted in all three species at power densities positively correlated with the body mass of the animals. The behavior of the squirrel monkeys was disrupted at the middle level of power densities. When the averages of these power densities (280, 450, and  $670 \text{ W/m}^2$ ) are plotted as a function of body mass (0.3, 0.7, and 5 kg), a semilog relationship becomes evident. Extrapolation along the resulting curve could permit prediction of the power densities needed to disrupt ongoing operant behavior in larger animals. The power densities associated with behavioral disruption approximated those power densities that produced an increase in colonic temperature of at least  $1^\circ\text{C}$  above control levels in the corresponding animals. These data support the need for scaling factors to extrapolate from small animals to larger animals and it becomes clear that different power densities are necessary to produce equal SARs in different size animals.

A number of studies evaluating the cognitive performance of humans exposed to RF, primarily using mobile phone signals and frequencies, have been recently conducted. Most of the investigations are for short time period exposures of 1 h or less. Several of these acute studies found changes in cognitive performance, in learning and memory tasks, in volunteers exposed to mobile telephone RF (Preece et al. 1999; Koivisto et al. 2000a,b; Lee et al. 2001; Edelstyn and Oldershaw 2002). However, there was a marked lack of consistency across studies, even within the same laboratory, and a number of

the studies had methodological problems that precluded drawing conclusions with confidence. A subsequent replication study by Haarala et al. (2003) failed to replicate the positive results of earlier studies in that laboratory (Koivisto et al. 2000a,b), so the determination of cognition effects in humans has not yet been validated for MW exposures.

With new industrial and military applications the issue of high peak, low repetition rate (and low average power) exposure has been studied. The safety standards of the 1980s (ANSI/IEEE 1982; NCRP 1986) did not limit peak power, setting standards only for temporal average powers. Thomas et al. (1982) reported disruption of operant behavior in rats at whole body average SARs of 0.2 to 3.6 W/kg and peak SARs of 0.2 to 3.6 kW/kg, 500 pps, 2  $\mu$ s pulse widths, 30 min/week exposure just before operant testing. However, Lebovitz (1983) found no effect on operant behaviors of pulsed exposures with whole body average SAR of 6.7 W/kg and peak SAR 11.0 kW/kg, 600 pps, 1  $\mu$ s pulse width, 3 h exposure daily during behavior. D'Andrea et al. (1989) also found no effects on monkeys, utilizing operant behavioral studies. In these studies in which tested behaviors were concurrent with exposures, whole body average SARs ranged from 0.05 to 0.80 W/kg and peak whole body average SAR was 8.3 W/kg.

In a later study, D'Andrea et al. (1994) exposed rhesus monkeys to high peak power 5.62 GHz microwave pulses during the performance of an operant task. Two different peak powers at each of three different SARs were evaluated. At a whole body average SAR of 2 W/kg and peak powers of 560 W/m<sup>2</sup> or 5.18 kW/m<sup>2</sup>, no alterations in behavior were found. At 4 W/kg with 1.28 and 12.7 kW/m<sup>2</sup> peaks, and also at 6 W/kg with 2.77 and 25.2 kW/m<sup>2</sup> peaks, decrements in performance were found. The differences in peak power at the two higher SARs did not produce any differences in behavior. Performance returned to baseline levels following exposure and the animals exhibited no discomfort during the exposure.

Hjeresen et al. (1990), utilizing a source capable of producing peak power densities up to 1.8 kW/cm<sup>2</sup>, 10  $\mu$ s pulse widths, 10 pps, at 1.3 GHz with resultant whole body average SARs from 1.8 to 26.2 W/kg, studied a number of behaviors in rats and concluded that disrupted behaviors correlated only with increased colonic temperature and therefore average SAR. Hjeresen and Umbarger (1989), utilizing a high-power microwave source with frequency range of 2.01 to 2.57 GHz producing peak power densities up to 241 MW/m<sup>2</sup> in pulses 85 ns wide, with 50 such pulses delivered over a 5 min period, produced no interference in behaviors in rats. No aversive properties were found and the authors suggested that peak power is apparently not as important as average power in causing deleterious biological effects.

Akyel et al. (1991), utilizing a high peak power 1.25 GHz source, which could produce a peak SAR of 0.21 MW/kg and SA of 2.1 J/kg per pulse, exposed rats for 10 min and then subjected them to an operant schedule. By varying the pulse repetition rate, a range of whole body average SARs from 0.84 to 213 W/kg could be produced. The highest SAR produced a colonic temperature elevation of 2.5°C. No interference with operant behavior was found at the lower dose levels. At the highest dose, animals failed to respond to the operant schedule. No specific effect of pulse power could be found and the interference with behavior appeared to be of thermal origin.

In further extension of this research, Raslear Akyel et al. (1993) studied high peak power pulsed exposures with short pulse widths and slow repetition rates (irregularly shaped pulse with a 2 MW/m<sup>2</sup> peak and an average of 500 MW/m<sup>2</sup> over the pulse period, 2 W/m<sup>2</sup> average, 80 ns pulse width, repetition rate of 0.125 Hz (200 pulses over 26 min), 3 GHz, average SAR of 0.07 W/kg, peak SAR of 7 MW/kg. These were the highest peak powers and peak SARs found in the literature, and degraded performance of rats was found in a number of test protocols, including Y-maze negotiation and treadmill running.

The authors concluded that at such high peak powers impairments can be found despite the low average SAR. In all of the pulse power studies described, the dosimetry suggests production of the auditory effect. What role it might have played in studies revealing effects is not clear. These studies generally show that pulsed MW are generally, but not always more effective in producing behavioral responses than CW at comparable time averaged, whole body exposure levels. The effects of high peak power pulses have been reviewed by Pakhomov and Murphy (2000) and Pakhomov et al. (2003).

Most of the research on the nervous system and behavior have been carried out in rodents and other lower animals. Behavior among animal species reflects adaptive brain–behavior patterns. Behavioral thermal regulation is seen as an attempt to maintain a nearly constant internal thermal environment. Changes in body temperature bring about not only autonomic drives, but also behavioral drives (Stolwijk and Hardy 1977). The MWs can influence behavioral thermoregulation and has been shown by Stern et al. (1979) and Adair and Adams (1982). This subject is treated in greater detail in [Chapter 5](#) of this volume.

Behavioral responses are not necessarily the manifestations of specific changes in the CNS and may be a function of direct or indirect action of MWs on other body systems. Extrapolation of brain–behavior functions from lower animals to man is thus subjected to many difficulties.

In assessing the significance of the reported behavioral changes, it is important to recognize certain fundamental factors. The resting metabolic rate for rats is approximately 7 W/kg. When this level is exceeded, disruption of behavior could be elicited. In most of the reports, alterations in the behavior of rats were observed with exposures at average energy absorption rates of 5 to 8 W/kg or greater, i.e., at exposure levels similar to those that produce increases in circulating corticosterone (CS) concentrations in rats (Lotz and Michaelson 1978). Behavioral changes may be related to more subtle heat alterations within the body. Heat may produce a general debilitation effect or a decreased motivation for food, since it has been shown that rats maintained in hot environments eat less food (Hamilton 1963) and rats show decreased response and food reinforcement frequency on an operant schedule when environmental temperature is 35°C, but not 25°C. Behavioral responses certainly may be influenced by the interaction of the organism with the environment. Nevertheless, it appears that changes in behavior definitely result from exposure to MWs of varying frequencies and intensities. Yet to be demonstrated with certainty are that observed behavioral responses can occur without the thermal increases associated with the MW exposure.

### **3.7.2 Behavioral Thermoregulation**

The regulation of body temperature can be accomplished by complex thermoregulatory patterns of responses of the skeletal musculature to heat and cold, which modify the rates of heat production and heat loss, for e.g., by exercise, change in body conformation, the thermal insulation of bedding, and by the selection of an environment which reduces thermal stress (Bligh 1975). In humans, optimal temperature in the body is also regulated by thermophysiological responses. Human body temperatures range from 35.5 to 40°C and include exercise, food intake, environmental conditions, and circadian variations. Age can also play a role in thermoregulation (Makrides et al. 1990).

Thermoregulation is part of a complex control system involving circulation, metabolism, and respiration, as well as neural structures. Temperature signals from cutaneous thermoreceptors reach the somatosensory region of the cerebral cortex. The main processing of thermal signals and generation of a controlling signal for the effector part of thermoregulation takes place in the hypothalamus. Adair et al. (1984) found minimal

changes in hypothalamic temperature in conjunction with alteration of thermoregulatory behavior in squirrel monkeys exposed to 2.45 GHz, 40 to 200 W/m<sup>2</sup> (SAR 0.2 to 1 W/kg). The whole subject of thermoregulatory behavior in response to energy absorption during MW exposure has been nicely reviewed and summarized by Adair and Black (2003; see also Chapter 5 of this volume).

Successful strategies or mechanisms to maintain body temperatures in a narrow and desirable range in a complex and varying thermal environment are termed thermoregulatory. Although such strategies or mechanisms are found in great variety, they fall into two main categories: voluntary and behavioral adjustments, and involuntary physiological adjustments. The limits of effectiveness of involuntary physiological thermoregulation are rather narrow and we must rely on behavioral methods of thermoregulation over most of the range of environmental temperatures to which we are often exposed. Changes in body temperatures bring about not only autonomic drives, but also behavioral drives (Stolwijk and Hardy 1977).

Thermal motivation arises in situations of thermal stress. On the warm side, it is the uncomfortable feeling of excessive warmth and the desire for temperature reduction; on the cold side, it involves the unpleasant feeling of remaining too cold and the desire for temperature increase. The biological significance of thermal motivation is that, by acting in such a way as to minimize thermal discomfort and maximize thermal comfort, the organism tends to escape from situations of thermal stress and to locate itself in a physiologically neutral thermal environment, thereby solving the problem of physiological temperature regulation (Corbit 1973).

Smialowicz et al. (1981a) made unrestrained, unanesthetized mice hypothermic by injecting them with 5-hydroxytryptamine (5-HT) in a controlled environment of 22°C and 50% relative humidity. Mice injected with saline were used as controls. Colonic temperatures were measured prior to injection. Following injection, groups of mice were exposed to 2.45 GHz CW RF for 15 min at 100, 50, or 10 W/m<sup>2</sup> (equivalent SARs 7.2, 3.6, and 0.7 W/kg) or were sham-exposed, after which their colonic temperatures were measured again. The experiments were performed with BALB/c and CBA/J mice. For saline-injected mice of either type, there were no significant colonic temperature differences between mice exposed at 100 W/m<sup>2</sup> and sham-exposed mice. For BALB/c mice made hypothermic with 5-HT, colonic temperatures were significantly higher for those exposed at all three power densities than those sham-exposed, and the differences increased monotonically with power density. The results for the CBA/J mice were similar, but the increases were statistically significant only at 50 and 100 W/m<sup>2</sup>. The investigators conclude that subtle heating by RF can alter the thermoregulatory capacity of hypothermic mice, whereas the colonic temperature of normal (saline-injected) mice was not significantly altered by exposure at 100 W/m<sup>2</sup>.

Adair and Adams (1980a, 1982) exposed squirrel monkeys to 2.45 GHz CW RF for 10 or 90 min in ambient temperatures of 15, 20, or 25°C. The power densities ranged from 25 to 100 W/m<sup>2</sup> (SARs 0.4 to 1.5 W/kg). Skin and colonic temperatures were monitored continuously during exposure. The metabolic heat production was calculated from the oxygen deficit in the expired air of each monkey. At all three ambient temperatures, 10 min exposure of two monkeys to a threshold power density of 40 W/m<sup>2</sup> and one monkey to 20 W/m<sup>2</sup> reliably initiated a reduction of their metabolic heat production, and the magnitudes of the reduction were a linear function of the power density above the threshold values. At exposure termination, the metabolic heat production often rebounded sharply and overshot normal levels. For the 90 min exposures at 20°C, the initially large reduction of metabolic heat production gradually diminished towards normal levels, apparently to ensure precise regulation of internal body temperature at the normal value.

Adair (1981) also exposed four squirrel monkeys to 2.45 GHz CW RF in warm ambient temperatures ranging from 32 to 35°C. After an initial equilibration period of 90 min or longer, each monkey was exposed for ten periods to power densities in an increasing sequence from 25 to 200 W/m<sup>2</sup>, with sufficient time between exposures for reestablishment of equilibrium. The colonic temperature and the skin temperature at the abdomen, tail, leg, and foot were monitored continuously. As in the previous investigation, the metabolic heat production was determined from the oxygen deficit in the expired air. In addition, thermoregulatory sweating from the foot was determined by sensing the dew point of the air in a special boot over the foot. The results indicate that at ambient temperatures below about 36°C, at which sweating in a sedentary monkey may occur spontaneously, the threshold power density (or SAR) for initiating thermoregulatory sweating from the foot decreased with decreasing ambient temperature.

Adair and Adams (1980b) equilibrated squirrel monkeys for a minimum of 2 h to constant environmental temperatures (22 to 26.5°C), which was cool enough to ensure that the cutaneous blood vessels in the tail and extremities were fully vasoconstricted, an effect produced by the thermoregulatory system to minimize heat loss. The monkeys then underwent 5 min exposures to 2.45 GHz RF at successively higher power densities, starting at 25 to 40 W/m<sup>2</sup>, until vasodilation in the tail occurred, as evidenced by an abrupt and rapid temperature increase in the tail skin. For example, a monkey equilibrated to 25°C, exhibited tail vasodilation when exposed to RF at 100 W/m<sup>2</sup> (whole body SAR 1.5 W/kg), whereas it did not when exposed to IR radiation at the equivalent power density, an indication that the effect resulted from stimulation of thermosensitive elements of the thermoregulatory system by the RF rather than from heating of the tail skin. RF exposure at higher power densities was required to cause tail vasodilation in monkeys equilibrated to lower environmental temperatures. Specifically, an increase of 30 to 40 W/m<sup>2</sup> was found necessary for every 1°C reduction in environmental temperature.

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## 3.8 Neuroendocrine Effects

### 3.8.1 Mechanisms of Interaction

Many of the studies of the endocrine system have involved whole body RF exposures of the rat. The issue has been the level of organization of the system at which thermal interactions exert an effect and the nature of the effect. Some investigators believe that endocrine changes result from stimulation of the hypothalamic–hypophysial system at the hypothalamic or adjacent level of organization; others have advanced evidence that effects are mediated at the hypophysis or the particular endocrine gland or end organ under study. The effects detected are not necessarily pathologic, because the function of the neuroendocrine system is to maintain homeostasis and hormone levels will fluctuate to maintain such organismic stability.

Effects of RF exposure on endocrine function are generally consistent with both immediate and long-term responses to thermal input and nonspecific stress, which can also arise from thermal loading. Changes found in plasma levels of corticosterone (CS) and growth hormone are typical reactions of animals to nonspecific stress; indeed, great care is required in performing experiments to ensure that the changes in hormone level do not result from stress caused by handling of the animals or the novelty of the experimental situation. The topic has been reviewed by Lu et al. (1980b) and also by Black and Heynick (2003).

### 3.8.2 Hypothalamic–Hypophysial–Adrenal Response

Several investigators have reported biochemical and physiological changes as a result of MW exposure, which suggest an adrenal effect. Indeed, the evidence of increases in plasma steroid levels in rats exposed to MWs is fairly reliable. Furthermore, sufficient research has been performed to determine exposure thresholds for such responses in rats. Demokidova (1974) reported increased adrenal and pituitary gland weight in rats exposed 1.5 months to 69.7 MHz, 12 V/m, 1 h/d and increased adrenal weight in infant rats exposed to 48 V/m, 4 h/d. The same author, however, reported decreased weight of the adrenal glands in infant rats exposed to 0.01488 GHz at 70 V/m. The pituitary gland in female mice retained its gonadotropic function when exposed to 3 GHz (100 W/m<sup>2</sup>) twice daily for 5 months, although its activity was reduced in comparison with that of non-exposed animals (Bereznitskaya and Kazbekov 1974). Tolgskaya and Gordon (1960) noted the reversibility of changes in the neurosecretory function of the hypothalamus when exposure was terminated. In another study, however, rats exposed to MWs of varying intensity showed no quantitative changes in CS were found in the adrenals and blood plasma (Mikolajczyk 1972). Prepubescent hypophysectomized rats displayed no differences in adrenal growth rate when treated with pituitary homogenates collected either from rats exposed to MWs or from control rats.

Rats exposed to 2.45 GHz (CW), 100 W/m<sup>2</sup> for 4 h showed no change in adrenal weight, phenylethanolamine-*N*-methyl transferase (PNMT) activity, or epinephrine levels (Parker 1973). After 16 h of exposure, however, decrease in adrenal epinephrine (32%) was significant and PNMT activity was elevated (25%). There were no statistically significant differences in adrenal or plasma CS levels between exposed and sham-exposed animals. It should be noted, however, that similar alterations in epinephrine levels can occur in rats subjected to a stressor such as immobilization or acute exposure to cold.

Rabbits exposed to 3 GHz (500 to 600 W/m<sup>2</sup>) 4 h daily for 20 d tended to show a decline in the amount of urinary 17-hydroxycorticosteroids (17-OHCS) at the beginning of exposure, followed by a gradual return to normal (Lenko et al. 1966). No change was evident in the excretion of 17-ketosteroids in the urine. According to Petrov and Syngayevskaya (1970), 3 and 24 h after dogs were exposed to 3 GHz, 100 W/m<sup>2</sup>, the serum corticosteroid content had increased by 100 to 150% above the original level. Serum potassium was decreased by 5 to 10% and sodium was increased by the same amount. They also noted that the susceptibility of rats to MW exposure was sharply increased 1 week after bilateral adrenalectomy. Chronic exposure of animals to MWs (CW or PW) was accompanied by reduced cholinesterase activity and an increased amount of 17-ketosteroids in the urine, reduced ascorbic acid, and reduced weight of the adrenal glands.

Increased adrenal function due to MW exposure has been correlated with colonic temperature increase in rats (Lotz and Michaelson 1979). Plasma CS levels in hypophysectomized rats exposed at 600 W/m<sup>2</sup> for 60 min were below control levels. When rats were pretreated with dexamethasone before exposed at 500 W/m<sup>2</sup> for 60 min, the CS response was suppressed. These results suggest that the MW-induced CS response observed in intact rats is dependent on adrenocorticotrophic hormone secretion by the pituitary, i.e., the adrenal gland is not the primary endocrine gland stimulated by MW. The evidence obtained in these experiments is consistent with the hypothesis that the stimulation of the adrenal axis in MW-exposed rats is a systemic, integrative process due to a general hyperthermia (Lotz and Michaelson 1978). Consistent with these results were data obtained by Lotz (1985) in which rats exposed to 255 MHz CW for 4 h (SAR 3 to 4 W/kg) showed no change in serum cortisol levels. The boundary between effects and no effects for corticosteroid levels appears to be approximately 4 W/kg in monkeys as

well as rats (Lotz 1983). The subject of effects of MW on the adrenal cortex has been reviewed by Lu et al. (1986).

### 3.8.3 Hypothalamic–Hypophysial–Thyroid Response

The literature offers comparatively few experimental studies of the effect of RF or MWs on the thyroid. No alterations in thyroid structure or function attributable to MW exposure were noted in rats subjected to 2.45 GHz, CW, 10 W/m<sup>2</sup> continuously for 8 weeks or 100 W/m<sup>2</sup>, 8 h/d for 8 weeks (Milroy and Michaelson 1972). On the other hand, a stimulatory influence of 50 W/m<sup>2</sup> on the trapping and secretory function of the thyroid gland of rabbits has been reported (Baranski et al. 1972). These functional changes were in agreement with altered histology of the thyroid.

In rats exposed for 16 h to 2.45 GHz (CW) at 100 to 250 W/m<sup>2</sup>, tests of thyroid function in general showed no statistically significant deviations from the norm, except that in animals with a 1.0 to 1.7°C increase in colonic temperature there was a reduction in the ability of the thyroid to concentrate iodide (Parker 1973). Decreased thyroid gland weight was noted in infant rats exposed to 0.0697 GHz, 48 V/m, 4 h/d, 1.5 months.

Increased thyroid hormone secretion has been correlated with MW-induced thyroid temperature increase in dogs (Magin et al. 1977). Vetter (1975) found that serum protein levels increased as a function of power density, indicating an alteration of protein synthesis or catabolism; levels of thyroid hormone decreased as power density of 1.45 GHz CW was increased from 50 to 250 W/m<sup>2</sup>. In agreement is the finding of Lu et al. (1977) who reported that serum thyroxine levels were transiently elevated after exposure of rats at 10 W/m<sup>2</sup> (2.45 GHz) and were depressed after exposure at 200 W/m<sup>2</sup>. None of the reported alterations was irreversible or resulted in morbidity.

Perturbation of the thyroid gland may be the result of an indirect effect, the thermal stress on the body producing a hypothalamic–hypophysial response. This is consistent with MW-induced thermal stimulation of hypothalamic–hypophysial–thyroid (HHT) activity (Michaelson et al. 1961). McLees and Finch (1971) pointed out that temperature elevation and heat stress have been associated with alterations in radioactive iodine (RAI) turnover rate. The HHT axis has been shown to be sensitive to environmental temperature (Michaelson 1961). Differences in rate of temperature change or alteration in thermal gradients could also result in qualitative differences in endocrine response.

In a study described as a delineation of acute neuroendocrine responses to MW exposure, Lu et al. (1980a) exposed gentled rats to the same frequency at power densities ranging from 10 to 700 W/m<sup>2</sup>, up to 8 h, at an environmental temperature maintained at 24°C. Sham-exposed rats were used as controls. After treatment, the rats were decapitated, colonic temperatures were taken, and blood was collected for assays of thyroxine (T<sub>4</sub>), thyrotropin (TSH), growth hormone (GH), and CS. For exposure of 1 h, colonic temperature increased with power density at 200 W/m<sup>2</sup> and higher, but consistent elevation of serum CS did not occur below 500 W/m<sup>2</sup>. Lower serum TSH and GH levels also occurred at this and higher power densities. Significant serum T<sub>4</sub> elevations were noted at 400 and 700 W/m<sup>2</sup>, but they were not consistently related to power density. For sham exposures and exposures at 10 to 200 W/m<sup>2</sup> for longer durations (2 to 8 h), the results were equivocal, presumably, because such exposures encompassed significant portions of the circadian cycle. Specifically, in the sham-exposed rats, the level of T<sub>4</sub> did not change significantly under sham conditions with exposure duration; and significant increases of CS and decreases of TSH and GH were seen, so it was difficult to discern consistent differences in these hormones ascribable to RF exposure. The investigators suggest that the divergent responses may be due to two different mechanisms that are dependent on RF intensity and the time of the exposures relative to circadian rhythms.

Abhold et al. (1981) exposed rats to 2.45 GHz CW RF for 8 h (continuously) at 20 or 100 W/m<sup>2</sup> (SAR 0.44 or 2.2 W/kg with the long body axis of the rat parallel to the electric component of the RF). Other rats were sham-exposed for the same period, and still others served as untreated controls. Within 15 min after treatment, the rats were euthanized and their blood was assayed for serum T4, triiodothyronine (T3), and T3 uptake. In addition, serum CS concentrations were measured. The results indicated that concentrations of T4, T3, and T3 uptake were not altered by the treatments. However, the rats that were sham-exposed or exposed to RF at 20 W/m<sup>2</sup> had higher levels of CW than the cage control rats, whereas the rats exposed to 100 W/m<sup>2</sup> had values similar to those of the untreated rats. The changes observed were more likely ascribable to stress and other factors in the experimental protocols than the RF exposure.

In a review article, Lu et al. (1980b) discussed evidence for the existence of threshold intensities for various RF-induced neuroendocrine effects. They indicate that such thresholds are dependent on the intensity and duration of exposure and can be different for each endocrine parameter.

### 3.8.4 Melatonin Response

Interest has grown in possible effects of MW radiation on circadian function, mediated through the pineal hormone, melatonin. In a number of recent reports, nighttime levels of melatonin have been measured in response to RF exposure in both animals and humans. Burch et al. (2002) reported a marginal increase in melatonin in humans associated with mobile phone use. This report however, is countered by a number of human provocation studies in which no effects in melatonin levels or secretion was observed following RF exposures (de Seze et al. 1998, 1999; Mann et al. 1998b; Radon et al. 2001). Indeed, no changes were observed in exposed humans for a range of hormones as well, including growth hormone, lutinizing hormone, cortisol, melatonin, and others. Research in rats has shown results consistent with these general findings in that no demonstrable effects on hormones, including melatonin, have been reported with low-level RF exposure at 435 MHz (Bonasera et al. 1988; Toler et al. 1988) or at 900 MHz (Vollrath et al. 1997; Heikkanen and Juutilianen 1999).

In summary, although some effects of RF exposure on the endocrine system appear to be relatively straightforward and predictable from physiological considerations, other more subtle effects require further study, notably those related to the interactions among the pituitary, adrenal, thyroid, and hypothalamus and their secretions. Part of the problem in interpreting results appears to arise from uncertainties regarding stressors inadvertently introduced into the experimental design and the response of the animal to such stressors. Animals placed in novel situations are much more prone to exhibit stress responses than animals that have been adapted to the situation. However, there may be large variations in adaptation among animals in a given situation or among experimental situations in different laboratories. Moreover, the use of sham-treated controls may not always reduce the problem. A review of the effects of RF on physiologic regulation has been published by Michaelson (1982).

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## 3.9 Cardiovascular Effects

Low-intensity MW exposures at nonthermalizing levels and clearly thermal levels have been studied. The role of heating and the possibility of unique MW-induced effects even



at thermalizing levels of exposure have been addressed. The database is anything but straightforward and not easily distilled into a brief summary. Some aspects of cardiovascular function during exposure to MW are reviewed by Black and Heynick (2003).

Clinicians in the former Soviet Union identified a so-called “microwave neurasthenia,” or “microwave radiation sickness,” the consequence of chronic occupational exposure to low levels of MW (Sacchikova 1974). This included bradycardia, tachycardia, hypertension, hypotension, T-wave changes, and other neurologic and behavioral changes. Such a syndrome is not considered adequately documented or well defined in Western medicine. The possible hemodynamic changes in the cardiovascular system have been studied in animals ever since. The chronotropic effects (bradycardia or tachycardia) have been of special interest. At nonthermal levels, both bradycardia and tachycardia have been found in various studies of animals but the results have been inconsistent overall. Some investigators have proposed that the heart rate response of rabbits depended on exposure technique, distribution of energy as a function of location, and the possibility of differing regional reflexes producing differing outcomes (Pressman and Levitina 1962). This has not been verified and it is just as likely that biologic variability among animals or the numerous sources of error could account for the findings. Even the distinction between thermal and nonthermal effects is not clear cut; and minor changes in temperature, perhaps less than 1°C, at the right anatomic location could affect hemodynamics by a neural mechanism even though the exposure is reported as nonthermal.

Hyperthermia of non-RF origin produces tachycardia and a decrease in total peripheral resistance caused by vasodilation, a heat-dissipating response to the thermal burden. This is true whether heat is imparted by environmental heating, water bath, or even a general whole body hyperthermia of RF origin (Basset and Kenny 1979).

Evidence that hemodynamic changes produced by MWs might be very sensitive to a precise dosimetric configuration was presented by Lu et al. (1992). Rats were subjected to ventral head–neck exposures in a waveguide at 1.25 GHz, PW and CW at 2.0 and 6.4 W average powers, 10  $\mu$ s pulses, 400 kW peak power, brain average SAR 9.5 and 30.4 W/kg, neck SAR 34.3 and 110.0 W/kg, for 5 min. Both tachycardia and bradycardia were observed but a decreased stroke volume was also found with consistency. This produced a consistent subnormal cardiac output. Mean arterial pressure was unaltered. From the data, it was possible to conclude that total peripheral resistance had increased. This would be an anomalous or unphysiologic response to the insertion of thermal energy into the body. This kind of a response is not observed in experimental animals subjected to whole body microwave exposure (Jauchem et al. 1984). The investigators concluded that the targeting of the head and neck, even in the presence of generalized hyperthermia, may have elicited specific signals at receptor or effector sites in that area to contravene the normal physiologic response. The baroreceptors were identified as a possible locus of stimulation of efferents, which raised blood pressure by increasing peripheral resistance. A hypothesis that the baroreceptors might respond to pressure waves induced by pulsed MW was not supported, in that both pulse and CW produced the anomalous effect at thermalizing levels.

Rasbury and Seaman (1992) also studied exposures of the head and neck in the rat under different conditions from those of Lu et al. The carotid sinus was surgically exposed and rats exposed to the radiation of a coaxial probe under anesthesia for brief periods, 10 s, and local SARs up to 70 kW/kg. At the highest SARs, where effects were found, approximately half of the animals exhibited decreased blood pressure and heart rate and half the animals exhibited increased blood pressure and heart rate. No changes in colonic or tail temperature occurred for any exposures. The authors could not account for the divergent results, but noted that the inconsistent findings were consistent with other studies on hemodynamic changes associated with MW exposure.

Additional studies in animals at a wide range of MW frequencies have reported definite effects on blood pressure and heart rate, but all at power levels clearly associated with increases in temperature within the body (Phillips et al. 1975; Jauchem et al. 1984, 1985b, 1988; Frei et al. 1988, 1989a,b; Frei and Jauchem 1989). Investigations of ultra-wideband and 94 GHz MW exposures showed no significant changes in the heart rate or mean arterial blood pressure in rats (Jauchem et al. 1999a,b), respectively. However, in another study of rats exposed to ultra-wideband fields, Lu et al. (1999) found a consistent hypotension, which persisted following exposure. This occurred with no changes observed in heart rate.

Cooper et al. (1961, 1962) and Pinakatt et al. (1963, 1965) studied the influence of various drugs in pentobarbital-anesthetized rats exposed to 2.45 GHz, 800 W/m<sup>2</sup> for 10 min, which resulted in a 40.5°C colonic temperature. Pyridoxine and digitoxin did not alter blood pressure or heart rate. Reserpine, vagotomy, and pharmacological ganglioplegia diminished the reaction to MW-induced hyperthermia. Jauchem (1985) also examined the influence of drugs on thermal responses in animals exposed to high-level MW.

A human study reported increases in blood pressure and heart rate in humans exposed to 900 MHz RF (Braune et al. 1998). The same group, however, could not replicate the positive findings in a subsequent study (Braune et al. 2002). Other human studies at much higher intensity RF found no effects of exposure on heart rate (Lu et al. 1992; Adair et al. 2001b). With regard to human exposures, a review by Jauchem (1997) stated that if a tissue is not heated during exposure, current flow appears to be required for significant cardiovascular effects to occur.

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### 3.10 Effects on Hematopoiesis and Hematology

Extensive investigations have been carried out to examine MW exposure effects on the hematopoietic system in animals. The preponderance of this work was performed in the two decades from 1960 to 1980 with the overwhelming evidence that exposed blood cells and blood-forming tissues are not affected by MW exposure except when the target is heated. White cells (leukocytes) are more sensitive than red cells (erythrocytes); however, even the white cell effects are consistent with thermally induced changes. In the absence of significant MW heating or specific electric currents, cardiovascular tissue also does not provide evidence of RF-induced changes, nor is blood pressure changed by MW exposure. Much of this work has been recently reviewed by Black and Heynick (2003).

Several papers from the early literature of Eastern European research, as indicated below, reported effects on blood cells, even at low MW exposures. Kitsovskaya (1964) subjected rats to 3 GHz, 100 W/m<sup>2</sup>, 60 min/d for 216 d; 400 W/m<sup>2</sup>, 15 min/d for 20 d; 1 kW/m<sup>2</sup>, 5 min/d for 6 d. At 400 W/m<sup>2</sup> and 1 kW/m<sup>2</sup>, total RBC, WBC, and absolute lymphocytes were decreased; granulocytes and reticulocytes were elevated. At 100 W/m<sup>2</sup>, total WBC and absolute lymphocytes decreased, and granulocytes increased. Bone marrow examination revealed erythroid hyperplasia at the higher power densities. Decreased leukocyte count and phagocytic activity had been reported in rats and rabbits exposed to 50 MHz, 0.5 to 6 V/m, 10 to 12 h/d, 180 d (Serdiuk 1969).

Baranski (1971a,b) exposed guinea pigs and rabbits to 3 GHz (PW or CW), 35 W/m<sup>2</sup> for 3 months, 3 h daily. Increases in absolute lymphocyte counts, abnormalities in nuclear structure, and mitosis in the erythroblastic cell series in the bone marrow and in lymphoid cells in lymph nodes and spleen were observed. No alteration in the granulocyte series was noted. Baranski suggests that extrathermal complex interactions seem to be the

underlying mechanism for the changes. Unfortunately, minimal information on exposure conditions was provided making determinations on thermal and nonthermal parameters very difficult.

Exposure of mice to 2.45 GHz, 1 kW/m<sup>2</sup> for 5 min resulted in a decrease followed by an increase in <sup>59</sup>Fe uptake in the spleen and bone marrow (Vacek 1978). Alteration in ferrokinetics was also found in rabbits and guinea pigs exposed to 3 GHz at 10 or 30 W/m<sup>2</sup>, 2 to 4 h daily for 14 to 79 d (Czerski et al. 1974a,b).

Miro et al. (1974) exposed Swiss albino mice to pulsed 3.105 GHz at 200 W/m<sup>2</sup> average power density (4 kW/m<sup>2</sup> peak) for 145 h. Stimulation of splenic lymphopoiesis and increased <sup>35</sup>S methionine incorporation in spleen, thymus, and liver were found. The authors interpret these results as a sign of stimulation of cells belonging to the reticuloendothelial system. Possible alteration in the circadian rhythm of bone marrow mitoses in guinea pigs and mice was noted. No effects were seen on precursors of granulocytes and only minimal effects were found on the erythroid series, but pronounced phase shifts were noted in the pool of stem cells. In inbred Swiss albino mice exposed once for 4 h at 5 W/m<sup>2</sup> to pulsed 3 GHz MWs, the diurnal rate of proliferation of the stem cell population was amplified and the phase shifted from that of controls (Czerski et al. 1974b). A comparable phase shift in the circadian rhythm of body temperature was observed in rats exposed to 2.45 GHz, CW, 10 W/m<sup>2</sup>, 1 to 8 h, suggesting an interrelated example of physiologic regulation (Lu et al. 1980a).

Subsequent studies, however, have not confirmed such effects. Peterson (1979) investigated both rabbit and human RBCs to 2.45 GHz and demonstrated no hemolysis or changes in exposed samples. Studies of mouse erythroleukemic cells exposed to 1.18 GHz for 48 h at 18.5, 36.3, or 69.2 W/kg also showed no significant differences between exposed and sham control cultures (Marshall 1984). Hyde and Friedman (1968) exposed anesthetized female CF-1 mice to 3 GHz, 200 W/m<sup>2</sup> and 10 GHz, 170, 400, or 600 W/m<sup>2</sup> up to 15 min. No significant effect on total or differential leukocyte count or hemoglobin concentration was noted immediately, 3, 7, or 20 d after exposure. There were no changes in femoral bone marrow other than a variable, but slight, increase in the eosinophil series of the exposed animals, which was not reflected in peripheral blood counts.

Spalding et al. (1971) exposed mice to 800 MHz, 2 h daily for 120 d in a waveguide at an incident power density of 430 W/m<sup>2</sup>. RBC and WBC count, hematocrit, hemoglobin, growth, voluntary activity, and life span remained normal. Smialowicz et al. (1981d) conducted long-term continuous exposures of rats for 69 to 70 d to 970 MHz at 2.5 W/kg. No differences were found between exposed and control animals in hemoglobin concentration or hematocrit, mean cell volume of erythrocytes, erythrocyte count, or total and differential leukocyte counts.

In a controlled temperature experiment at 100 MHz, Cleary et al. (1985) exposed rabbit neutrophils for 30 or 60 min and observed no changes due to RF exposure in either viability or phagocytotic ability. Hamrick and Fox (1977) exposed rat lymphocytes to 2.45 GHz MWs and did not demonstrate any significant differences between control and exposed cells.

In dogs exposed whole body to 2.8 GHz pulsed, 1 kW/m<sup>2</sup> for 6 h, there was a marked decrease in lymphocytes and eosinophils (Michaelson et al. 1967). The neutrophils remained slightly increased at 24 h postexposure, while eosinophil and lymphocyte values returned to normal levels. Following 2 h of exposure at 1.65 kW/m<sup>2</sup>, there was a slight increase in leukopenia and decrease in neutrophils. When the exposure was of 3 h duration, leukocytosis was evident immediately after exposure and was more marked at 24 h, reflecting the neutrophil response. After exposure to 1.285 GHz pulsed 1 kW/m<sup>2</sup> for 6 h, there was an increase in leukocytes and neutrophils. At 24 h, the neutrophil level was still noticeably increased. Lymphocyte and eosinophil values were moderately depressed

initially, but at 24 h slightly exceeded their initial value. An exposure of 6 h to 0.2 GHz (CW),  $1.65 \text{ kW/m}^2$  resulted in a marked increase in neutrophils and a mild decrease in lymphocytes. On the following day, the leukocyte count was further increased, and the lymphocytes markedly increased. Such shifts in the WBC picture are consistent with focal thermal lesions to be expected under these exposure conditions.

Work with human cells has demonstrated comparable results. Roberts et al. (1983) exposed human leukocyte cultures for 2 h to 2.45 GHz MW at 0.5, and 4 W/kg. No changes were observed in a number of end points between the groups. In a subsequent study, using cell cultures treated with mitogens or infected with an influenza virus, no significant differences were observed in exposed samples (Roberts et al. 1987). Kiel et al. (1986) also examined human lymphocytes for production of active oxygen species and observed no significant differences for the exposed cells.

In evaluating reports of hematological changes, one must be cognizant of the relative distributions of blood cells in a population of animals or humans and the thermal influence on these alterations. Early and sustained leukocytosis in animals to thermogenic levels of MWs may be related to stimulation of the hematopoietic system, leukocytic mobilization, or recirculation of sequestered cells. Eosinopenia and transient lymphocytopenia with rebound or overcompensation, when accompanied by neutrophilia, may be indicative of increased hypothalamic–hypophysial–adrenal function as a result of thermal stress (Michaelson 1974).

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### 3.11 Effects on the Immune Response

Some interest has continued in the effect of MW exposure on the immune system. A number of articles published in the 1970s and 1980s (Stodolnik-Baranska 1974; Hamrick et al. 1977; Liburdy 1977, 1979, 1980; Wiktor-Jedrzejczak et al. 1977a–c; Smialowicz et al. 1979a,b, 1981b,c 1982a–c; Huang and Mold 1980; Schlagel et al. 1980; Sulek et al. 1980; Roberts et al. 1983; Yang et al. 1983; Smialowicz 1987) were motivated by the possibility of enhancing the immune response with MW exposure with therapeutic goals in mind. By the late 1980s and early 1990s, with a redirection of interest to ELF investigation, the rate of output of studies on immune system effects had declined. Reports of lymphoblastoid transformation by MW were confirmed by some studies and not others (see below). The hope of exploiting such a phenomenon to augment beneficial immune responses in certain disorders was tempered by the concern that there might be a hazard involved if such processes led to autonomous and unregulated lymphoid proliferations. There is no evidence that the latter concern has any validity. On the other hand, no consistent and robust evidence has been elicited to date that MW-induced lymphoblastoid responses can be used to augment immunotherapy of infectious disease or cancer.

#### 3.11.1 Lymphocyte Kinetics

Many reports on effects of MW on lymphocytes have not produced a clear and consistent picture amenable to concise summary. Although lymphocytes can be straightforwardly identified with standard hematologic stains and mature lymphocytes give the appearance of small, uniform, metabolically inert cells, lymphocytes are composed of immunologically distinct subpopulations and, in response to antigenic stimulation or exposure to many chemical substances, can quickly enlarge, exhibiting a diameter two to five times

that of the resting cell, with a striking increase in amount of cytoplasm and transformation of the nucleus from a dark, dense mass of chromatin material to fine, lacy, or decondensed structure. This process, known as lymphoblastoid transformation, has been intensively studied immunologically and biochemically. The lymphocyte may or may not proceed to mitosis depending on the nature of the challenge or stimulus. A number of plant lectins have been used to stimulate lymphoblastoid transformation, protein, RNA, and DNA synthesis *in vitro* to study immune status. In immunologically competent animals, unequivocal, vigorous responses can be elicited. Responses to RF have been reported but of a much less robust nature.

Lymphoblastoid transformation, *in vitro*, after free field exposure to 3 GHz pulsed MWs, 70 W/m<sup>2</sup>, for 4 h daily and 200 W/m<sup>2</sup> for 15 min daily, 3 to 5 d, has been reported (Smialowicz 1976). At this power density, the temperature of the media increased by 0.5°C after 15 min, and after 20 min the increase was 1°C. Changes in the mitotic index were dependent on the exposure time. Although a 5 min exposure did not influence the proportion of dividing cells, slight differences compared with controls were observed after 10 and 15 min exposures and significant differences were seen following 3- or 4-h exposures at 70 W/m<sup>2</sup>. The same research group has examined the proliferative capacity of lymphocytes that are responsible for cellular immune responses (T cells) and humoral immune responses (B cells) following 2.45 GHz exposure *in vitro*. The ability of mouse spleen lymphocytes to undergo blast transformation in response to mitogens that selectively stimulate either T or B cells was measured by the incorporation of <sup>3</sup>H-thymidine into DNA. No consistent difference was found between the blastogenic response of exposed (100 W/m<sup>2</sup>, 19 W/kg, 1 to 4 h) and control cells. In additional work, Smialowicz et al. (1982a–c) exposed mice to CW and PW MW at 2.45 GHz at SARs up to 8.6 W/kg. No differences in mitogen-stimulated responses of lymphocytes or in primary antibody levels in sensitized animals were observed between exposed and control mice in either the CW or PW exposures.

Czerska et al. (1992) studied human peripheral blood lymphocytes *in vitro*, comparing conventional heating to CW to pulsed (PW) exposures at 2.45 GHz, with each type of exposure evaluated at temperature elevations up to 2°C. Average SARs ranged up to 12.3 W/kg for exposures continuous over 5 d. At nonheating levels, CW had no effect on transformation. At heating levels, both conventional and CW heating produced a small but significant increase in blast transformation; at nonheating levels, PW enhanced transformation significantly and increasingly so at heating levels. The authors speculated that pulse power might act via the larger field amplitudes or by microacoustic shock effects.

Cleary et al. (1990b) presented evidence that lymphocytes are stimulated to take up <sup>3</sup>H-thymidine following exposure at 27 MHz or 2.45 GHz *in vitro* under isothermal conditions, with and without phytohemagglutinin (PHA). Maximal uptake occurs at SARs in the range of 25 to 40 W/kg depending on frequency. The authors argue in favor of a nonthermal mechanism.

Kiel et al. (1986) investigated the effects of 2.45 MHz CW MW, SAR of 103.5 W/kg from the point of view of impact on oxidative activity in mononuclear cells, primarily lymphocytes, using a chemiluminescence assay for level of nonphosphorylating oxidative activity. Exposure to MWs at 37°C does not appear to affect oxidative metabolic activity of mononuclear cells.

Huang et al. (1977) reported that lymphocytes from Chinese hamsters exposed to 2.45 GHz CW, from 50 to 450 W/m<sup>2</sup> 15 min/d for 5 d showed changes in blast transformation and mitosis. No chromosomal aberrations were evident. These studies noted increased but reversible transformation of lymphocytes without mitogenic stimulation. These authors, noting temperature changes of less than 2°C, were among the first to suggest

that hyperthermia could be exploited to manipulate the immune response, assuming the changes were thermally mediated. Further investigations by the same group (Huang and Mold 1980), in which mouse spleen cells were exposed to MW with or without T- or B-cell mitogens, showed responses that varied cyclically with time. The control samples, however, also showed the cyclical variations so it was unclear whether the MW exposure was the causative agent for the observed responses. Zafra et al. (1988) reported that MW at 2.45 GHz and power density of  $320 \text{ W/m}^2$  for 15 min enhanced a number of activities of murine macrophages *in vitro* normally elicited in the immune response.

MWs have been reported to induce an increase in the frequency of complement receptor-bearing lymphoid spleen cells in mice (Wiktor-Jedrzejczak et al. 1977c), which may represent a maturation of B lymphocytes. Wiktor-Jedrzejczak et al. (1977a,c) exposed adult male mice to 2.45 GHz CW at an absorbed dose of 12 to 15 W/kg for 30 min in an environmentally controlled waveguide facility and then measured the function of different classes of lymphocytes *in vitro*. Such exposure failed to produce any detectable changes in function of T lymphocytes or increase in DNA, RNA, or protein synthesis, as measured by incorporation of tritiated-thymidine, -uridine, and -leucine by spleen, bone marrow, and peripheral blood lymphocytes *in vitro*. However, the maturation of B lymphocytes from the spleen of exposed mice was stimulated. Consistent with this effect on B lymphocytes are the results reported by Czerski and associates (Czerski et al. 1974a,b; Czerski and Siekierzynski 1975).

Roberts et al. (1987) exposed influenza-virus-infected human mononuclear leukocytes to 2.45 GHz RF, CW or PW at 16 or 60 Hz at an SAR of 4 mW/mL. No alteration by RF of mitogen-stimulated DNA synthesis occurred either in virus-infected leukocytes or uninfected leukocytes.

Mice exposed to 2.45 GHz CW, 50 to  $350 \text{ W/m}^2$  (SAR of 4 to 25 W/kg) for 1 to 22 consecutive days (15 to 30 min/d) showed no consistent significant alterations in several parameters such as mitogen-stimulated response of T- and B-splenic lymphocytes, and the primary antibody response of mice to sheep erythrocytes (Smialowicz et al. 1979b). Subsequently, Schlagel et al. (1980) reported that RF-induced complement-receptor-positive (CR+) lymphocyte increase was under genetic control. Smialowicz et al. (1981b) showed, in addition, the age and strain of the mouse, the RF exposure characteristics (waveguide versus far field), and the environmental conditions are all sources of variation that affect the CR+ cell appearance.

Smialowicz et al. (1979a) exposed rats *in utero* and neonatally through 40 d of age (4 h/d, 7 d/week) in a controlled environment to either 2.45 GHz (CW,  $50 \text{ W/m}^2$ , SAR of 1 to 5 W/kg) or to 0.425 GHz (CW,  $100 \text{ W/m}^2$ , SAR 3 to 7 W/kg). At 40 d of age significant increases in the response of lymphocytes from exposed rats to *in vitro* stimulation with several mitogens was observed in several experiments. While these results have not been consistently reproduced, the trend in the results suggests that chronic exposure during fetal and neonatal development may change either the frequency or responsiveness of lymphocyte subpopulations. The biological significance of these observed changes is unknown. The mechanism by which these changes are initiated may be related to a thermally induced stress response. Similar responses have been observed in animals following prolonged exposure to nonspecific stressors.

Gildersleeve et al. (1987b) exposed Japanese quail eggs to 2.45 GHz MW at  $50 \text{ W/m}^2$  for 12 d. After hatching an immunologic challenge did not elicit any modification of a cell-mediated or humoral immune response.

Hamrick (1973) examined the response of mammalian lymphocytes exposed to 2.45 GHz (CW) in cultures at  $200 \text{ W/m}^2$  (7 W/kg) for 48 h. Changes in the stimulation caused by PHA were not affected by MW exposure.

Decrease in natural killer (NK) activity in lymphocytes of hamsters after exposure to 13 W/kg, 2.45 GHz CW for 1 h was reported by Yang et al. (1983). Roberts et al. (1983) examined the effects of exposure to 2.45 GHz continuous wave RF on human mononuclear synthesis after exposure to RF at SARs up to 4 W/kg. They also measured effects on synthesis of specific host defense proteins, namely interferons, and examined for morphological lymphoblastoid transformation as well as changes indicated by incorporation of radiolabeled precursors. Leukocytes were exposed in 37°C chambers without attempts to counteract RF-induced heating, and final culture temperatures were approximately 0.9°C higher than those of sham-exposed cultures, similar to changes induced by Stodolnik-Baranska (1974). Such exposures resulted in no detectable effects on viability or on unstimulated or mitogen-stimulated DNA, RNA, total protein, or interferon synthesis by the human mononuclear leukocytes (Yang et al. 1983).

There can be considerable difficulty in comparing reports, analyzing the literature, and extrapolating from *in vitro* to *in vivo* effects. Immunological responses to MW irradiation have also been recently reviewed by Black and Heynick (2003).

### 3.11.2 Adaptation

Czerski et al. (1974a,b) have reported that inbred Swiss mice, immunized with sheep RBCs and exposed 2 h/d for 6 or 12 weeks to 5 W/m<sup>2</sup> at 2.95 GHz, showed increased serum hemagglutinin titers and antibody-producing cells in lymph node homogenates. The increase was greater in mice exposed 6 weeks than in those exposed 12 weeks. Similar results were obtained in rabbits exposed 2 h/d to 30 W/m<sup>2</sup> for 6 months with the maximum increase occurring after exposure of 1 or 2 months, and then returning to control values. According to Czerski (1975), this may indicate that after a period of response, the animals become adapted to the MWs. The phenomenon of physiologic adaptation or decreased reaction as a result of repeated exposure to microwaves has also been reported by others (Gordon 1970; Michaelson 1974; Phillips et al. 1975; Varma et al. 1976).

Baranski (1972b) exposed adult guinea pigs 3 h/d for 3 months to 35 W/m<sup>2</sup> at 3 GHz and found an increase in lymphopoiesis over controls, as indicated by increased incorporation of <sup>3</sup>H-thymidine and increased mitotic indices. No differences could be detected between pulsed or CW MWs of the same average power level. Twofold increases in lymphocyte numbers were found in the spleen and lymph nodes.

### 3.11.3 Influence of Hyperthermia

RF-induced hyperthermia in mice has been associated with transient lymphopenia and neutrophilia, with a relative increase in splenic T and B lymphocytes, and with decreased *in vivo* local delayed hypersensitivity (Liburdy 1977). The latter was not affected by a comparable increase in core temperature produced by warm air. Reduced thymic mass and cell density (Smialowicz et al. 1979b) and suppressed inflammatory response (Phillips et al. 1975; Smialowicz et al. 1979b) have been reported. Such alterations in lymphocyte distribution and function are concomitant with a state of immunosuppression. Qualitatively, similar changes can be induced by administration of synthetic glucocorticoids or CS (Smialowicz et al. 1979b; Roberts et al. 1983). Liburdy (1979) reported elevated plasma CS levels in mice following exposure to RF energy sufficient to cause hyperthermia. A similar response has been reported by Lotz and Michaelson (1979), who showed a correlation between MW-induced body heating and CS levels in the blood of rats. These

studies suggest that exposure to MWs of sufficient intensity results in stimulation of the hypothalamic–adrenal axis, which could affect the immune system (Liburdy 1979).

It would thus appear that RF exposure initially causes a general stimulation of the immune system, but if the exposure continues, the stimulatory effect disappears, suggesting a phase of adaptation to continued RF exposure. There is a body of literature on the influence of heat *per se* on immunity. Significant influences of microwaves on immune responsiveness would be expected on the basis of the known effects of hyperthermia. Although there has been some uncertainty whether fever enhances host resistance to infection (Bennett and Nicastrì 1960; Atkins and Bodel 1972), recent evidence suggests that fever may enhance survival after infection in an animal model (Kluger et al. 1975). Cell-mediated immunity plays a role in defense against facilitative intracellular bacteria, viruses, and certain other infectious agents (Frenkel and Caldwell 1975; Mandell 1975). Roberts and Steigbigel (1977) have shown that increased temperature (38.5°C) enhances human lymphocyte response to mitogen (PHA) and antigen (streptokinase–streptodornase) and enhances, but does not accelerate, certain bactericidal functions of human phagocytic leukocytes. One, therefore, has to be circumspect in assessing the mechanisms of MW exposure related to alterations in immune processes. It may be that if MWs do, in fact, increase the proportion of lymphocytes undergoing transformation, it may not in itself be harmful, but actually beneficial. The immune system has a considerable redundancy and adaptability. Perturbations of the immune system may not have clinical significance.

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### 3.12 Carcinogenesis

There are no laboratory studies in humans with cancer as the direct end point of investigation. Such studies would clearly cross-ethical boundaries and would not be acceptable. Furthermore, RF fields lack sufficient energy to disrupt chemical bonds so there is little theoretical basis for suspecting that such fields would cause mutations or other genotoxic effects. There are a few human studies investigating possible indirect and nongenotoxic effects relevant to cancer that are discussed in the section on endocrine function studies. Additionally, accompanying a rising concern regarding exposure of large numbers of people to mobile telephone signals, a number of recent epidemiologic studies have been performed evaluating RF exposures at cellular phone frequencies.

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#### 3.12.1 Long-Term Animal Studies

Long-term animal bioassays, often conducted in two species (usually rats and mice) and in both male and female animals for 2 y, provide a reasonable surrogate for human lifetime exposure. A relatively small number of long-term animal bioassays have been performed exposing rats and mice to RF signals. Unlike many other areas of biological investigation of MW exposures, long-term animal cancer bioassays have almost all been conducted since the last edition of this Handbook was issued. These more recent studies, performed at nonthermal levels, have indicated no pathological or carcinogenic effects. This includes studies with a focus on brain cancer at 836 MHz, 1.1 to 1.6 W/kg (Adey et al.



1999, 2000), 900 MHz at ~1 W/kg (Zook and Simmens 2001), and 1.6 GHz (Anderson et al. 2004). Complete histopathology in life span and hematologic studies were performed at 835/847, 1600, 800, and 2450 MHz at 1.3 W/kg (La Regina and Roti Roti 2003), up to 1.6 W/kg (Anderson et al. 2004); up to 12.9 W/kg (Spalding et al. 1971), and at 0.3 W/kg (Frei et al. 1998), respectively. Some pathological effects have been reported at thermal levels (Roberts and Michaelson 1983). Prausnitz and Susskind (1962) reported the induction of leukemia in mice exposed to 9.27 GHz RF at 1 kW/m<sup>2</sup>, 4.5 min/d, 5 d/week for 59 weeks. This report is viewed as a flawed study, principally with regard to interpretation of the histology of the hematopoietic abnormality. Szmigielski et al. (1980) described accelerated development of spontaneous and benzopyrene-induced skin cancer in mice exposed to 2.45 GHz, 150 W/m<sup>2</sup> (6 to 8 W/kg). At 50 W/m<sup>2</sup> (2 to 3 W/kg), the cancer induction was comparable to that which occurs with chronic stress resulting from confinement. Preskorn et al. (1978), however, reported decreased tumor growth and greater longevity in mice exposed to intense levels of 2.45 GHz energy, i.e., sufficient to make the mice hyperthermic.

The only report of an increased tumor incidence with long-term RF exposure at clearly nonthermal levels was reported by Chou et al. (1992). They reported a small increase in overall tumor occurrence in rats exposed for 24 months to 2450 MHz (SAR of 0.15 to 0.4 W/kg). There was no effect in the Chou et al. study on a number of other parameters including metabolism, immune function, hematology, serum chemistry, thyroxine levels, protein parameters, growth or open-field behavior. In another bioassay cancer study at 435 MHz, no overall increase in cancer in animals exposed to 1.0 mW/cm<sup>2</sup> was found (Toler et al. 1997). Some organs (specifically the adrenal glands) did show slight trends toward increased cancer in RF-exposed animals; however, the number of tumors was small and no statistically significant differences could be determined between the exposed and control groups.

Shorter term but repeated exposures of C3H/HeJ mice to ultra-wideband MW pulses showed no effects on the development of mammary tumors (Jauchem et al. 2001). A 2 week exposure experiment at 900 MHz with average whole body SARs of 75 and 270 mW/kg showed no significant effects on tumor development or growth rate or time of tumor onset (Chagnaud et al. 1999).

### 3.12.2 Radiation or Chemically Initiated and Transgenic Animal Bioassays

Similar to long-term animal bioassays, studies in which tumors have been initiated by means other than RF have been mostly negative. Many different initiation models have been used in these studies in which rodents have been exposed to RFs between 800 and 1500 MHz. The experimental models used include brain tumors initiated in rats with ethyl nitrosourea (836 and 860 MHz, approximately 1 W/kg) (Adey et al. 1999, 2000; Zook and Simmens 2001), benz[*a*]pyrene-initiated sarcomas in rats (900 MHz, 0.075 and 0.27 W/kg) (Chagnaud et al. 1999), dimethyl benzanthrane (DMBA)-initiated rat mammary tumors (900 MHz, SARs from 0.017 to 0.07 W/kg) (Bartsch et al. 2002), diethyl nitrosamine-induced hepatomas in rats (929 and 1500 MHz) (Imaida et al. 1998a,b), and radiation-induced mouse lymphomas (902 MHz, 0.35 W/kg) (Heikkinen et al. 2001). In all of these cited cancer studies, no adverse effects of RF exposure were noted. Mason et al. (2001) studied mice exposed to 94 GHz for 12 weeks. Treatment of both exposed and control mice with DMBA resulted in papilloma development, as expected, but no differences were observed between the groups.

In addition to chemicals and radiation, genetically initiated animal models (transgenic mice) have also been studied in RF carcinogenicity testing. No effects of RF exposure on mutagenicity or tumor development were found using pKZ-1 transgenic mice (900 MHz,

4 W/kg) (Sykes et al. 2001). Another study by Repacholi et al. (1997) in Pim-1 mice reported an association between long-term RF exposure (900 MHz, 0.13 to 1.4 W/kg) and mortality from a certain subtype of lymphoma. A subsequent study performed at multiple dose levels with more uniform and more fully characterized exposure (900 MHz, multiple levels to 4.0 W/kg) did not confirm the positive effects reported in the original study (Utteridge et al. 2002). Chronic exposure of C3H/HeJ mammary tumor-prone mice has also been investigated. The mice were exposed or sham-exposed to 1 mW/cm<sup>2</sup> at 435 MHz for 21 months. No significant effects were observed in any of the tumor-related indices (Toler et al. 1997).

### 3.12.3 Tumor Cell Injection Bioassays

A few studies of tumor progression, using nonthermal RF exposure levels, have been conducted by injecting tumor cells into mice and determining growth rate, survival, and metastatic progression. Although increased survival of the host, as well as inconsistent evidence of either augmentation or suppression of immune function, has been reported in response to thermal levels of RF exposure, no such effects were observed in studies using lower levels of exposure: at 915 MHz, up to 8.3 W/kg (Salford et al. 1993b) and at 836 and 847 MHz, 0.75 W/kg (Higashikubo et al. 1999).

### 3.12.4 Summary of Animal Cancer Bioassays

Most animal bioassay studies have not demonstrated increased cancer risk resulting from long- or short-term RF exposure at nonthermal levels. In the very few studies at thermal levels of MW exposure, only inconsistent evidence of effects have been reported, and those have not been confirmed in similar or replicate studies. Recent reviews have been published on cancer studies in MW-exposed animals (Elder 2003; Heynick et al. 2003).

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## 3.13 Other Specialized Organ Response

### 3.13.1 Auditory Response

A phenomenon called “microwave hearing” or “RF hearing” is a well-established biological effect resulting from exposure of the head to pulsed microwaves. RF hearing is sensed in the head at rather low-power densities (Frey 1961, 1962). In and of itself this phenomenon, characterized by the sound of clicking, buzzing, knocking, or chirping in the head, has no known adverse health consequences (Frey 1985; Roschmann 1991; Elder and Chou 2003). The fundamental MW frequencies that are involved vary from 2.4 MHz to 10 GHz (Frey 1962, 1963; Putthoff et al. 1977; Cain and Rissmann 1978; Khizhnyak et al. 1980). However, the MWs must be modulated to produce the hearing phenomenon, generally in the 7 to 10 kHz range (Chou et al. 1977, 1982b; Watanabe et al. 2000), or 8 to 15 kHz (Lin 1977a,c).

The mechanism responsible for eliciting such an effect appears to be thermoelastic expansion (Foster and Finch 1974; Chou et al. 1975, 1982a; Guy et al. 1975a; Lin 1976, 1977a; [see also Chapter 10](#) of *BBA*). It is probable that RF-generated waves of thermoelastic expansion result in cochlear hair cell distortions, mimicking the sonic waveforms of acoustic stimuli (Foster and Finch 1974; Taylor and Ashleman 1974; Chou et al. 1975,

1985; Guy et al. 1975a; Lebowitz and Seaman 1977a,b; Lin 1977b; Chou and Guy 1979; Lin et al. 1979a, 1982, 1988; Olsen and Lin 1981, 1983). There are studies, however, that indicate the RF-hearing phenomenon can also occur after the middle ear has been ablated (Taylor and Ashleman 1974; Guy et al. 1975b; Chou and Galambos 1979; Wilson et al. 1980). Other studies have described thresholds for this RF response in animals (Guy et al. 1975a; Lebowitz and Seaman 1977a,b; Cain and Rissmann 1978; Seaman and Lebowitz 1989).

Comprehensive reviews are available discussing the MW-hearing effect and research directed to an examination of the mechanisms involved in producing such effects (Chou et al. 1976, 1982a; Lin 1978, 1981, 1989, 1990, 2001, 2002; Elder and Cahill 1984; Postow and Swicord 1996; Stewart 2000; Elder and Chou 2003; [see also Chapter 10](#) of *BBA*).

### **3.13.2 Ocular Effects**

During the past 40 y, numerous investigations in animals and several surveys among human populations have been devoted to assessing the relationship of MW exposure to the subsequent development of cataracts. It is significant that of the many experiments on rabbits by several investigators using various techniques, a power density above 1 kW/m<sup>2</sup> for 1 h or longer appears to be the lowest time–power threshold in the tested frequency range of 0.2 to 10 GHz. In other species of animals such as dogs and nonhuman primates, the threshold for experimental MW-induced cataractogenesis appears to be even higher. This threshold is a time–power threshold, that is, the higher the power density, the shorter is the time threshold and vice versa, down to a certain minimum power density. All of the reported effects of MW radiation on the lens can be explained on the basis of thermal injury.

#### **3.13.2.1 Threshold for Opacity in Rabbits**

The most extensive investigations in this area were performed by Carpenter and Van Ummersen (1968) and Carpenter et al. (1960). Later work by Guy et al. (1975b) and Kramar et al. (1975, 1987) has shown that in rabbits exposed to 2.45 GHz, the threshold for cataract production is 1.5 kW/m<sup>2</sup> for 100 min or  $\geq 150$  W/kg for  $\geq 30$  min. The data suggest that an intraocular temperature of at least 41°C (Guy et al. 1975b) must be obtained to induce cataracts, although exceptions may be found in the literature. These investigators also found that single potentially cataractogenic exposures will not injure the eye under conditions of controlled general hypothermia, and exposure to 1 kW/m<sup>2</sup>, 2 h/d, for 4 to 9 d produced no cataracts, as evidenced by periodic examinations for 6 months after exposure. Guy et al. (1974) exposed rabbits to 0.918 GHz, 4.66 kW/m<sup>2</sup> for 15 min and 1.17 W/m<sup>2</sup> for 100 min with no evidence of cataract and concluded that the threshold for cataractogenesis is higher for this frequency in comparison to 2.45 GHz. Williams et al. (1975) discussed the ultrastructural changes that occur in rabbit eyes when RF exposure fields are intense. Additional studies using high intensity exposures were conducted in the eyes of the dog (Daily et al. 1950; Ballie 1970; Ballie et al. 1970). At the same average power, both pulsed and continuous RF exposures were equally effective in producing cataracts in rabbits (Birenbaum et al. 1969). These results support the indication of a thermal mechanism for the damage.

Appleton et al. (1975) exposed rabbits to 3 GHz, 1 or 2 kW/m<sup>2</sup> for 15 to 30 min. Examining daily for 14 d, weekly for 1 month, and monthly for a year revealed no ocular changes. At power densities of 3, 4, or 5 kW/m<sup>2</sup> for 15 min, acute ocular changes involving the conjunctiva and iris occurred during exposure. In a comparable study by Hirsch et al. (1977), rabbits were repeatedly exposed to 3 GHz once daily for a month.

Clinical examinations were carried out for 1 y afterward. No changes occurred at power densities under  $3 \text{ kW/m}^2$ .

#### **3.13.2.2 Biochemical Changes**

Reports of decreased enzymatic activity (Kinoshita et al. 1966; Carpenter and Van Ummersen 1968) may quite likely be due to thermal inactivation with resultant alterations in metabolism. Decreases in ascorbic acid concentration in the lens have been cited as the first biochemical indication of opacity formation (Merola and Kinoshita 1961; Weiter et al. 1975); and progressive clouding of the lens is associated with decreases in ascorbic acid below  $60 \mu\text{g/g}$  of lens tissue. All of these effects could be fully repairable until the altered metabolism has produced a permanent opacification of the lens. Latent periods and time-power thresholds would be in agreement with a mechanism of this nature.

#### **3.13.2.3 Thermal Aspect of Microwave Cataractogenesis**

Guy et al. (1974, 1975a,b), Kramar et al. (1975), and Taflove and Brodwin (1975) have computed the MW energy deposition and induced temperatures in the eyes of rabbits and a model of the human eye. They have indicated that a distinct hot spot exceeding  $40.4^\circ\text{C}$  probably occurs deep within the eye at a frequency of 1.5 GHz, when the power density would be cataractogenic (i.e., greater than  $2 \text{ kW/m}^2$ ).

Paulsson (1976) measured the absorption of 0.915, 2.45, and 9.0 GHz MW energy in a model of the human head. The absorbed power showed an essentially exponential decrease with the distance from the cornea at 9.0 GHz. At 2.45 and 0.915 GHz, maximum absorbed power occurred within the eyeball.

#### **3.13.2.4 Pulse Wave Exposures**

Kues and Monahan (1992; Kues et al. 1985, 1992) investigated effects of pulsed MW on the eyes of monkeys *in vivo*. Exposures were at 2.45 GHz ( $10 \mu\text{s}$ , 100 pps) at an average incident power of  $100 \text{ W/m}^2$  (SAR of  $2.65 \text{ W/kg}$ ) on three consecutive days for 4 h/day. Damage to the corneal endothelium and an increase in iris vascular permeability was found persisting for more than 72 h after exposure. This report suggests that PW exposures might have significantly lower thresholds for damage than CW exposures, especially high peak power exposures with low SAR resulting from a sufficiently slow repetition rate. Johnson et al. (1992) also exposed monkeys to 1.25 GHz MW,  $0.5 \mu\text{s}$  wide pulses, 16 pps at an average ocular SAR of 3.5 to  $4.0 \text{ W/kg}$ , seven 4-h exposures spread over several weeks. At this high peak, low repetition rate exposures retinal damage was found by electroretinography, suggesting cone photoreceptor damage. However, Kamimura et al. (1994) failed to confirm the corneal lesions reported by Kues et al. after CW exposure, and Lu et al. (2000) did not replicate the PW effects of exposure. Furthermore, no functional changes in vision were observed (McAfee et al. 1983). Kues et al. (1999) did not see corneal damage or changes in vascularity permeability of the iris or lens opacity in rabbit or monkey eyes exposed to 60 GHz fields at  $100 \text{ W/m}^2$ . Nor were histological changes observed at  $2250 \text{ W/m}^2$  (Williams and Finch 1974). Creighton et al. (1987) have also found evidence of an enhanced effect of pulsed over continuous MW at equivalent average power level by observing cataract formation in the rat lens *in vitro*.

#### **3.13.2.5 Cumulative Effect**

The possibility of a cumulative effect on the lens from repeated subthreshold exposures of the rabbit eyes to MW was proposed. Such an effect presumably would result from

clinically inapparent damage from any given exposure, which would eventually manifest clinically after repeated exposures.

McAfee et al. (1979) trained monkeys to face an RF source and then exposed them to 9.3 GHz pulsed RF at an average power density of  $1.5 \text{ kW/m}^2$ . Each of 12 monkeys was exposed for up to 20 min/d for 30 to 40 sessions over several months. A total of 75 monkeys, nonexposed and sham-exposed, served as controls. No cataracts or corneal lesions were seen in any of the 12 exposed animals up to 12 months after exposure.

Chou et al. (1982b) exposed two groups of six rabbits to 2.45 GHz CW/PW (10  $\mu\text{s}$  pulses, 100 pps, peak power density  $150 \text{ kW/m}^2$ ) at  $150 \text{ W/m}^2$  for 2 h/d over several months; a third group was sham-irradiated. Periodic eye examinations for cataract formation yielded no statistically significant differences among the three groups. Guy et al. (1980) and Chou et al. (1982b, 1983) exposed rabbits to 2.45 GHz RF at  $100 \text{ W/m}^2$  (maximum whole body SAR of  $17 \text{ W/kg}$ ) 7 d/week, 23 h/d for 6 months. For controls, other rabbits were sham-exposed for the same durations. Periodic eye examinations with a slit lamp microscope showed normal aging changes in the lenses and no significant differences between the exposed and control groups.

Although it is difficult to identify the time–power threshold precisely, varying the times and powers to produce such a curve, a general reading of the literature suggests that no one has been able to produce cataracts in animal models at power densities below 800 to  $999 \text{ W/m}^2$  even by repetitive exposures. These values are well above those of the most widely used safety standards (ANSI/IEEE 1982; NCRP 1986).

In general, the results from animal studies indicate that RF cataractogenesis is essentially a gross thermal effect that has a threshold power density at which the difference between the rates of heat generation by RF and heat removal is large enough to result in damage to the lens of the eye. The case of very high peak powers at low repetition rates may prove to be an exception.

### **3.13.2.6 Extrapolation to the Human**

After 40 y of studies of the effects of MWs on the ocular lens, primarily in the rabbit and monkey, the principal conclusions are:

- The acute thermal insult from high intensity MW fields is cataractogenic in the rabbit if intraocular temperature is greater than  $43^\circ\text{C}$ .
- The MW exposure threshold is between  $1$  and  $1.5 \text{ kW/m}^2$  applied for about 60 to 100 min.
- There does not appear to be a cumulative effect from MW exposure unless each single exposure is sufficient to produce some irreparable degree of injury to the lens.

That opacity of the ocular lens can be produced in rabbits by exposure to MW is well-established. Extrapolating results from animal studies to humans is difficult, because the conditions, durations, and intensities of exposure are usually quite different. Reports of adverse reactions to exposures are necessarily uncontrolled and exposure conditions are generally impossible to determine or reconstruct. It is also difficult to relate cause and effect, because lens imperfections do occur in otherwise healthy individuals, especially with increasing age. Numerous drugs, industrial chemicals, and certain metabolic diseases are associated with cataracts. Because of the many uncertainties, workmen's compensation and negligence cases are usually settled out of court with the details of the settlement withheld from the public. Lipman et al. (1988) have reviewed the conditions under which MW might produce injury to the lens.

There have been a few epidemiological studies that specifically address the issue of MW exposure effects in the human eye. The principal end point has been risk of cataract formation or other opacities in the eye. Difficulties arise in almost all of these studies in that exposure frequencies are seldom specified, nor are exposure levels known. These studies are reviewed in Elder (2003) with the strong conclusion that no evidence of changes in the eye are apparent. One might assume that at MW exposure levels high enough to produce heating in the eye (as in animal studies) that one might observe opacities develop. However, only two cancer assessment studies in humans show effects. Holly et al. (1996) found a twofold risk increase for uveal melanoma in RF-exposed workers. Stang et al. (2001) also reported an increased risk of eye cancer from a case-control study of mobile phone users. The limitations of the methodologies used, however, prevented clear interpretation of the data. Johansen et al. (2001, 2002) attempted to replicate these observations in a Danish study and found no association between mobile phone use and eye (or brain or other) cancers.

### 3.13.3 Hypersensitivity

A number of studies have investigated claims by individuals of an association between exposure to electrical fields and a number of subjective end points, including dizziness, perspiration, irritability, and other bioeffects (COMAR 2002). This condition has been termed "hypersensitivity" and has been reported to occur with exposure to video display terminal (VDT) instruments (Sandstrom et al. 1995; Stenberg et al. 1995), with occupational RF fields (Bini et al. 1986), or other external RF sources (Choy et al. 1986; Flodin et al. 2000; Sandstrom et al. 2001). A study of RF-exposed workers in Poland claimed health effects but study design limitations make interpretation of results difficult (Bielski 1994). Some carefully conducted research has reported no association between mobile telephony exposure and hypersensitivity in both provocation studies (Kovisto et al. 2000a,b) and self-identified sensitive subjects (Heitanen et al. 2002). However, other studies report links between hypersensitivity and cell phone exposure (Hocking 1998; Hocking and Westerman 2000). Kimata (2002) observed increased allergic reactions in sensitive subjects with cell phone exposure for 60 min. A follow-on study, however, indicated that the actual field exposure may not be the causative factor (Kimata 2003).

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## 3.14 Critique

Elucidation of the biological effects of RF exposure requires study and analysis of the available literature. This literature has been accumulating, in a sustained way, since the late 1940s with the development and application of radar in World War II. The literature is not particularly user-friendly in that comparisons of studies must often rest on assumptions of uncertain validity. Over the last half century, there has been a steady improvement in measurement capabilities, dosimetric measurements, and standards of quality in the performance of investigations. This improvement has been so dramatic that it makes contemporary investigators mistrustful of the older literature, resting as it did on a less-sophisticated technology and approach. The older literature also contained a major contribution from the closed societies of Eastern Europe, which was hobbled by barriers to free and open communication and which may have been tainted by political interference. It was commonly the case that reports from countries dominated by the former

Soviet Union were inadequately documented and the results slanted to conform to expectations of authorities. This may have had the effect of endowing RF effects at low power with greater and perhaps more sinister efficacy in producing specific biological effects, especially on the nervous system, than the contemporary consensus would support.

The general improvement in the quality of research has produced areas of agreement on effects and potential hazards, but also left areas of disagreement. The issue of window effects produced by modulated RF on calcium exchange in the nervous system is still permeated with disagreement. The issue of an increase in intrinsic permeability of the BBB at nonthermal levels of RF has been resolved to the satisfaction of a majority of students of the subject but some dissent still remains. General consensus is that there is no such increase at nonthermal levels and that brain temperature must rise to 41 to 43°C, rapidly producing tissue necrosis and morbidity, in order to see transfer of hydrophilic molecules into brain parenchyma. Studies of effects on the neuroendocrine system always pose interpretative problems because of the labile response of the system to stress. Even the most gentled animals may be responding to the stress of handling and this may potentiate an RF effect which otherwise may not be observed.

Even if the effects can be validated and the dose–response worked out quantitatively in a manner to pass the most stringent review, there remains the problem of what constitutes a hazard. One objective definition of injury is an irreversible change in biological function producing a functional deficit as observed at the organ or system level. With this definition, it is possible to define a hazard as a probability of injury on a statistical basis. It is important to distinguish between the intensities of RF energy at which injury may be sustained and those that produce nonharmful effects with no further pathophysiologic consequences. All effects are not necessarily hazards. Some effects may have beneficial application under appropriately controlled condition. For that reason therapeutic application of RF energy by use of diathermy technology to produce frank heating is excepted from safety standards prescribing permissible exposure limits. In this case, risk–benefit analyses are applied.

It is important to determine whether an observed effect is irreparable, transient, or reversible, disappearing when the electromagnetic field is removed either immediately or after some interval of time. Of course, even reversible effects are unacceptable if they transiently impair the ability of the individual to function properly or to perform a required task.

Although there is a much greater appreciation in recent years of the need for rigorous experimental design, the challenges are still formidable. An investigator may think he is observing a low level or nonthermal effect in one animal because the incident power is low, while in actuality the animal may be exposed to as much absorbed power in a specific region of the body due to resonance or focusing effects as another larger animal is with much higher incident power at certain frequencies. A detailed understanding of interspecies scaling factors is essential.

The review of the literature as a whole has its own problem. The process of comparing studies is usually not straightforward, especially if the studies seem to contradict each other. Even an investigation, which has as a specific goal the confirmation or nonconfirmation of a previous study, is inevitably different in some ways. The significance of the differences is debated at scientific meetings and these debates are usually not reflected in the literature. If a follow-up study does not use exactly the same frequency, the question of the ability to extrapolate from one frequency to the other arises.

Pickard and Foster (1987) proposed that it may be time to limit further RF biological effects research by formulating guidelines to restrict further work. They advanced this proposal because of the inherent noisiness of the field and the apparent inability to

resolve controversies in a timely manner. In subsequent meeting forums many scientists took issue with the proposal, arguing that more (and more careful) research was needed, not less.

As mentioned in the introduction of this chapter, the rate of RF research output declined significantly during the 1990s. The U.S. Defense Department, the major underwriter of RF research prior to about 1995, dramatically reduced its support of MW research. Public concerns through the late 1980s and early 1990s directed more support to ELF research. However, in the mid-1990s concerns that the RF output of cellular telephones might play a role in the initiation of tumors caused some return of funding to RF effects research. In assessing the literature, it will be evident throughout this chapter that research conducted since the last edition of this Handbook has concentrated in particular areas. A focus on potential RF effects on cancer, certain neurophysiological effects such as investigations of the BBB, and further studies at the cellular level predominate in the recent literature.

Although the public policy rationale undergoes periodic shifts, overall support for continuing research in electromagnetic effects will undoubtedly continue, motivated substantially by safety considerations and exploration of potential medical use of MWs in diagnoses and therapies.

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# 4

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## *Behavioral and Cognitive Effects of Electromagnetic Field Exposures\**

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Sheila A. Johnston and John A. D'Andrea

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## 4.1 Introduction

The human brain is comprised of more than 100 billion individual nerve cells that are functionally interconnected into systems. These systems construct our perceptions and cognitions of the world, and control how we react to the external world (Kandel et al., 2000). This chapter presents an overview of the interaction of nonionizing electromagnetic fields (EMFs) as external stimuli to the nervous systems and the behavior of humans and laboratory animals. This overview of the scientific literature specifically includes the detection of EMFs and effects of EMFs on behavioral performance and cognition. EMF detection by humans and responses of laboratory animals suggests that detection could motivate changes in behaviors. The chapter is divided into two parts, which are distinguished simply by frequency: microwave (MW) radio frequency and extremely low-frequency (ELF). This chapter extends the findings of recent reviews of the animal and human research literature (Hermann and Hossmann, 1997; D'Andrea, 1999; Cook et al., 2002; D'Andrea et al., 2003a,b; Hossmann and Hermann, 2003).

Biological effects of MWs occur due to absorption of energy in the body. Microwave absorption by water molecules has a resonant frequency in the region of 2.45 GHz that causes heating by molecular vibration and is the result of the interaction of MWs and tissue of the body. Water is the molecular component of tissue known to have a resonant frequency that could contribute to the absorption of MW energy. Heating (absolute temperature rise) is the best understood mechanism for the effective transfer of MW energy into tissue. Other tentative mechanisms for the transfer of energy from MWs into human tissue are not established as potential sources for health effects or the modification of human behavior.

The accepted unit of measurement is specific absorption rate (SAR). The IEEE (Std C95.1-2005) defines "specific absorption rate (SAR) as the time derivative of the incremental energy absorbed by (dissipated in) an incremental mass contained in a volume element of given density." The ICNIRP (1998) defines "specific energy absorption rate (SAR) as the rate at which energy is absorbed in body tissues, in watt per kilogram (W/kg). SAR is the dosimetric measure that has been widely adopted at frequencies above about 100 kHz." Absolute rise in temperature in the tissues of the body is also a measure of MW absorption of energy.

Biological effects of ELF are known to be due to electrostimulation which is defined as "the induction of a propagating action potential in excitable tissue by an applied electrical stimulus; electrical polarization of pre-synaptic processes leading to a change in post synaptic cell activity" (Reilly, 1998; IEEE, Std C95.6-2002). The unit of measurement is slightly different for IEEE and ICNIRP. For the IEEE, the unit is the induced *in situ* electric field stimulation of the nervous system (volts per meter: V/m) (IEEE, Std C95.6-2002) and for the ICNIRP the unit is the induced current density (ampere per square meter: A/m<sup>2</sup>) that results in stimulation of the nervous system (ICNIRP, 1998).

The body generates thermal noise and also electrical noise and against that background the input of low power MW stimulation may be indistinguishable from the endogenous biological thermal noise while core temperature remains stable (Adair, 2003) and the electrical power stimulation may be indistinguishable from the endogenous electrical noise below the threshold for the generation of action potentials of nerves (Reilly, 1998). Establishing scientific evidence of the time course and dose effects for MW-induced temperature rise and ELF-induced electrical stimulation of nerves are essential. Reviewed in this chapter, these are reflected in the animal and human gradients from biological sensory detection of MWs and ELF exposures up to the level of adverse health effects on behavioral and cognitive responses.

EMF may affect a biological system, causing a biological effect, without necessarily causing an adverse change in health. We feel it is very important and relevant in this chapter to see the full picture that is the gradient of established biological effects up to adverse effects following the international criteria for scientific evidence. Several papers, chapters of books, and Web sites are available that list the research criteria required for evaluation of the scientific literature (Repacholi, 1998; Erdreich and Klauenberg, 2001; Gajšek et al., 2002; ICNIRP, 2003; Johnston, 2003, 2004/2005; Kheifets et al., 2003). An essential part of the full research picture is establishing scientific evidence of the thresholds for MW\* and ELF<sup>†</sup> adverse effects on behavior and cognition for setting international standards and guidelines to protect our health. The scientific evidence exists in its own right, independent of the standards, and reciprocally the standards review bodies review all the scientific evidence, not just adverse effects.

Confirmed and replicated scientific results of MW and ELF exposure are essential to establish scientific evidence. The results of single studies, even when they are of good quality, are not considered as scientific evidence on their own because single results may fail to be reproduced. Only when the results are repeated and confirmed or replicated independently or when the results from well-conducted studies are consistent with the weight of evidence from other good quality research data, are they accepted as scientific evidence.

Within the scientific literature, it is important to distinguish between replication and confirmation studies because they may have different implications for results that fail to be reproduced. Often published studies have incomplete or poor protocols to test the hypothesis and thus only confirmation studies could be considered to investigate the same hypothesis. In this instance, the lack of confirmation could be due to different research designs testing different underlying mechanisms. The failure to confirm single results in this instance would be inconclusive and difficult to resolve. This is especially true when the underlying mechanism for a reported single effect is unknown. But, it is feasible that confirmation studies with slightly different protocols could test the same known underlying mechanism. One such example is different maze protocols that are known to test spatial memory (Morris et al., 1982; Gray and McNaughton, 1983; Kandel et al., 2000; Maguire et al., 2000, 2003a,b; Burgess et al., 2002; see Section 4.2.4). Such studies that test the same hypothesis with slightly different protocols but report the same result are considered as confirmation studies. Single experiments with well-defined protocols, when replicated independently, suggest both experiments are testing the same underlying mechanism. In this instance, if replication fails then the single result is rejected. Well-conducted and published confirmation and replication studies that produce the same result contribute to the weight of scientific evidence. These central concepts will be referred to in this chapter.

This chapter explores the failure of U.S. scientists to replicate some of the MW exposure effects on the nervous system, the blood–brain barrier (BBB), and cognition as reported by

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\*Established adverse MW effects are associated with whole body heating at levels that usually increase temperature by approximately 1°C or more (IEEE Std C95.1-2005). "Available experimental evidence indicates that the exposure of resting humans for approximately 30 minutes to MWs producing a whole-body SAR of between 1 and 4 W/kg results in a body temperature increase of less than 1°C". [ICNIRP, 1998]. Animal data indicate a threshold for behavioral responses in the same SAR range [ICNIRP, 1998; D'Andrea et al., 2003b; IEEE Std C95.1-2005]. These data form the basis for an occupational exposure restriction of 0.4 W/kg, which provides a 10× margin of safety [ICNIRP, 1998; IEEE Std C95.1-1999]. The public standard has an additional 5x margin of safety (0.08 W/kg) for both the IEEE Std C95.1-2005 and ICNIRP guidelines [1998].

<sup>†</sup>Harmonisation of the ELF basic restrictions between IEEE and ICNIRP is in progress [Reilly, 2005; ICNIRP, 2003]. For public exposure at 60 Hz for the brain the IEEE exposure limit is set at 0.018 V/m and for ICNIRP at 0.01 V/m and for other tissue for IEEE at 0.70 V/m and ICNIRP at 0.01 V/m. The ICNIRP current density was converted to E field, based on  $\sigma = 0.2 \text{ S/m}$  [Reilly, 2005].

Russian scientists. Within the area of behavioral and cognitive effects investigated are changes in attention, simple and complex memory tasks, sleep and awake electroencephalogram (EEG), and changes in the BBB (Kandel et al., 2000). This overview explores the past research and extends the findings of recent reviews of the animal and human behavioral and cognitive research literature (Hermann and Hossmann, 1997; D'Andrea, 1999; Cook et al., 2002; D'Andrea et al., 2003a,b; Hossmann and Hermann, 2003) to newly published papers in the MW and ELF fields. One question considered is whether cognitive performance disruption might be more "sensitive," that is have a lower threshold for disruption by MW and ELF exposure, than performance disruption of simple tasks.

The ICNIRP (1998) and IEEE (Std C95.1-1991/1999, Std C95.1-2005) safety standards that limit human exposure to MWs differ tremendously from those of the former Soviet Union (FSU), which are based on Soviet study results on fixed durations of exposure (Hossmann and Hermann, 2003). Since the 1970s, the maximum permissible exposure in the Soviet block countries has been reported to be 1000 times lower than most other countries. Due to these large differences in MW safety standards, a program was established in 1975 for the collaborative study of the biological effects of low-level MW exposures. A leading area of study was the effect of nonionizing radiation on the central nervous system (CNS) and behavior (Shandala et al., 1979). The presumption was that microwave fields might alter ongoing electrical activity in the brain by induction of fields and currents that might alter the resting membrane potential of neurons, synapses, and sensory organs. However, Chou and Guy (1978) failed to replicate the result of Kamenskii (1964) of a MW effect on the isolated nerves exposed in air and parallel to the E-field. When the nerve was kept in a temperature-controlled waveguide filled with physiological solution, no effect on action potentials was observed other than those of thermal origin, even if the nerve was exposed to very high pulsed power levels with peak SAR up to 220 kW/kg (Chou and Guy, 1978). Support for thermal effects on action potentials reported by Chou and Guy (1978) comes from electrophysiological recordings by Andersen and Moser (1995), who reported that "various bioelectrical signals are more sensitive during warming, axonal conduction is speeded up and stimulus elicited transmitter release becomes faster and more synchronized." The effects in the original study (Kamenskii, 1964) appeared to be due to the particular *in vitro* exposure condition. When the proper temperature control was applied, the effects disappeared.

However, at that time most research efforts were focused on electroencephalography, evoked responses, and behavioral changes. D'Andrea et al. (2003b) reviewed the details of many of these studies recently. After initial experiments in the United States and FSU failed to mutually confirm low-level effects, an agreement was made to use similar experimental designs and research protocols (McRee et al., 1979). The results of the joint project, which lasted only a few years, found some alteration in spectral power of the EEG, but these results were not the same between the experiments in both countries (Mitchell et al., 1989). The collaborative project ended without mutual confirmation by both research groups of low-level, microwave-induced biological effects. This outcome illustrates the paramount importance of replication of experimental studies. Replication is important because some estimate of natural variation is needed in order to know if any observed effect can be attributed to the experimental treatment or due to just random change. Any experiment should have sufficient statistical power to detect an effect that is biologically meaningful.

An example of low-level exposure studies that have often yielded positive results, but were not confirmed in replication attempts, is a study by D'Andrea et al. (1986b), which was designed to test previously reported low-level behavioral effects. They exposed rats intermittently to 0.5 mW/cm<sup>2</sup> 2450 MHz MWs for 90 d and reported

changes in time-related lever-pressing behavior. However, a replication experiment by DeWitt et al. (1987) reported different behavioral changes and failed to replicate the lever-pressing findings reported in the D'Andrea et al. (1986b) study. Interestingly, both of these experiments did not replicate the earlier behavioral changes following low-level MW exposures reported by Rudnev et al. (1983) and Shandala et al. (1979). D'Andrea et al. (2003b) concluded that these experiments were all below the threshold for reliable effects and could not be considered as established scientific evidence. However D'Andrea et al. (1986a) at 2.5 mW/cm<sup>2</sup> did show effects at a higher SAR although this study was never replicated. Other low-level MW exposure studies were reviewed in detail by D'Andrea et al. (2003b). Because these Russian low-level chronic exposure studies remain controversial and are not established as international scientific evidence, they have not served a role in health risk assessment and in safety standard setting for the international standard setting bodies.

The WHO EMF Project (1996; see also Veyret and Lagroye personal communication) in collaboration with ICNIRP and the IEEE have renewed efforts to evaluate the Russian literature and the Russian scientific approach for setting safety standards used in the FSU and allied European countries. As a result of these meetings, international scientists have translated and identified Russian BBB research of the Vinogradov group for replication. Because breaches of the BBB could lead to brain cell death and cognitive impairment, BBB studies are reviewed later in this chapter.

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## 4.2 Microwave Effects

### 4.2.1 Detection of Microwaves

Humans and laboratory animals have shown the capability to detect fairly low levels of MW exposure. There are two well-established thermal mechanisms that may convey detection of MWs by mammals. One is the auditory hearing effect. MW-induced auditory sensations are thought to be thermal events based on very small areas of heat-related tissue expansion created on the surface of the brain by pulsed MWs (Foster and Finch, 1974). The thermoelastic expansion, created by each MW pulse propagates as a small acoustic wave along the surface of the brain, couples to the skull and is thought to be conducted to the cochlea of the inner ear as sound, transmitted to the auditory cortex and heard as a click (Chou et al., 1982; Elder and Chou, 2003). At first, the reports that persons standing next to a radar antenna could hear the radar pulses drew skepticism (Airborne Instruments Laboratory, 1956). However, the first scientific study by Frey (1961) confirmed the MW-hearing effect when experimental subjects reported hearing "clicks" or "buzzing" during delivery of the microwave pulses. In the case of microwave hearing, biophysical mechanisms (Foster and Finch, 1974) have been confirmed to predict the experimental data (Airborne Instruments Laboratory, 1956; Frey, 1961; Chou et al., 1982; Elder and Chou, 2003) (see also Chapter 10 in *BBA*).

The second well-established thermal mechanism is the heating of tissues by MW exposure that can be detected by thermal receptors in the skin and elsewhere in the body and CNS. The identification of a family of transient receptor potential (TRP) ion channels that are gated by specific temperatures has been an important advance in the elucidation of the molecular mechanisms of thermosensitivity. Research has revealed a family of TRP proteins that sense heat and cold at the cellular level (Patapoutian et al., 2003). These proteins show sensitivity in the range of degrees. A warmth-sensitive

(~34°C) TRP channel is concentrated in skin cells, suggesting that the skin itself senses heat and passes the message to neurons. There is also literature to suggest that mammals have neurons sensitive to temperature changes of fractions of a degree in the brain stem and spinal cord along the walls of the 3–4th ventricles and spinal cord canal (Hensel, 1981). These CNS neurons may be implicated in the autonomic rapid response sweating to MW exposure (100 and 220 MHz) SAR above the guideline limits (Adair et al., 2003, 2005; Allen et al., 2003, 2005; see also Chapter 5 in this volume) when no whole body temperature changes are evident. Further research in this area is ongoing at Brooks Air Force Base. Molecular mechanisms of action associated with MW stimulation of sweating need to be investigated further (Patapoutian et al., 2003).

A variety of studies have shown that humans detect warming by microwave heating at fairly low levels. Adair and Black (2003) described several archival studies that have documented thresholds for detection of microwave warming (Vendrik and Vos, 1958; Hendler and Hardy, 1960; Hendler et al., 1963; Hendler, 1968). The duration of microwave heating in all of these studies was 10 s or less and involved exposure of an area of the forehead or forearm. For example when a small area (37 cm<sup>2</sup>) of forehead was exposed for 4 s, the mean absolute threshold of warmth varied across frequency. At 3 GHz this threshold was 33.5 mW/cm<sup>2</sup>, at 10 GHz it was 12.6 mW/cm<sup>2</sup>, and at frequencies for far infrared (IR) it was 4.2 mW/cm<sup>2</sup>.

Justesen et al. (1982) studied the detection on the forearm of IR and 2450 MHz. The authors used a 15-cm diameter aperture in a wall of microwave-absorbent material to limit the exposed area of skin. Using a double-staircase psychophysical method, the subjects were given 10-s exposures. Thresholds of 26.7 and 1.7 mW/cm<sup>2</sup> were documented for 2450 MHz and IR exposures, respectively. The authors noted that a high correlation within subjects between thresholds for detection of just-noticeable warming by IR and MW irradiation ( $r = 0.97$ ) allowed them to conclude that the same set of superficial thermoreceptors was stimulated.

A recent study by Blick et al. (1997) measured detection thresholds on the skin in the middle of the back of human subjects. They used long duration (10 s), large area (327 cm<sup>2</sup>) stimuli to minimize temporal or spatial summation. Frequencies of 2.45, 7.5, 10, 35, and 94 GHz as well as IR were tested. From 2.45 to 94 GHz, the detection thresholds decreased more than an order of magnitude. The 2450 MHz threshold (63.1 mW/cm<sup>2</sup>) and the IR detection threshold (5.34 mW/cm<sup>2</sup>) were roughly 2.5 times the thresholds found by Justesen et al. (1982). However, as Adair and Black (2003) pointed out, the total surface area of the exposures and duration of exposure as well as location of stimulation are important and easily account for the differences noted here.

Animal studies have also determined thresholds for microwave detection. A unique study by King et al. (1971) studied the detection of MWs by rats. They used the technique of conditioned suppression to determine the threshold of detection of 2450 MHz MWs by rats exposed in a multimode cavity. The rats were given dose rates of 0.5 to 6.4 W/kg. The authors described how several sources of artifactual cueing were controlled in this study and that MWs were reliably detected at dose rates as low as 0.6 W/kg. They pointed out that MWs lack the saliency of an auditory stimulus but were, nevertheless, readily detectable.

Ziriak et al. (1996) determined detection thresholds for 94 GHz MWs on the back of a nonhuman primate model (*Macaca mulatta*) and found a threshold of 7.7 mW/cm<sup>2</sup>. Likewise, D'Andrea et al. (1999) determined threshold detection for exposure of the face of rhesus monkeys at 94 GHz to be 8 mW/cm<sup>2</sup>. These are slightly higher than the power densities detected on the back of humans at 94 GHz (4.5 mW/cm<sup>2</sup>) by Blick et al. (1997) but as stated above procedural differences easily account for the discrepancies.

## 4.2.2 Microwave Performance Disruption

The studies of warmth detection by humans and responses of laboratory animals to similar exposures provide confidence that behavioral changes in animal studies are indeed thermally motivated. Thresholds to disrupt simple operant behaviors during acute whole body exposure were determined to be near  $\sim 4$  W/kg (de Lorge, 1976, 1979, 1983; D'Andrea et al. 1977; de Lorge and Ezell, 1980; see D'Andrea et al., 2003b). Several studies have shown that the disruption of ongoing behavior during acute MW exposure is generally associated with  $1^\circ\text{C}$  or greater increase of body temperature. This is a statistically reliable measure that is associated with whole body SARs in a narrow range between 3.2 and 8.4 W/kg, across differences in carrier frequency (225 MHz–5.8 GHz), species (rodents to rhesus monkeys), and exposure parameters (near and far field, CW and pulse-modulated). These changes in behavior are highly associated with the thermal increase produced by the MW exposures and have withstood the test of replication. Several of the studies cited above form an important part of the core database of scientific evidence from which safe whole body exposure standards have been derived (see IEEE, Std C95.1-2005). To date, however, none of the published MW research on performance disruption has been conducted on humans. To truly understand the nature of a MW hazard equivalent, behavioral experiments should be conducted on human volunteers. Such experiments would confirm or change the threshold hazard level of 4 W/kg currently used in safety standards.

## 4.2.3 Microwave Cognitive Effects: Animal Studies

### 4.2.3.1 Microwave Cognitive Performance Disruption in Animals

Nelson (1978) determined the effects of pulsed MWs on the learning of new behaviors by male squirrel monkeys in a repeated acquisition task. The estimated threshold whole body SAR range was 3.2–3.6 W/kg. The results of this experiment were compatible with the hypothesis that behavioral changes were directly related to hyperthermia in the monkey. D'Andrea et al. (2003b) operationally defined performance disruption as a significant rate change in behavior from a well-defined baseline. Work stoppage is simply the point at which the animal ceases performing the trained behavior for a predetermined time period. The performance disruption and work stoppage paradigms, described above, have provided a threshold (4 W/kg) for potential hazards of MW radiation absorption. However, cognitive components have been employed in many of the nonhuman primate studies. For example, the behavioral tasks used by de Lorge (1976) and D'Andrea et al. (1994) are vigilance tasks and involve attention processes by monkeys measured by simple reaction time or choice reaction time to either auditory or visual stimuli. In most cases reaction time was altered by MW exposures, which produced a whole body SAR of  $\sim 4$  W/kg and above.

D'Andrea (1999) postulated that performance of cognitively mediated tasks might be disrupted at levels of exposure lower than that required to cause performance disruption: "Unlike disruption of performance of a simple task, a disruption of cognitive functions could lead to profound errors in judgment due to alteration of perception, disruption of memory processes, attention, and/or learning ability, resulting in modified but not totally disrupted behavior. Any of these hypothesized deficits can compromise personnel operating in critical industrial or military occupations. Such deficits, which may be subclinical, can be amplified depending on the requirements of the occupation."

However, a "weight of evidence" approach indicates that the animal studies of cognitive performance disruption that have been conducted, to date, have not confirmed the implied concept of more "sensitive" to MW exposure than performance disruption



of a simple task (see D'Andrea, 1999). For example, Mickley et al. (1994) and Mickley and Cobb (1998) investigated memory deficits in rats exposed to MWs and determined that the threshold for memory effects was 10 W/kg. Similarly, Luttges (1980) evaluated the effects of MW exposure on learning and memory in mice and found an enhancement of performance (estimated whole body SAR 13 W/kg). The MW memory facilitation was found in both automated active avoidance testing and in single trial, passive avoidance tests. Beel (1983) repeated the study by Luttges (1980) and again found significant enhancement of learning and memory following 15 min exposure, both with five consecutive days of multiple trial, active avoidance training and with single trial, passive avoidance training.

Raslear et al. (1993) exposed rats to high peak power pulsed MWs produced by the TEMPO (Transformer Energized Megavolt Pulsed Output) virtual cathode oscillator at a whole body average SAR of 0.072 W/kg. The authors concluded that significant effects on cognitive function in rats were observed, particularly in the decision-making process. But in a subsequent experiment, Raslear and colleagues reported that the microwave pulse inhibition and enhancement of startle were similar to previously reported effects of sensory stimuli delivered at similar lead times, indicating the possibility that action was mediated by sensory stimulation (Seaman et al., 1994).

From the descriptions of the studies described above, an overall conclusion can be made that measures of cognitive-based performance disruptions in animals are not more sensitive to MW exposure (see D'Andrea, 1999) than simple performance tasks. However, the different cognitive and simple performance tasks, different exposure systems, modulation parameters, and differences in irradiation frequency make a more detailed conclusion difficult. This is true also because of the different test animals and exposure durations that make easy interpretation of this sparse literature difficult. However, there is support for this conclusion from basic neuroscience. The results are in line with the demonstrated conservation of the memory mechanisms across simple tasks and more complex tasks such as spatial memory (Kandel et al., 2000, p. 1265). This means that neurophysiologically simple and complex memory mechanisms are similar, and as such we would expect them to be similarly disrupted by the same rises in temperature induced by similar levels of SAR.

#### **4.2.4 Animals: Spatial Memory Replications and Confirmations**

##### **4.2.4.1 Introduction**

When considering the effects of MW exposure on spatial memory experiments in rats, it is relevant and essential to understand the established scientific evidence in the spatial memory field over the past three decades (e.g., O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978; Morris et al., 1982; Andersen and Moser, 1995; Kandel et al., 2000; Bast et al., 2005; O'Keefe and Burgess, 2005). The established scientific evidence in the spatial memory field includes the homology of spatial memory in the hippocampus of rats and humans, a common neural hippocampal component for spatial memory in various maze paradigms and activation of the same molecular mechanism for establishing spatial memory. There is a homology between the use of the hippocampus for spatial memory in rats in a maze and humans performing similar navigation tasks (Maguire et al., 2000, 2003a; Burgess et al., 2002). The importance of this homology is far-reaching and suggests a conservation of spatial memory across species (Kandel et al., 2000; Burgess et al., 2002). Thus there is strong support for the extrapolation of MW spatial memory results from animals to humans.

In animal spatial memory experiments, there are many versions of the maze experimental paradigm. Although the spatial paradigms and apparatus such as the T-maze,

**TABLE 4.1**

Effects of MW Exposure on Spatial Memory Performance in Animals

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
No overall effect for 20-min exposure but more errors each day in a 12-arm radial maze at 45-min exposure	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	20 min/d and 45 min/d exposure for 10 d	Lai et al. (1989)
Deficit in spatial working memory function reversed by pretreatment with physostigmine or naltrexone	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	45-min exposure	Lai et al. (1994)
These results show that acute exposure to pulsed MWs caused a deficit in the spatial "reference" memory in the rat	SD Rats	Avg. whole body 1.2 W/kg	2450 MHz	Pulsed width 2 $\mu$ s, 500 pps	Avg. power density 2 mW/cm <sup>2</sup>	For 1 h in a circular waveguide system	Wang and Lai (2000)
Low-level exposure to pulsed 900 MHz MW radiation does not cause deficits in the performance of a spatial learning task in mice	Male mice C57BL/6J	Whole body 0.05 W/kg	900 MHz	Pulsed at 217 Hz		45 min/d for 10 d	Sienkiewicz et al. (2000)
No deficits in spatial learning after "head-only" exposure of rats to GSM electromagnetic fields in the two spatial learning tasks	SD Rats	Head only 1 or 3.5 W/kg	900 MHz EMF	GSM modulation at 217 Hz, 1/8 duty factor		45 min over 10-14 d	Dubreuil et al. (2002)
Head-only exposure to GSM 900 MHz electromagnetic fields does not alter rat's memory in spatial and nonspatial tasks	SD Rats	Head only 1 and 3.5 W/kg	900 MHz EMF	GSM modulation at 217 Hz, 1/8 duty factor		45 min	Dubreuil et al. (2003a)
No significant effects on memory performance were found due to RF, avg. SAR within the brain was 7.4 W/kg and the whole body average SAR was 1.4 W/kg	SD Rats	1: Brain (B) 7.4 W/kg and whole body (WB) 1.4 W/kg, 2: Brain 25 W/kg and WB 4.5 W/kg	1439 MHz	PDC (Japan)		I: 1 h/day for 4 d; II: 45 min/day for 4 d	Yamaguchi et al. (2003)

*continued*

**TABLE 4.1 (continued)**

Effects of MW Exposure on Spatial Memory Performance in Animals

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
The results included no significant exposure effect, no significant drug effect, and no significant interactions between those two factors	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	45 min/d for 10 d	Cobb et al. (2004) (Lai et al., 1994 replication)
Response of MWs and temporally incoherent 60 mG on spatial learning in a water maze was similar to that of the sham-exposed animals	SD Rats	Avg. whole body 1.2 W/kg	2450 MHz	CW circular polarized	2 mW/cm <sup>2</sup>	1 h CW; 1 h CW + 60 mG	Lai (2004)
Whole body exposure to 2.45 GHz electromagnetic fields does not alter radial maze performance in rats.	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	45 min	Cassel et al. (2004) (Lai et al., 1994 replication)
Whole body exposure to 2.45 GHz does not alter anxiety responses in rats: a plus maze study including test validation	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	45 min before testing	Cosquer et al. (2005a)
Whole body exposure to 2.45 GHz electromagnetic fields does not alter 12-arm radial maze with reduced access to spatial cues in rats	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 $\mu$ s pulses, 500 pps	1 mW/cm <sup>2</sup> but very high peak power	45 min before testing	Cosquer et al. (2005b)
MW exposure had no effects on learning in a radial arm maze.	Adult male mice C57BL/6J mice	1. Whole Body 0.1, 0.6 or 3 W/kg 2. headmainly exposure 0.1, 0.4 or 2.2 W/kg	1. 900 MHz	1. GSM talk		1.1 h/d over 15 days	Jones et al., 2005

radial arm maze, and water maze differ, spatial learning in these various maze paradigms requires a common hippocampal memory component since hippocampal lesions impair spatial learning in all types of mazes (Morris et al., 1982; Gray and McNaughton, 1983).

In the field of spatial memory, the underlying established molecular mechanism of spatial memory has been demonstrated to be located in the CA1 region of the hippocampus and requires glutamate neurotransmission in the various spatial memory maze paradigms (Morris et al., 1982; Gray and McNaughton, 1983; Kandel et al., 2000; Maguire et al., 2000, 2003a,b; Burgess et al., 2002). Because spatial memory confirmation and replication paradigms address the same molecular mechanism in the hippocampus their results are comparable. It is well established that spatial memory requires glutamate neurotransmission in Schaffer collateral fibers released to glutamate\* receptors of CA1 pyramidal cells of the hippocampus. Long-term potentiation in the CA1 cells results in synapse alteration and spatial memory and learning. Even though brain along with body temperatures fluctuates 2–3°C in normal physiological conditions, spatial memory is quite robust and occurs at brain temperatures between 30 and 39°C in rats (Andersen and Moser, 1995).

Research over the years on Alzheimer's disease, which is associated with memory loss and destruction of acetylcholine (ACh) neurons in the basal forebrain, has focused on attempts to find the association between cholinergic systems and spatial memory. ACh neurotransmission has not been established as a mechanism for spatial memory storage. There is no evidence that new synapses have formed on ACh neurons during spatial learning (Hoh et al., 2003). One hypothesis is that place (spatial) learning occurs in the hippocampus through glutamate release and response learning occurs later through ACh release from the striatum and may supersede place learning (Chang and Gold, 2003). Hippocampal ACh is derived entirely from projections of cholinergic neurons of the diagonal band and medial septum (Woolf and Butcher, 1981; Woolf, 1991; Calabresi et al., 2000). ACh neurotransmission may modulate the use of spatial memory possibly through the perforant fiber pathway in the dentate region of the ventral hippocampus (Levin, 1988; Levin et al., 2003, 2005; Dong et al., 2005). It is important to keep in mind that this key established evidence on spatial memory when reviewing the microwave literature on spatial memory.

#### 4.2.5 Microwave Spatial Memory Experiments

Effects of MW exposure on spatial memory have been investigated in animal replication and confirmation experiments in six laboratories ((i) Lai et al., 1989, 1994; Wang and Lai, 2000; Lai, 2004; (ii) Sienkiewicz et al., 2000; Jones et al., 2005; (iii) Dubreuil et al., 2002, 2003a,b; (iv) Yamaguchi et al., 2003; (v) Cobb et al., 2004; (vi) Cassel et al., 2004; Cosquer et al., 2005a–c; [see also Table 4.1](#)). These experiments were undertaken to establish scientific evidence on the effects of MW exposure on spatial memory.

It is notable that the Lai et al. (1989, 1994), Cobb et al. (2004), Cassel et al. (2004), and Cosquer et al. (2005b,c) groups used a 12-arm radial maze and Dubreuil et al. (2002) and Sienkiewicz et al. (2000) used an eight arm radial maze. Glassman (1999) pointed seven as the number of different digits or radial arms, etc., we can normally hold in short-term memory (Miller, 1956). Eight is within this region. However, the 12-arm radial maze suggests a memory task beyond the “normal” short-term retention range. Maguire et al. (2003b) confirmed this when they investigated the superior and

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\*The glutamate receptors are identified as NMDA (N-methyl-D-Aspartate) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [see reviews by Kandel et al., 2000, Chapters 62–63; Day et al., 2003; Bast et al., 2005].

normal mnemonic memory of humans. However, they found the superior memory persons used a spatial learning strategy that also employed the hippocampus. This reaffirms the scientific evidence that performance in the 12-arm radial maze requires the same underlying spatial memory molecular mechanism in the hippocampus as performance in the eight arm radial maze (see Section 4.2.4.1).

The MW dosimetry of the experiments of Lai et al. (1989, 1994), Cobb et al. (2004), Cassel et al. (2004), and Cosquer et al. (2005a,b) are similar. They used the circular waveguide (Guy et al., 1979) to expose the whole body of rats to MWs at 0.6 W/kg. The circular waveguide (Guy et al., 1979) was designed initially for experiments on animals in the MW oven frequency range (2450 MHz) with whole body exposures like those in MW ovens. The exposure is from two sources of “circularly polarized, guided waves that provide a relatively constant and easily quantifiable coupling of microwave energy to each animal, regardless of their position, posture or movement” (Guy et al., 1979). For the same average incident power density, the average SARs in the heads of these rats were about two times higher in the circular waveguide ( $0.6 \text{ W/kg} \times 2$ ) than for other exposures, such as in an anechoic chamber (Chou et al., 1985). In the circular waveguide, Guy et al. (1979) and Cassel et al. (2004) have estimated the brain average SAR to be  $0.8 \text{ W/kg} \pm 4 \text{ dB}$  when the whole body SAR is  $0.6 \text{ W/kg}$  in rats of 250–300g. Wang and Lai (2000) and Lai (2004) also used the circular waveguide (Guy et al., 1979) to expose the whole body of rats to MWs, but used a whole body SAR of  $1.2 \text{ W/kg}$ . As well, Cosquer et al. (2005c) used the same exposure chamber but used a whole body SAR of  $2 \text{ W/kg}$ .

This circularly polarized wave, dual source exposure used in the experiments above is in contrast to the plane wave exposure from a single source, typical of the mobile telecommunications industry, used to expose animals in the other spatial memory experiments ((i) Sienkiewicz et al., 2000 (GSM 900 MHz average whole body SAR of  $0.05 \text{ W/kg}$ ); Jones et al., 2005 (900 MHz GSM whole body exposure SARs of 0.1, 0.6, or  $3 \text{ W/kg}$ , or exposure mainly of the head, local SARs of 0.1, 0.4, or  $2.2 \text{ W/kg}$ ); (ii) Dubreuil et al., 2002, 2003a (GSM 900 MHz, head only exposure, SAR 1 and  $3.5 \text{ W/kg}$ ); (iii) Yamaguchi et al., 2003 (pulsed 1439 MHz time division multiple access (TDMA) brain average SAR  $7.5 \text{ W/kg}$  or  $25 \text{ W/kg}$ )).

The MW exposure experiments on spatial memory of Lai and colleagues over the years have used two maze paradigms, the 12-arm radial maze (Lai et al., 1989, 1994), and more recently the water maze (Wang and Lai 2000; Lai, 2004) and unlike the other five laboratories, they have consistently reported positive results. Lai et al. (1989, 1994) investigated the effects of MW exposure (circular waveguide paradigm, 2450 MHz, 2 s pulses, 500 pulse per second (pps), whole body average SAR  $0.6 \text{ W/kg}$ ) on learning in the 12-arm radial arm maze. Rats were trained in the maze to obtain food reinforcements immediately after 20 or 45 min of MW exposure. Lai et al. (1994) did not report on the blindedness of their procedures. Exposure to MWs for 20 min prior to training had no significant main effect on maze learning but affected the shape of the learning curve, whereas 45-min exposure significantly retarded the performance of the rats. Wang and Lai (2000) and Lai (2004) found that acute exposure to pulsed 2450 MHz MWs affected water maze performance of rats. Lai et al. (1989, 1994) and Wang and Lai (2002) maze studies have been reported to have statistical problems and the exposed and sham animals exhibited no apparent interaction between testing days and treatment (IEGMP, 2000; Sienkiewicz, 2002; Dubreuil et al., 2002).

Cobb et al. (2004) attempted to replicate the results of Lai et al. (1994) that showed a working memory deficit in rats exposed to 2450 MHz pulsed MW fields (2 ms, 500 pps, whole body average SAR of  $0.6 \text{ W/kg}$ ) and also attempted that this deficit was reversed by a pretreatment with physostigmine, an acetylcholinesterase inhibitor, or with naltrexone hydrochloride, an opioid antagonist that acts on both CNS and peripheral receptors, but

not with the peripheral opioid antagonist, naloxone methiodide. Cobb et al. (2004) study was conducted with a double blind procedure. Cobb's analyses of error rates revealed no significant exposure effect, no significant drug effect, and no significant interaction between the two main factors. There was a significant difference in test days, as expected, with repeated test-trial days, which indicates that learning was accomplished. Cobb et al. (2004) failed to replicate the work of Lai et al. (1994). They concluded that there is no evidence from their current study that exposure to MWs under parameters examined caused decrements in the ability of rats to learn the spatial memory task.

Replication studies by Cassel et al. (2004) and Cosquer et al. (2005b) also failed to confirm the MW exposure effects of Lai et al. (1994) on spatial learning in the 12-arm radial maze. Cosquer et al. (2005a) reported that similar MW exposure did not alter anxiety responses assessed in the elevated plus maze. These elevated plus maze, anxiety responses are hypothesized to be associated with changes in benzodiazepine receptors (Lai et al., 1992: "benzodiazepine receptors in the brain are responsive to anxiety and stress"). Cosquer et al. (2005a) failed to confirm the work of Lai et al. (1992) about 2450 MHz exposure altering anxiety and the hypothetically associated benzodiazepine receptors in the rat brain.

Lai (2005a) has written a letter suggesting that the rats of Cobb et al. (2004) overlearned the spatial memory task, while his rats did not, and thus she failed to replicate his work. He also suggested that there was a "typo" in his 1994 paper and that his dose of physostigmine was 0.1 mg/kg, i.p. instead of 1 mg/kg i.p. as reported in 1994, and as such Cobb et al. (2004) who used 1 mg/kg did not replicate his work. Lai (2005a) has theorized that his reported microwave-induced learning deficit was caused by a decrease in cholinergic functions in the brain. Jauchem (2005) and Cassel (2005) have replied to Lai's (2005a) comments and Lai (2005b) has replied to them. Cassel (2005) presented a graphed comparison of the radial maze performance curves of Cobb et al. (2004) and Lai et al. (1994), showing parallel trends, especially after the third day; and Cassel also pointed out on the last day Cobb et al.'s (2004) rats' mean errors approached 2, whereas Lai et al.'s (1994) rats' mean errors were less than 0.5. Lai (2005b) continues to contend that no one has replicated his results because both Cobb and Cassel gave the rats more chance to learn the maze than he did. Lai (2005b) does concede that Cassel et al. (2004) replicated Cobb et al.'s (2004) results.

There is no confirmatory (see below) or replicated evidence to support Lai and his colleagues' papers (1989, 1994, 2000, 2004), suggesting MW exposure effects on spatial memory. Although Lai (2005a,b) has indicated that other experiments were not exact duplications, his contentions are dismissed by Cassel (2005). Indeed if we refer to the scientific evidence (see Section 4.2.4.1) we know that no matter what the spatial memory paradigm, spatial memory always involves glutamate neurotransmission in the CA1 pyramidal cells of the hippocampus. Therefore slight differences in training procedure in the 12-arm radial maze would not make a difference to the location in the brain of spatial memory or the molecular mechanism forming spatial memory and thus, the possible effects of MW exposure on spatial memory. Furthermore, there is no established neuroanatomical location or molecular mechanism for Lai's cholinergic hypothesis for his MW-induced spatial memory deficit (see Section 4.2.4.1).

As for Lai's (2005b) contention that there may be task or MW-related genetic differences within the substrains of the Sprague-Dawley rat species he used (1994) and those used by Cobb et al. (2004) and Cassel et al. (2004), this seems unlikely since spatial memory occurred in all three experiments in the rats during control conditions. Also the groups of rats of both Cobb et al. (2004) and Lai et al. (1994) show a parallel trend in the graphed curves of their radial maze performance for both control and MW conditions (Cassel, 2005). The lack of a MW effect on spatial memory is replicated or confirmed in

possibly five different sources of Sprague-Dawley rat subspecies ( (i) Dubreuil et al., 2002: Charles River Laboratories, IFFA, Credo, France; (ii) Yamaguchi et al., 2003: Charles River, Yokohama, Japan; (iii) Cassel et al., 2004: R. Janvier-St. Berthevin, France; (iv) Cobb et al., 2004: Charles River Laboratories, Portage, MI; (v) Cosquer et al., 2005a–c: R. Janvier Le Genest St. Isle, France). Perhaps the MW positive results of Lai and his colleagues have to do with undisclosed methodological shortfalls or those suggested previously (IEGMP, 2000; Dubreuil et al., 2002; Jauchem, 2005).

There were three groups that investigated spatial memory using plane wave exposures. Sienkiewicz et al. (2000) failed to find any memory deficits in mice repeatedly exposed to 900 MHz fields in a GHz Transverse electromagnetic (TEM) cell. This may be due to the fact that the average whole body SAR was low (0.05 W/kg). Dubreuil et al. (2002) found no significant spatial memory difference between MW- and sham-irradiated rats that were exposed at relatively high local SARs of 1 and 3.5 W/kg. They exposed the heads of the rats in a TEM cell at 900 MHz.

Yamaguchi et al. (2003) reported that pulsed TDMA fields (1439 MHz, brain average 7.5 and 25 W/kg) affected performance of rats in a T-maze task only when body temperature was elevated (at 25 W/kg). Sprague-Dawley rats were exposed for either 1 h daily for 4 d or for 4 weeks to a pulsed 1439 MHz TDMA field in a carousel-type exposure system. When the brain, average SAR was 7.5 W/kg, the whole body average SAR was 1.7 W/kg. Other subjects were exposed at the brain average SAR of 25 W/kg with the whole body average SAR of 5.7 W/kg for 45 min daily for 4 d. Learning and memory were evaluated by reversal learning in a food-rewarded T-maze, in which rats learned the location of food (right or left) by using environmental cues. The animals exposed to MW with the brain average SAR of 25 W/kg for 4 d showed statistically significant decreases in the transition in number of correct choices in the reversal task, compared to sham-exposed or cage control animals. However, rats exposed at the brain average SAR of 7.5 W/kg for either 4 d or for 4 weeks showed no T-maze performance impairments. Intraperitoneal temperatures, as measured by a fiber optic thermometer, increased in the rats exposed to the brain average SAR of 25 W/kg but remained the same for the brain average SAR of 7.5 W/kg. These results suggest that the exposure to a TDMA field at levels about four times stronger than emitted by cellular phones (2 W/kg limit) does not affect the learning and memory processes when there is no whole body temperature rise.

#### **4.2.6 Conclusions: Animals: Spatial Memory Replications and Confirmations**

The weight of evidence from the six research groups using two species of mammals (Sprague-Dawley rats and C57BL/6J mice) is that there is no effect on spatial learning as tested by six spatial learning paradigms at whole body or local (head) MW exposures within guideline limits and also well above the whole body and partial body limits, when whole body temperature did not measurably rise. Five groups ( (i) Sienkiewicz et al., 2000; Jones et al., 2005; (ii) Dubreuil et al., 2002, 2003a,b; (iii) Yamaguchi et al., 2003; (iv) Cobb et al., 2004; (v) Cassel et al., 2004; Cosquer et al., 2005a–c), using spatial memory animal experiments confirmed or replicated each others' results indicating that MW exposures at frequencies from 900 to 2450 MHz had no effect on spatial memory. The positive results of Lai and colleagues (Lai et al., 1989, 1994; Wang and Lai, 2000; Lai, 2004) are not supported by the weight of scientific evidence. Sienkiewicz's group (Jones et al., 2005) have recently reconfirmed and extended their results on spatial memory in mice exposed to MWs lending further support to this weight of scientific evidence conclusion.

Extensive neurophysiological and neuroanatomical research (see Kandel et al., 2000) has established that the critical neurotransmission for spatial memory to occur is glutamate binding to glutamate receptors of pyramidal cells in the CA1 region of the

hippocampus. Newer research in the literature continues to reconfirm this (Maguire et al., 2000, 2003a,b; Burgess et al., 2002; Bast et al., 2005).

If the strong evidence for homology of memory is accepted at this time, there does not seem to be any further requirement for studies in this area in other strains and species of animals and possibly in man (Kandel et al., 2000; Burgess et al., 2002; Maguire et al., 2000, 2003a,b). However, it is generally preferred to have confirmatory MW exposure, spatial memory experiments in humans for health risk assessment. Thus, we recommend testing spatial memory in humans during MW exposure. Human cognitive studies are reviewed in the following section.

In conclusion, MW exposure appears to disrupt spatial memory in rats at a dose at or above the whole body threshold of 4 W/kg in a fashion similar to simple tasks of perception. This is in line with the mechanism of whole body temperature increases of 1°C or higher.

#### **4.2.7 Microwave Cognitive Effects: Human Studies (Table 4.2)**

In recent years, the mobile phone has become extremely popular and is widely used by about 1.4 billion members of society. This rapid increase in use of a relatively new technology has resulted in concerns over alleged effects because of exposure of the head to MW radiation from the phone antenna. During the period 1999–2005, a variety of studies have been done, both to measure the dose rate to the head and to evaluate the effects of mobile phone irradiation on cognitive processes in humans (see Table 4.2) (Preece et al., 1999, 2005; Van Leeuwen et al., 1999; Koivisto et al., 2000a,b; Krause et al., 2000a,b, 2004; Lee et al., 2001, 2003; Croft et al., 2002; Edelstyn and Oldershaw, 2002; Hamblin and Wood, 2002; D'Andrea et al., 2003b; Haarala et al., 2003b, 2004, 2005; Curcio et al., 2004; Maier et al., 2004; Hamblin et al., 2004; Besset et al., 2005). The studies that examine human memory processes and mobile phone usage appear to show no established evidence of memory deficits. Rather, some results have been reported of memory and attention facilitation. Preece et al. (1999) reported an improvement in reaction time of humans during exposure to cell phone radiation at 915 MHz while performing a “working” memory task. This finding could be viewed as a cognitive enhancement. However Preece et al. (2005) have failed to replicate this work in 18 children of 10–12 y of age.

A Finnish group has performed a number of experiments testing human cognitive effects. Koivisto et al. (2000a,b) reported that exposure to GSM 902 MHz radio frequency energy emitted by cellular telephones was associated with a facilitation of brain functioning during memory tasks, including faster response times, thus suggesting an improvement in cognitive function. Haarala et al. (2003b, 2004) recently reported a failure to replicate the effects (Koivisto et al., 2000a,b) of GSM 902 MHz EMF emitted by cellular telephones on response times and short-term memory in humans, using double blind conditions in two independent laboratories. Indeed Haarala et al. (2005) have done a similar double blind study on adolescents (32 children: 16 boys, 16 girls) of 10–14 y old (mean 12.1 y, SD 1.1) and found no effect of MW exposure on cognition.

Krause et al. (2004) reported that exposure to similar fields did not alter resting EEG in humans, but caused a decrease in cortical activity in human subjects performing auditory memory tasks. One interpretation of this finding is that more efficient brain function was attained with less effort. Krause et al. (2000b) found that such exposure altered event-related desynchronization and synchronization responses of all EEG frequency bands studied during the performance of a visual working memory task. The biological significance of this finding, if any, was not stated. But recently, the term “desynchronized” for the activated states of waking and REM has been rendered obsolete by the discovery of



**TABLE 4.2**

## Effects of MW Exposure on Human Cognition

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration of Exposure	Ref.
They calculated a maximum rise in brain temperature of 0.11°C for an antenna with an average emitted power of 0.25 W, the maximum value in common mobile phones, and indefinite exposure	Human bioheat head model	Max SAR 0.91 W/kg for 10 g	916 MHz		0.25 W	Indefinite exposure	Van Leeuwen et al. (1999)
There was evidence of a significant increase with the analog but not with the digital simulation, in choice reaction time only. No effects on memory	Humans		915 MHz and CW	217 Hz pulsed 12.5% duty cycle	1 W mean power	25–30 min	Preece et al. (1999)
No effect of 902 MHz mobile phone transmission on cognitive function in children. Double blind	18 children 9 m., 9 f.; 10.2–12.2 y	Estimate worst case 0.28 W/kg	902 MHz GSM Nokia 3110	217 Hz pulse width 577 μs	X: 0 W, Y: 0.2 W peak (0.025 W mean), Z: 2 W peak (0.25 W mean)	30–35 min over 2 out of 3 d, (Y,Z) left side	Preece et al. (2005)
No effect on nine tasks and 11 comparisons choice reaction tasks. Facilitatory effect on tasks requiring attention and manipulation of information in working memory. Single blind	48: 24 m., 24 f.; 18–49 y, avg. 26 y, R. handed		GSM 902 MHz, Nokia 6110	217 Hz pulse width 577 μs	0.25 W mean power	About 1 h/d 2 d left ear	Koivisto et al. (2000a)
Memory load was varied from 0 to 3 items in an n-back test. Significantly speeded up response times on three items (on increased memory load). Single blind	Humans	0.683 W/kg avg. over 10 g	GSM 902 MHz, Nokia 6110	217 Hz pulse width 577 μs	0.25 W mean power	30 min left ear	Koivisto et al. (2000b) (SAR measurement for Koivisto et al., 2000b done in replication Haarala et al., 2004)

902 MHz mobile phone exposure had no effect on performance of nine human cognitive tasks: Failed replication of Koivisto et al. (2000a) in Finland and Sweden double blind	Humans: 32 m., 32 f.	0.88 W/kg avg. over 10 g	GSM 902 MHz, Nokia 6110	217 Hz pulse width 577 $\mu$ s	0.25 W mean power	65 min left ear	Haarala et al. (2003b)
A PET study showed no rCBF changes were in the area of maximum 902 MHz mobile phone SAR. During scanning, the subjects performed a visual working memory task	14 R. handed healthy males		GSM 902 MHz, Nokia 6110	217 Hz pulse width 577 $\mu$ s	0.25 W mean power		Haarala et al. (2003b)
902 MHz mobile phone does not affect short-term memory in humans. Failed replication in Finland and Sweden: Koivisto (2000b). Double blind	64: 32 m., 32 f.; 20–42 y, Finland avg. 24.19 y. Sweden avg. 29.34 y	0.99 W/kg avg. 10 g	GSM 902 MHz, Nokia 6110	GSM	0.25 W mean power	Mean duration 65 min/d over 2 d left ear	Haarala et al. (2004)
No effect of 902 MHz mobile phone transmission on eight cognitive function tests in children. Double blind. Temperature of face skin was same with and without MW transmission. No subject could detect if the phone was on or off above chance	32 children 16 m., 16 f.; 10–14 y; R. handed; mean 12.1 y	Mean 0.99 W/kg over 10 g, Peak 2.07 W/ kg	GSM 902 MHz	217 Hz pulse width 577 $\mu$ s	0.25 W mean power	60 min over 2 d; tested same time each day. Left side	Haarala et al. (2005)
On three measures of attention mobile phone users performed significantly better than controls on one test (trail making)	72 bright students 16 y		Mobiles Hong Kong	GSM		Median 3712.5 min of use	Lee et al. (2001)
The differential effect of the duration of exposure to the electromagnetic field emitted by mobile phones was on only one of two tests of human attention	78 univ. students R. handed		Mobiles Hong Kong	GSM		30 min	Lee et al. (2003)

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*continued*

**TABLE 4.2 (continued)**

## Effects of MW Exposure on Human Cognition

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration of Exposure	Ref.
Significant effects were found for digit span forward, spatial span, and serial subtraction tasks, three of six tests for exposed versus unexposed conditions	38 humans 21 y avg.		900 MHz			30 min left ear	Edelstyn and Oldershaw (2002)
Modulated 450 MHz decreased variance in errors in two visual memory tasks and increased error in two others	100: 37 f., 63 m.		450 MHz	7 Hz	0.158 W/cm <sup>2</sup>	10–20 min	Lass et al. (2002)
Mobile phone use facilitates word recall memory in male, but not female, subjects only in the short term. Single blind	62: R. handed 33 m., 29 f.; avg. 26.5 y, 18–53 y	0.79 W/kg	Ericcson A2618s 1800 MHz			Left ear 15 min	Smythe and Costall (2003)
A pilot study on pulsed field interference with auditory discrimination task. Single blind	11 m., 11 f; 23– 48 y, 39.1 avg.		GSM 902 MHz Motorola 920	Pulsed at 217 Hz pulse width 577 μs	1 mW/cm <sup>2</sup>	50-min exposure then testing	Maier et al. (2004)
Effect of time course (25–30 min) of GSM 902.40 exposure on repeated simple and choice reaction times and increase of local tympanic temperature. No effect on speed and accuracy measures, visual search task, and descending subtraction task. Double blind	20: 10 m., 10 f.; 22–31 y; mean 26.4 y, R. handed	Max 0.5 W/kg	GSM 902.40 MHz Motorola Timeport 260	Pulsed at 217 Hz pulse width 577 μs	Max power 1 W; avg. power 0.25 W/cm <sup>2</sup>	45 min left ear	Curcio et al. (2004)
No effect on cognitive function from daily mobile phone use tested after 13-h rest periods. Double blind	55: 27 m., 28 f.; avg. 24.3 y, 18–40 y	0.54 W/kg	GSM 900 MHz	217 Hz pulse width 577 μs		2 h/day over 5 d/ week, total 27 d (preferred hand/side)	Besset et al. (2005)
No effects of mobile phone emitted EMFS on human event-related potentials and performance	120 volunteers		895 MHz	217 Hz, pulse width 577 μs	250 mW	N100, P300	Hamblin et al. (2005)

highly synchronized gamma frequency (30–80 Hz) activity in these states (Hobson and Pace-Schott, 2002). Krause et al. (2004) have recently failed to replicate their 2000 study. All eight of the significant changes in the earlier study were not significant in the present double blind replication.

Among other groups that have studied this problem, Edelstyn and Oldershaw (2002) found that human volunteers exposed to the fields of GSM mobile phones showed improvement in the performance of three cognitive function tests: (i) immediate verbal memory capacity, (ii) immediate visual spatial working memory capacity, and (iii) sustained attention. Lee et al. (2001) performed three measures of attention to compare responses of mobile phone users (Hong Kong, PCS 1900 MHz) with those of nonusers. Mobile phone users performed better on one of the measures, a “trail making test.” The authors noted that the results could have been confounded by self-selection. Lee et al. (2003) repeated their previous results of enhanced attention following mobile phone exposure and discovered that duration of the exposure may be important for the development of differential effects on attention processes.

Curcio et al. (2004) reported an improvement of both simple and choice reaction times that was accompanied by an increased temperature of the area irradiated during exposure from a GSM mobile phone. They reported a positive relationship with duration of MW exposure and changes in temperature and reaction time performance. They stated that a minimum of 25 min of EMF exposure is needed to show appreciable changes. These studies (Lee et al., 2001, 2003), however, did not observe increased error rates on the attention and cognitive tests. Also, increases in the temperature of the head during exposure (Curcio et al., 2004) may be largely due to the local insulation of the head by the physical proximity of the phone, rather than the MWs emitted from the phone. This confounding heat needs to be controlled for in further research (Van Leeuwen et al., 1999; Straume et al., 2005).

Besset et al. (2005) investigated the effects of daily exposure to GSM 900 MWs on cognitive function in a double-blinded design. Fifty-five subjects (27 males and 28 females, 18–33 y) were divided into two groups: a group with mobile phones switched on and a group with them switched off. The groups were matched according to age, gender, and IQ. Over 45 d, there were three testing periods: baseline (3 d), exposure (28 d), and recovery (14 d). Subjects were exposed or sham-exposed for 2 h/d, 5 d/week. The neuropsychological test battery composed of 22 tasks screened four cognitive categories: information processing, attention capacity, memory function, and executive function. For the most part, these 22 tasks are well-validated in the scientific literature. This neuropsychological battery was performed four times, once during baseline, twice during exposure, and once during recovery. Their results indicate that daily mobile phone (MP) use has no effect on cognitive function after a 13-h rest period.

Maier et al. (2004) in their pilot study found an increase in discrimination time on an auditory discrimination task after 50 min of pulsed GSM fields (902 MHz) exposure from a programmable mobile phone as compared to sham exposure. Maier used an auditory discrimination task based on the auditory order threshold (OT). They defined OT as the minimum time needed (a) to recognize that two auditory stimuli are presented just separately and (b) to decide without error on what side the first of both stimuli was presented. Out of 11 MW-exposed subjects, two showed shorter discrimination time, and nine showed an increase in discrimination time. There was no indication of the normal variation in discrimination time and no clear verification of the reliability and validity of the measurement. No other laboratory has reported MW exposure results using this paradigm.

Hamblin et al. (2004) studied auditory event-related potentials (ERP) during an auditory task performed during 1 h of exposure to a GSM mobile phone (SAR at the head 0.87 W/kg). In this pilot study with 12 subjects (eight males and four females, 19–44 y), they found reduced amplitudes and latency for the N100 component (stimulus detection) and only delayed latency for the P300 component (thought to represent cognitive processes). Differences during the auditory task were found at cortical sites closest to the active mobile phone. The authors questioned that whether this could be due to stronger MW at those sites or whether these sites were the most MW sensitive to the performance of the task. The authors reported that reaction times were significantly longer for the exposed condition than for sham. These preliminary results suggest that exposure may affect neural activity. The ERP literature was previously reviewed (D'Andrea et al., 2003b), leaving doubt as to the validity of the various ERP experimental paradigms used. That conclusion still holds in relation to the Hamblin pilot paper (Hamblin et al., 2004). Hamblin et al. (2006) at a conference presented a large, double blind, replication study of 120 subjects and concluded that no MW effects on ERP were observed. Any effects they saw were reported as weak and were dismissed as chance. Hamblin et al.'s (2006) study appears to have been conducted with improved methodology; and perhaps firm conclusions about ERP effects, at the low SARs produced by mobile phones, may now be drawn.

The studies that examine human cognitive processes and mobile phone usage appear to show no established evidence of memory deficits. Replication studies with standardized protocols, larger samples (multicentered replications; Haarala et al., 2003b), better experimental controls, double blind conditions, and Bonferroni or other statistical corrections for multiple comparisons appear to have eliminated the “false positives.”

#### **4.2.8 Microwave Effects on the Electroencephalogram**

Included in cognitive effects are the EEG and BBB studies that were previously reviewed by D'Andrea et al. (2003b). The reviews by D'Andrea et al. (2003a,b) concluded that the evidence for mobile phone effects on human cognitive performance was very weak. And they concluded that until more studies could be conducted with improved methodology and standardized protocols, firm conclusions about cognitive effects at the low SARs produced by mobile phones could not be drawn. Since D'Andrea et al.'s (2003a,b) review of EEG publications (see [Table 4.3](#)), there are a few new studies of note including Huber et al. (2003, 2005), showing effects of pulsed signals on EEG, and Hinrikus et al. (2004), reporting the normal EEG is too variable to be able to measure possible small MW effects.

The Borbély, Huber, and Ackerman group papers (Borbély 1999; Huber et al., 2000, 2002, 2003, 2005; Achermann et al., 2005; see [Table 4.3](#)) on the effects of MW exposure on nighttime and daytime sleep EEG, heart rate, and positron emission tomography (PET) scans are very difficult to follow because they publish preliminary studies (1999, 2000, 2002), followed years later by further analysis and reanalysis of previous results without addressing discontinuities in methodological details, and in the results. The preliminary sleep EEG studies in 1999 (Borbély et al., 1999) on the effects on EEG (and electrocardiogram) of subjects' nighttime sleep episode due to an intermittent exposure schedule (by three antennas, 900 MHz; SAR 1 W/kg consisting of alternating 15-min on and 15-min off intervals) and in 2000 (Huber et al., 2000) on the effects on EEG of a unilateral 900 MHz GSM exposure for 30 min prior to 3 h of daytime sleep, were reanalyzed in the data given by Huber et al. (2003), finding MW effects on different sleep bands after reanalysis. Borbély et al. (1999) reported effects on nonrapid eye movement (NREM) episodes 1–3, but Huber et al. (2003) reported only effects on the first NREM episode (see [Table 4.3](#)).

**TABLE 4.3****Effects of MW Exposure on Electroencephalography (EEG)**

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
Increase number of spindle shape activity firings and slow waves; enhance evoked responses in the visual cortex neurons to single light flashes	Rabbits (unanesthetized)		2400	CW	40	1 min	Chizhenkova et al. (1988)
Significant effects in the power spectral analysis of EEG frequency, but not at the same frequency	Rats	2.7	2450	CW	10	7 h	Mitchell et al. (1989)
Significant elevations of EEG asymmetry in 10–14 Hz range during the first 20 s	8 Rats		945 MHz	(AM-4 Hz) 2 ms pulse duration	0.1–0.2 mW/cm <sup>2</sup> antenna 28–30 cm above rat	1-min on–1-min off alternating for 10 min	Vorobyov et al. (1997)
Increase total power of spectra for (ii) 30 mW/cm <sup>2</sup> condition only	40 Rats	WB	2450 CW	CW	(i) 10 (ii) 30	10 min	Thuroczy et al., 1994
Increase in the power of the delta waves (thermal)	40 Rats	42 mW/g	4000 CW	AM 16 Hz		30 min	Thuroczy et al., 1994
Increase in the power of beta waves	40 Rats	8.4 mW/g	4000	AM 16 Hz		30 min	Thuroczy et al., 1994
Increase in cerebral blood flow	40 Rats	8.4 mW/g	4000 CW	CW		30 min	Thuroczy et al., 1994
Magnetic fields mimic the behavior effects of REM sleep deprivation in humans, enhancement of motivational and drive related behaviors	Human with two cases multiple sclerosis and Parkinsons		2 Hz		7.5 pT	6 min	Sandyk et al. (1992)

**Borbély/Huber/Achermann Group (1994–2005)**

EMF Effect	Species Human	Antenna	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Exposure Duration	Electrodes	Time Duration of EEG Recording	Ref.
Significant sleep-inducing effect	52: 32 f., 20 m. 18–53 y, median 24 y	A LEET Low energy emission therapy device intrabuccally	<10 in oral mucosa: 100 mW/kg in brain	27.12 MHz	Intrabuccal amplitude-modulated 42.7 Hz		3-s on 1-s off for 15 min	16 recoding electrodes eyes closed in a chair in a dark room	Between 15:00 and 20:00 EEG for 15-min post-EMF	Reite et al. (1994)

*continued*

TABLE 4.3 (continued)

## Effects of MW Exposure on Electroencephalography (EEG)

EMF Effect	Species Human	Antenna	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Exposure Duration	Electrodes	Time Duration of EEG Recording	Ref.
Significantly reduced sleep onset and spectral power of NREM slow wave sleep increase in the 10–11 and 13.5–14 Hz bands in initial part of sleep, declined but still significant in 2nd and 3rd NREM episodes	24 R. handed men 20–25 y; mean 22.6 y	Three $\lambda/2$ dipole antennas 30 cm away from back of head	Spatial peak avg. 1 W/kg over 10 g	GSM 900 MHz	Linear		1 Night 11 p.m.–7 a.m.; 15-min on–15-min off	EEG electrodes were placed according to international 10–20 system	23:00–7:00	Borbély et al. (1999)
Spectral power of the EEG in NREM sleep was increased in first 30 min of sleep only $p < .01$ . This was significant for the entire 8-h sleep. The maximum rise occurred in the 9.75–11.25 Hz and 12.5–13.25 Hz bands in first 30 min of NREM sleep (alpha-band). No effect on sleep latency or sleep stages or REM sleep spectrum. Same effect whether exposure was from the left or right side. Double blind	16 R. handed men 20–25; avg. 22.3 y	Two planar antennas, unilateral left or right side 11 cm from the ear	Spatial peak avg. 1.0 W/kg over 10 g. Hemi-brain 0.14 W/kg	900 MHz: GSM	Linear		30 min prior to 3-h sleep begin at 9:45 or 10:15 (night before only 4-h sleep)	EEG (F3, C3, P3, O1, F4, C4, P4, O2) 30-min preceding	3-h daytime sleep episode and night before	Huber et al. (2000)
EMF Effect	Species Human	Antenna number	SAR (W/kg)	Freq. (MHz)	Modulation H <sub>2</sub>	Intensity (mW/cm <sup>2</sup> )	Exposure Duration h	Electrodes location	Time Duration of EEG Recording; h	Reference
1. Reanalysis of 1999 (Borbély et al.). Effects on sleep stages: duration of waking after sleep onset reduced. Spectral power of NREM slow wave sleep increase in the 11.5–12.25 and 13.5–14 Hz bands in initial part of sleep only.	24 R. handed men 20–25 y; mean 22.6 y	Three $\lambda/2$ dipole antennas 30 cm away from back of head	Spatial peak avg. 1 W/kg over 10 g	GSM 900 MHz	Linear		1 Night 11 p.m.–7 a.m.; 15-min on–15-min off	EEG electrodes were placed according to international 10–20 system	23:00–7:00	Huber et al. (2003). Part 1: Reanalysis of Borbély (1999) data

<p>Notice the change. Waking EEG increase in power of 11–11.5 Hz</p> <p>2. Reanalysis of 2000 (Huber et al.). No effects on sleep variables. The sleep EEG in first 30 min of NREM sleep increase in power more pronounced in the left hemisphere for 9–13.5 Hz and 12.5–13.25 Hz bands irrespective of exposure side <math>p &lt; .05</math>. Waking EEG reduction in power in 10.5–11 Hz and 18.75–19.5 Hz bands <math>p &lt; .05</math></p>	<p>16 R. handed men 20–25; avg. 22.3 y</p>	<p>Two planar antennas, unilateral left or right side 11 cm from the ear</p>	<p>Spatial peak avg. 1.0 W/kg over 10 g. Hemi-brain 0.14 W/kg</p>	<p>900 MHz: GSM</p>	<p>Linear polarized 2, 8, 217, 1736 Hz 87.5% duty cycle cocktail. Synthesized base station-like signals (cocktail)</p>	<p>30 min prior to 3-h sleep begin at 9:45 or 10:15 (night before only 4-h sleep)</p>	<p>EEG (F3, C3, P3, O1, F4, C4, P4, O2) 30-min preceding</p>	<p>3-h daytime sleep episode and night before</p>	<p>Huber et al. (2003). Part 2: Reanalysis of Huber et al. (2000) data</p>
<p>3A. Analysis of heart rate data from 1999 (Borbély et al.). Heart rate was not affected. But heart rate variability (spectra of RR intervals) was. But none of the all night spectra was significantly different that controls</p>	<p>24 R. handed men 20–25 y; mean 22.6 y</p>	<p>Three <math>\lambda/2</math> dipole antennas 30 cm away from back of head</p>	<p>Spatial peak avg. 1 W/kg over 10 g</p>	<p>GSM 900 MHz</p>	<p>Linear polarized 2, 8, 217, 1736 Hz 87.5% duty cycle. Base station cocktail</p>	<p>1 Night 11 p.m.–7 a.m.; 15-min on–15-min off</p>	<p>ECG was recorded with a polygraphic amplifier</p>	<p>23:00–7:00</p>	<p>Huber et al. (2003). Part 3A: Analysis of Borbély et al. ECG (1999) data</p>
<p>3B. Analysis of heart rate data from 2000 (Huber et al.). During the 30 min of MW exposure no difference in heart rate between the three conditions was observed. Heart rate variability was altered during the 3-h sleep spectra but not during first 30-min NREM sleep. Observed effects were weak</p>	<p>16 R. handed men 20–25; avg. 22.3 y</p>	<p>Two planar antennas, unilateral left or right side 11 cm from the ear</p>	<p>Spatial peak avg. 1.0 W/kg over 10 g. Hemi-brain 0.14 W/kg</p>	<p>900 MHz: GSM</p>	<p>Linear polarized 2, 8, 217, 1736 Hz 87.5% duty cycle cocktail. Synthesized base station-like signals (cocktail)</p>	<p>30 min prior to 3-h sleep begin at 9:45 or 10:15 (night before only 4-h sleep)</p>	<p>ECG was recorded with a polygraphic amplifier</p>	<p>3-h daytime sleep episode and night before</p>	<p>Huber et al. (2003). Part 3B: Analysis of Huber et al. ECG (2000) data</p>

*continued*



TABLE 4.3 (continued)

## Effects of MW Exposure on Electroencephalography (EEG)

EMF Effect	Species Human	Antenna	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Exposure Duration	Electrodes	Time Duration of EEG Recording	Ref.
4A. EMF exposure dosimetry of Borbély et al. (1999), including the assessment of the exposure variability and uncertainties (SAR variations up to 40% higher)	Head and body phantoms numerical model	Three $\lambda/2$ dipole antennas 30 cm away from back of head	DASY 3 Measurement in free space and inside head and I body phantoms	GSM 900 MHz	Linear		During testing	Measurements of induced currents of electrodes on surface of phantom shell with diff. orientations (SAR variations up to 40% higher)		Huber et al. (2003). Part 4: Analysis dosimetry. Borbély et al. (1999)
EMF exposure dosimetry of the brain and body areas of Huber et al. (2000) including the assessment of the exposure variability and uncertainties. Measurements of induced currents of electrodes on surface of Phantom shell (8%) and ear and eye electrodes inside the shell (35% higher, 1 g)	Head and body phantoms numerical model	Planar antennas, unilateral left or right side 11 cm from the ear	DASY 3 Measurement in free space and inside dielectric phantoms	900 MHz: GSM	Linear		During testing	Measurements of induced currents of electrodes on surface of phantom shell (8%) and ear and eye electrodes inside the shell (35% higher, 1 g)		Huber et al. (2003). Part 4: Reanalysis dosimetry. Huber et al. (2000)
5. RE electrosensitivity: subjective assignment of experimental condition was not better than chance	16 R. handed men 20–25; avg. 22.3 y	Two planar antennas, unilateral left or right side 11 cm from the ear	Spatial peak avg. 1.0 W/kg over 10 g. Hemi-brain 0.14 W/kg	900 MHz: GSM	Linear		30 min prior to 3-h sleep begin at 9:45 or 10:15 (night before only 4-h sleep)	EEG (F3, C3, P3, O1, F4, C4, P4, O2) 30-min preceding	3-h daytime sleep episode and night before	Huber et al. (2003). Part 5: Huber et al. (2000)

RECOMMENDATIONS: For exposure design of human studies addressing health risk evaluations of mobile phones important exposure parameters are the signal, field distribution, field strength, the setup & dosimetry. State uncertainty on all measurements

Cover entire half cortex, Double blind Temp load, Minimize intersubject variability

Patch antenna for local unilateral exposure R & L Minimize exposure variability modeling

Worst case. 10 W/kg

Mobile phone in use

Worst case cocktail & CW & intermittent exposures

Maximize spectral power

Not specified

Model electrodes increases in current density interference etc

Not specified

Kuster et al. 2004

EMF Effect	Species Human	Antenna Number/type	SAR (W/kg)	Frequency (MHz)	Modulation Hz	Intensity (mW/cm <sup>2</sup> )	Exposure Duration h	Electrodes location	Time Duration of EEG Recording h	Reference
The present results seem to show that (i) PM-EMF alters waking rCBF and (ii) pulse modulation of EMF is necessary to induce waking and sleep EEG changes. Double blind	16 Human male R. handed (20–25 y)	Planar antennas, unilateral left side 11 cm from the ear	Spatial peak 1 W/kg over 10 g (4× higher than base site ex.)	Example 1. 900 MHz handset signal Example 2. 900 MHz CW	Examples 1 and 2 12.5% duty cycle, 2, 8, 217, 1736 Hz and harmonic					Huber et al. (2002)
(1) Pet awake. Compared with the sham condition, PM-EMF exposure increased relative rCBF in the dorsolateral prefrontal cortex of the left hemisphere, the side of prior EMF exposure ( $p < 0.01$ , two-tailed paired $t$ -test) but not on the contralateral side. EMF-induced changes in the activity of dorsolateral prefrontal cortex may underlie EMF effects on working memory. On the other hand, an interaction between the counting task and EMF exposure cannot be excluded due to the lack of a performance measure of the counting task	16 Human male R. handed (20–25 y) mean 22.5 y reduced to $n = 13$	Planar antennas Unilateral left side 11 cm from the ear. Subject on a chair, head BTW two plates	SAR 1 W/kg 4× higher peak SAR but maintain same time avg. SAR as base station-like signals	900 MHz handset signal	Pulse-modulated 2, 8, 217, 1736 Hz and harmonics in awake subjects. Provide higher spectral power of the 2 and 8 Hz modulation components		Exposure for 30 min to left side of head then 10-min delay before PET scan	PET scan 3 scans 60 s, intervals of 10 min, using 300–350 mBq H <sub>2</sub> <sup>15</sup> O slow bolus. End 10-min transmission scan: re-photon attenuation correction	Between 8:00–14:00 h during scanning count from 1 to 60 slowly-cognitive activity	Huber et al. (2002) Part 1

TABLE 4.3 (continued)

## Effects of MW Exposure on Electroencephalography (EEG)

EMF Effect	Species Human	Antenna	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Exposure Duration	Electrodes	Time Duration of EEG Recording	Ref.
(2) Sleep study. Spectral power of the EEG sleep onset revealed that the alpha-frequency range was increased in the PM-EMF conditions in comparison with the sham condition. This effect was not present for the CW EMF condition. The sleep EEG was also modified after PM EMF exposure. In sleep stage 2, power in the 12.25–13.5 Hz range was increased relative to sham exposure. There tended to be a decrease, which was statistically significant in a single, slightly higher frequency bin. The extended duration of PM EMF-induced changes in the sleep EEG appeared to increase in the course of the night. Spindle amplitude was increased in the PM EMF condition compared with the CW EMF and sham conditions ( $p < 0.05$ , two-tailed paired $t$ -test). The duration and number of sleep spindles did not differ. The NREM sleep EEG was modified, but the REM sleep EEG was not affected and no differences were seen in sleep architecture, REM sleep latency or the	16 Human male R. handed (20–25 y) mean age 22.3 reduced to $n = 13$	Planar antennas unilateral left side 11 cm from the ear. Subject on a chair, head BTW two plates	SAR 1 W/kg 4 higher peak SAR but maintain same time avg. SAR as base station-like signals	1. 900 MHz hand set signal. 2. 900 MHz CW	1. Pulse modulated 2, 8, 217, 1736 Hz and harmonics in awake subjects. Provide higher spectral power of the 2 and 8 Hz modulation components		Exposure for 30 min to left side of head	EEG recordings C3A4, C4A1 derivations only	Exposure for at 22:20 then 10-min delay before sleep start at 23:00–07:00	Huber et al. (2002) Part 2

duration of NREM-REM sleep cycles											
Reanalysis of the PET studies (2002): Only 12 subjects analyzed. 1. Comp PET after handset versus sham-exposed. Significant increase in relative cerebral blood flow (rCBF) left dorsolateral prefrontal cortex (LDPFC). 2. Comp PET after Bstat versus sham-exposed. Not significant. 3. Comp PET handset versus base station. Significant increase rCBF LDPFC. Physiological effect may be dependent on spectral power in the amplitude modulation of MW carrier of handset signal	16 Human male R. handed (20-25 y) mean 22.5 y reduced to $n = 12$	Planar antennas, unilateral left side 11 cm from the ear. Subject on a chair, head BTW two plates	SAR 1 W/kg 4× higher peak SAR for handset signal but maintain same time avg. SAR as base station-like signals	1.900 MHz handset signal 2.900 MHz base station signal	1. Handset 1. 2, 8, 217, 1736 Hz and harmonics in awake subjects. Duty cycle 12.5% provide higher spectral power of components. 2. Base station 2, 2, 8, 217, 1736 Hz, 87.5% duty cycle cocktail	Ratio between pulse peak power and avg. power differed by 4×. 1.2. Base station and 4.8 for handset signal	Exposure for 30 min to left side of head then 10 min delay before PET scan	PET scan 3 scans 60 s, intervals of 10 min, using 300-350 mBq $H_2^{15}O$ slow bolus. End transmission scan: re-photon attenuation correction	Between 8:00-1400 h during scanning count from 1 to 60 slowly-cognitive activity	Huber et al. (2005). Reanalysis of Huber et al. (2002) Part 2	

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
Scalp electrodes (occipital) O1/O2. During and after exposure for some hours the O2 position alpha-wave is altered (increased energy)	Human 17 men and women; 20-27 y		150 MHz (magnetic field coils at the neck, 10 <sup>-8</sup> T)	217 Hz, pulse width 4.6 ms, interrupts 10 μs	At brain 6 cm depth: 1 μW/cm <sup>2</sup>	Exposed 2-3× for 15 min	von Klitzing et al. (1995)
In relaxed awake subjects there was increase in EEG power in α2: 9.75-12.5; β1 and 2 with a delay of 15 min after exposure (mega-wave caused increase in EEG power during and after exposure in α2: 9.75-12.5; β1 and 2)	Humans 36 males and females	SAR similar to 0.25 W phone	902.4 MHz GSM 8 W; (and 150 MHz MEGA Wave therapy device)	217 Hz pulse frequency (80 μs 9.6 Hz)	(Magnetic flux density in the range of 400 pT)	1 h 6 min	Reiser et al. (1995)

continued

**TABLE 4.3 (continued)**

## Effects of MW Exposure on Electroencephalography (EEG)

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
During MW exposure, significant suppressive effect on REM sleep and increased REM EEG spectral power (alpha mainly affected) density and shortening of sleep onset (NREM)	Human 14 males (mean 27 y)	?	900 GSM mobile phone	217 Hz, pulse width 580 $\mu$ s	0.05 at 40 cm	8 h, 3 night	Mann and Röschke (1996)
No short-term effects of digital mobile phone telephone on the awake closed eye EEG with special attention to the spectral power density of the alpha (8–13 Hz) EEG	Human 34 males (mean 27 y)		900 GSM mobile phone	217 Hz, pulse width 580 $\mu$ s	0.05 at 40 cm	3.5 min	Röschke and Mann (1997)
No (CNS-mediated) effects heart rate variability during human sleep	Human 12 males 21–34 y	?	900 GSM mobile phone	217 Hz, pulse width 580 $\mu$ s	0.05 at 40 cm	8 h, 1 night	Mann et al. (1998)
Suppression of REM sleep as well as a sleep inducing effect, previous results could not be replicated, might be due to dose-dependent effects of the EMF on the human sleep profile	Human 24 healthy males 18–37 y mean 26 y	0.3 to Max 0.6 W/kg	circular polarized 900 MHz GSM	217 Hz, pulse width 577 $\mu$ s	0.2 W/m <sup>2</sup>	8 h, 1 night 11 p.m. –7 a.m.	Wagner et al. (1998)
No effects on human EEG activity recorded in an awake, closed eyes situation. Exposure to one of the phones caused a statistically significant change in the absolute power of the delta recording probably due to statistical chance	Human 10 men 28–48 y and 9 women 32–57 y	?	5 cellular phones (analysis and digital 900 MHz or 1800 MHz).	900 NMT, GSM; PCN 1800 MHz	peak power 1–2 W, transmit at max power	20 min;	Hietanen et al. (2000)
Significant difference on the alpha frequency range for the EEG derivation between occupational and nonoccupational group exposure. No significant difference on EEG for long term user, short-term user, and nonuser	98 healthy male volunteers 25–45 y		Cellular phones Korea			30-min field exposure	Kim et al.
Effect with task-relevant target stimuli in the EEG band 18.75–31.25 Hz (Beta/gamma/awake). No effect with irrelevant standard stimuli	Human 13 healthy males 21–27 y		916.2 MHz	217 Hz pulse frequency pulse width 577 $\mu$ s radiated power of aerial 2.8 W		Around 10 min with exposure to left posterior temporal region	Eulitz et al. (1998)

Significant decrease in preparatory slow brain potentials (SP) in awake visual monitoring task at central and temporoparietal-occipital regions but not frontal. No effects on finger movement and contingent negative variation task	Human 16 healthy R. handed males 21–26 y	SAR 0.882 mW/g over 10 g	916.2 MHz	217 Hz pulse frequency pulse width 577 $\mu$ s radiated power of aerial 2.8 W		Around 10 min: telephone at left ear	Freude et al. (1998)
Significant decrease in awake slow brain potentials (SP) in a complex visual task. No effects on finger movement and contingent negative variation task	Human 20 healthy R. handed males 21–30 y	SAR 0.882 mW/g over 10 g	916.2 MHz	217 Hz pulse frequency pulse width 577 $\mu$ s radiated power of aerial 2.8 W		Around 10 min: telephone at left ear	Freude et al. (2000)
1-h exposure to mobile phone MWs EMG has no effect on auditory brain stem responses (ABRs) and distortion products otoemission (DPOE) recordings in the conditions of their protocol	10 men, 10 women, 20–30 y		900 MHz	217 Hz GSM	Full power peak power 2 W/8 duty cycle 1/8	60-min exposure	Thimonier et al. (1999)
Effects of MWs (900 MHz) on the cochlear receptor: exposure systems and preliminary results. No effect of exposure was found on otoacoustic emissions from the cochlea	S-D rats $n = 8$ per group	(a) Head 0.2 or 1.0 W/kg (b) 1.0 W/kg	(a) 950 MHz (b) 936 MHz	CW	far field exposure	(a) 3 h/day for 3 d or (b) 3 h/d for 5 d	Marino et al. (2000)
Results suggest that the exposure to EMF does not alter the resting EEG <i>per se</i> but increases EEG relative power of 8–10 Hz significantly during auditory memory tasks (words) during retrieval but not during resting	8 males and 8 females right handed mean 22 y		Digital 902 MHz GSM	217 Hz, pulse width 577 $\mu$ s, 0.25 W	Normal use position right side of head	Dimmed room, in chair, 30-min exposure right side	Krause et al. (2000a)
There was no main effect of EMF at any frequency band between the event-related synchronous (ERS) and desynchronous responses (ERP elicited by targets and non targets, reaction time or accuracy (a) visual sequential letter task n-back)	24 right handed adults M/F 20–30 mean 23 y		Digital 902 MHz GSM	217 Hz, pulse width 577 $\mu$ s, 0.25 W	Normal use position right side of head	Dimmed room, in chair, 30-min exposure right side	Krause et al. (2000b)
MP MWs may suppress the excessive sleepiness and improve performance while solving a monotonous cognitive task requiring sustained attention and vigilance	22 narcolepsy-cataplexy pts mean 48 y	0.06 W/kg over 10 g	900 MHz Motorola d520 MP	217 Hz, pulse width 577 $\mu$ s, 2.8 Hz 0.25 W		45 min right ear	Jech et al. (2001)

continued

**TABLE 4.3 (continued)**

## Effects of MW Exposure on Electroencephalography (EEG)

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
Mobile phone exposure decreased 1–4 Hz and 4–8 Hz activity and increased 8–12 and 30–45 Hz activity as a function of exposure duration recording an auditory discrimination task. Single blind	24 16 m, 8 f., 19–48 y. avg. 27.5 y. 20 R. handed		900 MHz Nokia 5110 and EMF attenuator	217 Hz pulse width 577 $\mu$ s		20 min 19 scalp electrodes	Croft et al. (2002)
EMF effects on the EEG and on the performance on memory tasks were variable and were not replicated from Krause et al. (2000a) for unknown reasons	24 normal subjects	0.648 mW/kg	Digital 902 MHz GSM Nokia 6110	217 Hz, pulse width 577 $\mu$ s, 0.25 W	Normal use position right side of head	Dimmed room, in chair, 30-min exposure right side	Krause et al. (2004)
Increase in error rate effects of 450 MHz exposure on human performance on visual memory tasks were reported by large variance between scores. Single blind	100, 63/37 m/f	0.35 W/kg est.	450 MHz	7 Hz 50% duty cycle	0.158 mW/cm <sup>2</sup>	10–20 min	Lass et al. (2002)
Cell phones exposure gives transient abnormal slow waves in EEG of awake persons	awake subjects		Mobile phones			16 channel telemetric EEG (ExpertTM)	Kramarenko and Tan (2003)
During sham and MW exposure changes in human EEG varied strongly between subjects. No effect of MWs was evident. Single blind	20, 11/9 m/f; 19–23 y	0.35 W/kg est.	450 MHz	7 Hz 50% duty cycle	0.16 mW/cm <sup>2</sup> at the scalp	60-s on–60-s off for 10 cycles	Hinrikus et al. (2004) (Lass et al., 1999, 2002)
Effects of GSM signals on correlation coefficients of the spectra of auditory evoked responses were observed but it was difficult to deduce relation to human health	9 Healthy and 6 epileptic people		Various GSM MWs			13–14 selected out of 32 electrodes	Maby et al. (2005)
GSM 1800 did not affect verbal memory encoding tested by recoding Event-related magnetic fields	12, 10 f., 18–30 avg. 23.6	0.16 W/kg (over 10 g)	GSM 1870	Pulsed at 217 Hz pulse width 577 $\mu$ s		30 min left ear	Hinrichs and Heinze (2004)

Huber et al.'s (2002) preliminary results on the effect of 900 GSM on waking regional cerebral blood flow (rCBF) and on waking and sleep EEG in humans (see Table 4.3) are reanalyzed by Huber et al. (2005). Although it is not stated in the 2002 paper that they did the base station-like exposures and the subsequent associated PET scans in 2002, which were analyzed in the 2005 paper, we deduce this from the same age range (20–25 y) and average age (22.5 y) of the subjects in the 2005 paper. If the base station-like exposures and PET scans had been done in 2005 with the same subjects they would have been 3 y older. As such they have presented incomplete PET methodology in both the 2002 and 2005 papers, making interpretation difficult. The coordinates of significant results from the first analysis on the PETs after handset-like exposure in 2002 do not match the coordinates in 2005 at all. The authors fail to explain the variance of their PET scores or details of how they computed them and how they controlled for scientific uncertainty in their measurements, and thus this data cannot be interpreted. Presently, PETs are considered unreliable on their own and can present spurious results, so interpretation of PETs must be accompanied by other brain scans such as the computer tomography scans (Wager and Smith, 2003). Computed tomography (CT) scans allow for cross-sectional views of brain tissues. Presently, we have no validation that Huber et al. (2002, 2005) are measuring what they say they are measuring.

Two conclusions can be made. Firstly, the normal EEG appears to be too variable to be able to be a reliable measure of possible small MW effects. For instance, changes in EEG during the night could be attributed to uncontrolled changing brain temperatures that are known to change spike latency of potentials (Moser et al., 1994). And the PET scans used to locate the brain site of significant effects of MWs on EEG are known to be unreliable for this purpose. Unless EEG recording and analysis methodology is improved and standardized and the scientific uncertainty in EEGs is carefully defined and controlled for, it appears that further studies should not be considered.

#### 4.2.9 Blood–Brain Barrier Studies

The BBB prevents high molecular weight substances in the blood from getting into the brain. This barrier protects the brain from foreign toxic substances, but allows the molecules that are necessary for metabolism to enter. Controversy has followed reports that MW exposure caused leakage through the BBB. In many follow-up studies, most researchers could not replicate the low-level BBB permeability changes or could show the effect only at high intensity levels, when the heating of the brain tissue was obvious. Now many researchers believe that the permeability change is associated with an increase in temperature-induced blood flow. D'Andrea et al. (2003b) reviewed 24 BBB studies since 1982 (see Table 4.4) and concluded that the question of MW-induced BBB permeability changes was still subjected to debate and required further experimentation. Presently, remaining BBB concerns related to MW exposures at low levels of SAR that require further study are the reported increase in binding of immunoglobulins to brain extracts (0.6 W/kg; see papers of the Vinogradov group, 1975–1993 in Table 4.4 and cited below), increased BBB permeability, and the occurrence of dark neurons in the brain (Salford et al., 1993, 1994, 2003; 2, 20, and 200 mW/kg). Replication and confirmation BBB experiments are currently in progress to address these uncertainties.

The Vinogradov group (1975–1993) (Vinogradov and Dumanskij, 1975; Dumanskii and Rudichenko, 1976; Vinogradov et al., 1981, 1983, 1985, 1991; Shandala et al., 1983, 1985; Vinogradov, 1993) has shown that exposure of rats to MW radiation led to an increased permeability of the BBB and a modification of the brain structure leading to the



**TABLE 4.4**

## Effects of MW Exposure on the Blood–Brain Barrier

Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
The pre-1982 (13) publications showed the specific barrier function against nonlipid-soluble macromolecules of the BBB, is permeated above 42–43°C							Oldendorf (1970), Polyaschuk (1971), Sutton et al. (1973), Frey et al. (1975), Oscar and Hawkins (1977), Merritt et al. (1978), Preston et al. (1979), Sutton and Carroll (1979), Albert (1979), Frey (1979), Lin and Lin (1980), Albert et al. (1981), Oscar et al. (1981)
Increase BBB permeability by Evans blue dye, which is related to intense MW hyperthermia	Rats	3.0	2450	PW		20 min	Lin and Lin (1982)
No significant increase in BBB leakage correcting for thermal effects of the MW, using [ <sup>14</sup> C] and [ <sup>3</sup> H] as tracers molecules	CD albino rats	0, 2, 4, or 6	2450	CW	0, 10, 20, or 30	30 min	Ward et al. (1982)
No increase in permeation effects on the BBB were observed, <sup>131</sup> I albumin as a tracer molecule	Mongrel dogs		1000	CW	2, 4, 10, 50, or 200	20 min	Chang et al. (1982)
Attempts to alter <sup>45</sup> Ca <sup>2+</sup> binding to brain tissue with pulse-modulated MW energy	Rat brain <i>in vivo</i> and <i>in vitro</i>	1.9–2.9	1000	PW	1	20 min	Merritt et al. (1982)

Absence of MW effects on BBB permeability to [ <sup>14</sup> C] sucrose in the conscious rat		0.3	2450 and 2800	CW and pulsed at 500 pps	10–40 CW 1–15 PW	30 min	Gruenau et al. (1982)
Increase BBB permeability to sodium fluorescein was found only in the brain made considerably hyperthermic by exposure to ambient heat or MW energy	Fisher-344 rats	13 (>41°C)	2450	CW	65	30 or 90 min	Williams et al. (1984a)
No increase in BBB permeability to HRP following exposure to ambient heat or MW, actually, a reduced uptake of the tracer by the brain was observed	Fisher-344 rats	13	2450	CW	0, 20, or 65	30, 90, 180 min	Williams et al. (1984b)
No increase in BBB permeability to tracer [ <sup>14</sup> C]	Rats	13	2450	CW	20 or 65	30 or 90 min	Williams et al. (1984c)
No change in uptake of either [ <sup>14</sup> C] or [ <sup>3</sup> H] tracer was found in any of the eight brain regions as compared with those of sham exposed animals	CD rats	0.1	1700	CW and PW		30 min	Ward and Ali (1985)
Increase of BBB permeability to [ <sup>86</sup> Rb] are associated with intense, MW-induced hyperthermia, and that the observed changes are not due to field-specific interaction	Wistar-derived rats	Avg. 3, a peak SAR: 240	2450	PW		5, 10, or 20 min	Goldman et al. (1984)
Ethanol inhibits MW-induced permeation of the BBB through reduced heating of the brain	Wistar rats	3.0	3150	CW		15 min	Neilly and Lin (1986)
MRI increases the BBB permeability to <sup>153</sup> Gd diethylenetriaminepentaacetic acid	Rats		6.25			23 min	Prato et al. (1996)
Increase uptakes of an intravascular molecule (Rh–F complex) through the BBB	Albino rats	~2	2450	PW	10	30–120 min	Neubauer et al. (1990)
Opening the BBB is due to MW-induced hyperthermia, not related to the nonthermal effect of MW	S-D rats	42.5 or 44.3°C	2450	CW		30 or 60 min	Moriyama et al. (1991)
Both CW and PW MW are able to open up the BBB for albumin passage	Fisher-344 rats	3.3	915	CW and PW 8, 16, 50 and 200/s			Salford et al. (1993)

*continued*

**TABLE 4.4 (continued)**

## Effects of MW Exposure on the Blood–Brain Barrier

Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm <sup>2</sup> )	Duration	Ref.
Extravasation was independent of SAR <2.5 W/kg but rose significantly for higher SAR and was not significantly different between pulse and CW exposure conditions	Fisher-344 rats	0.16–5 W/kg	915	CW and PW 8, 16, 50 and 200/s		120 min	Salford et al. (1994)
Nerve cell damage in mammalian brain after exposure to MW from GSM mobile phones	Fischer 344 m. and f. 12–26 weeks	WB 2, 20 and 200 mW/kg	862–960	GSM 217 Hz	Peak power densities 0.24, 2.4, and 24 W/m <sup>2</sup>	2 h	Salford et al. (2003)
Extravasation of Evans blue was observed in the regions where the temperature reached 43°C and above, but not in the areas where the temperature was 42°C and below	20 Wistar rats	<43°C	8	CW		30 min	Ohmoto et al. (1996)
Immunohistology against rat's own albumin: extravasation only at 7.5 W/kg	Rat	0.3–7.5 Brain	900	GSM		4 h	Fritze et al. (1997)
Immunohistology against rat's own albumin and fibrinogen. Avidin–biotin: extravasation at all SAR levels	Fischer m. and f. 344 rats 630 exposed various modulation and frequency and 372 controls	0.0012–12 WB	915 CW and GSM	217 Hz with 0.57 ms pulse width, or at 50 Hz with 6.6 ms pulse width		From 2 min to 960 min	Persson et al. (1997)
Immunohistology against rat's own albumin. Avidin–biotin Evan's blue: No extravasation	Rat	2 Brain	1439	GSM TDMA		1 h/d, 2–4 weeks	Tsurita et al. (2000)
<i>In vitro</i> model of BBB immunohistology against the cytosolic peripheral membrane proteins <sup>14</sup> C. sucrose flux: increased permeability	Mice	0.3	1800	GSM		4 d	Schirmacher et al. (2000)

<i>In vivo</i> immunohistology against mouse's own albumin. No extravasation	Mice	4.0 WB	898.4	GSM	60 min	Finnie et al. (2001)	
Effect of long-term mobile communication MW exposure on vascular permeability in mouse brain. No extravasation	Mice	0.25, 1.0, 2.0 and 4.0 WB	898.4	GSM	60 min, 5 days/week for 104 weeks	Finnie et al. (2002)	
New papers since D'Andrea review (2003)							
No leakage effect of 915 MHz exposure on the integrity of the BBB (Salford failed replication) except under conditions of heat. Double blind	S-D Fischer rats	20, 2, 0.2, 0.02, 0.002 W/kg (CW). 0.2, 0.02, 0.002 (pulsed 16). 2.5, 0.25, 0.025, 0.0025 (pulsed 217).	915	CW; Pulsed at 16 Hz and 217 Hz	30 min	McQuade et al. (2005)	
Lack of effect of 1439 MHz local exposure on the BBB in immature and young rats	Rats	0, 2, 6 W/kg	1439	TDMA	90 min/d for 1–2 weeks	Shirai et al. (2005)	
No BBB leakage due to exposure to 2.45 GHz as evidenced by no effects of scopolamine methylbromide on spatial memory during MW exposure or after MW exposure	S-D rats	Whole body 2.0 W/kg. Brain avg. 3.0 W/kg	2450	500 pps 2 $\mu$ s	45 min	Cosquer et al. (2005c)	
GSM 1800 MHz did not alter blood–brain barrier permeability to sucrose in BBB models <i>in vitro</i>	Porcine endothelial cell culture	Avg. 0.3 W/kg	1800	GSM	1–5 d	Franke et al. (2005a)	
No evidence of UMTS field-induced disturbance of the BBB cultures quantified by $^{14}$ C-sucrose, serum albumin permeation, transendothelial electrical resistance, permeation of transporter substrates and integrity of the tight-junction proteins	Porcine endothelial cell culture	Max 1.8 W/kg		UMTS	Avg. 3.4–34 V/m	84 h	Franke et al. (2005b)

increase in binding of immunoglobulins to brain extracts. Transfer of these brain extracts to other animals led to strong immune responses. The Vinogradov group reported this effect on the immune system in rats exposed to a radar-like signal at around 2700 MHz (pulse recurrence frequency of 400 Hz, 40 ms duration of a train of pulses). These Russian papers have been translated and are reviewed by Lagroye and Veyret (personal communication). They reported "the critical effect was found at  $500 \mu\text{W}/\text{cm}^2$  i.e.,  $5 \text{ W}/\text{m}^2$ . In rats, this incident power corresponds to ca.  $0.6 \text{ W}/\text{kg}$ . With a reduction factor of 10 the Russian workers limit is set at  $50 \mu\text{W}/\text{cm}^2$  and  $15 \mu\text{W}/\text{cm}^2$  for sensitive population" (Lagroye and Veyret, personal communication). Since the Vinogradov group studies have methodological limitations, replication studies are performed at PIOM, France where they have the exposure systems and the ability to test the immune response.

Because of the recent claims of Salford et al. (1993, 1994, 2003) of leaks in the BBB and the presence of dark neurons, reported to result from low MW exposures (whole body: 2, 20, and 200 mW/kg), replications of the Salford group studies are underway at the U.S. Air Force Research Laboratory (Mason), in Japan (Ohkubo) and within the French research program (Veyret).

Overall, the new BBB references in this chapter (Cosquer et al., 2005c; Franke et al., 2005a,b; McQuade et al., 2005; Shirai et al., 2005; see Table 4.4) indicate no effects of MW exposure in a wide range of exposures above and below the present ICNIRP and IEEE limits for either whole body or local exposure on the BBB where the body temperature does not rise above  $1^\circ\text{C}$ . McQuade et al. (2005), in a replication of the work of Salford et al. (1993, 1994, 2003), have reported at a conference no leakage in the BBB after low SARs (2, 0.2, 0.02,  $0.002 \text{ W}/\text{kg}$ ). At the same conference, Shirai et al. (2005) reported the lack of an effect of 1439 MHz local exposure on the BBB in immature rats. A published paper by Cosquer et al. (2005c) showed no BBB leakage after 2450 MHz MW exposure (2  $\mu\text{s}$  pulse width, 500 pps, whole body SAR of  $2.0 \text{ W}/\text{kg}$ ,  $\pm 2 \text{ dB}$  and brain averaged SAR of  $3.0 \text{ W}/\text{kg}$ ,  $\pm 3 \text{ dB}$ ) as evidenced by no spatial memory deficit after scopolamine methylbromide, administered i.p. This muscarinic antagonist substance does not cross the BBB unless there is leakage in the BBB. If it does, it is known to disrupt performance of spatial memory.

Franke et al. (2005a) had previously reported that GSM 1800 exposure increased sucrose permeation across the BBB *in vitro* (Schirmacher et al., 2000). The cell culture BBB model used in their previous study was of rat astrocytes in coculture with porcine brain microvascular endothelial cells. In this study (Franke et al., 2005a), after optimization of cell culture conditions, the GSM 1800-related effects on BBB permeability (Schirmacher et al., 2000) were no longer evident. Cell cultures were exposed for 1–5 d at an average SAR of  $0.3 \text{ W}/\text{kg}$ . They reported that they could not confirm the enhanced permeability of the BBB *in vitro* after the MW exposure of Schirmacher et al. (2000) since the *in vitro* barrier tightness in the Franke et al. (2005a) experiments was now more like that of the *in vivo* situation. Frank et al. (2005b) used this same optimized *in vitro* model of the BBB (Frank et al., 2005a) to investigate the effects of a generic Universal Mobile Telecommunications System (UMTS) signal on BBB tightness, transport processes, and the morphology of the porcine brain microvascular endothelial cells. This *in vitro* model of the BBB was exposed continuously for up to 84 h at an average electric field strength of 3.4–34 V/m (maximum  $1.8 \text{ W}/\text{kg}$ ). They reported that they did not find any evidence of the UMTS field-induced disturbances of the function of the BBB.

The BBB area of research remains open while we are awaiting the publication of results of further BBB replication studies.

#### 4.2.10 Electrosensitivity in Humans

The Netherlands Organization for Applied Scientific Research (TNO) study, known colloquially as COFAM, on the effects of GSM and UMTS signals on well-being and cognition, was released to the public on the internet at the Health Council of the Netherlands home page on September 9, 2003 (Zwamborn et al., 2003). COFAM suggested that GSM and UMTS base site-like signals might have a negative influence on “well-being” and cognition. One of the two groups of subjects was self-reported to be “electrosensitive.” It was found shortly after release, when international critical scientific comments on the statistical analyses were addressed through reanalyses of their data that the only effects were on “well-being” from UMTS exposure.

Following this, the Dutch Health Council EMF Committee was asked to carry out a critical review of the COFAM research methods (Roubos et al., 2004). The consultant psychometrist advised that there was no empirical evidence to indicate the metrological qualities of the subscales and tests of the “well-being” questionnaire and the cognitive tests used with the particular TNO subject groups under the conditions that prevailed for the TNO study. Since there was no measure of their validity, there was no proof to suggest the “well-being” questionnaire and the cognitive tests measured what they claimed to measure. The Dutch EMF Committee concluded that there was no objective evidence to justify mitigating measures. It is essential that, along with dosimetric and statistical expertise, international expertise in psychometrics be included in replication and follow-up studies (Roubos et al., 2004).

The Swiss Government authorized a TNO “replication” and expansion study in September 2004, to be completed in September 2005 (Achermann et al., 2005). Two similar studies are underway in the U.K. in the MTHR program, and several other European research groups including Denmark will carry out confirmation or replication studies of the TNO-COFAM study. The COFAM study is not published in a peer-reviewed scientific journal and hence is criticized by groups such as the WHO EMF Project as not fulfilling its criteria for inclusion in the research base for health risk assessment. The consequent public concern about the COFAM results has slowed down roll out of UMTS in Europe (BBC NEWS, 2003).

There are several studies of subjects’ self-reported symptoms (see Table 4.5; Bergqvist and Vogel, 1997; Hansson Mild et al., 1998; Hocking, 1998; Chia et al., 2000; Oftedal et al., 2000; Koivisto et al., 2001; Sandstrom et al., 2001; COMAR, 2002; Hietanen et al., 2002; Zwamborn et al., 2003; Schmid et al., 2005; Balik et al., 2005; Balikci et al., 2005; Belyaev et al., 2005; Markova et al., 2005; Rubin et al., 2005; Seitz et al., 2005). The scientific consensus is that this is an idiopathic environmental intolerance (Staudenmayer et al., 2003). There is no demonstrated relation between the self-reported sensitivity to MWs electrohypersensitivity (EHS) and the exposures of these people. However, more research is required to better characterize this self-reported EHS condition. Research is presently ongoing concerning subjects’ self-reported symptoms of EHS.

#### 4.2.11 Future Microwave Mobile Signals and Further Research

The SAR values are guaranteed by manufacturers to be within guideline limits (ICNIRP, 1998; IEEE, Std C95.1-2005). With frequency and power modulations different from the GSM signal and other “second generation” cell phone signals in third and fourth generation signals of MW telecommunications (Andersen, 2005), must we once again do biological tests for next generation telecommunications to investigate if the new modulations could have adverse biological effects? This is an important question. If the only established biological effects are from thermal mechanisms, then the existing standards

**TABLE 4.5**

## Effects of MWs on Self-Reported Human Hypersensitivity Ideopathic Environmental Intolerance

This review was unable to establish a relationship between low- or high-frequency fields and electromagnetic hypersensitivity or with symptoms typically occurring among such afflicted individuals	138 centers and 15 EU countries	Limit 2 W/kg or less	Mixed mostly, Analog signals in public use	Mostly CW	Questionnaires self-report	Bergqvist et al. (1997)
GSM users reported symptoms (headaches) warmth sensation on the ear and behind or around the ear less frequently than NMT users	Questionnaires 6379 GSM users; 5613 NMT users	Limit 2 W/kg or less	Analog and GSM phones in public use	Analog (CW) GSM (217 pulse width 577 $\mu$ s)	Self-report	Mild et al. (1998)
MW sickness. The syndrome involves the nervous system and includes fatigue, headaches, dysaesthesia, and various autonomic effects in MW radiation workers	40 self-reporting persons		Australia	Mobile phones	Self-report survey	Hocking (1998)
Subjective sensations of warmth around the ear. No effect on memory	Humans		Sweden 900 MHz	CW, GSM	Normal use	Oftedal et al. (2000)
Comparison of subjective symptoms of users of analog and GSM phones with adjusted odds ratios did not indicate any increased risk for GSM users with his larger sample (changed result from Mild (1998) above)	Sweden 6379 GSM users; 5613 NMT. Norway 2500 GSM and 2500 NMT		900 MHz	GSM NMT	Questionnaires, self-report	Sandstrom et al. (2001)
A review of EMF hypersensitivity. The symptoms reported by self-reported EMF hypersensitive (EHS) individuals—headache, fatigue, stress are common and nonspecific. EHS bears close resemblance to idiopathic environmental intolerances (IEI)						COMAR (2002)

Subjective symptoms in humans were not related to their MW exposure. Single blind	1. 48: 24 m., 24 f., 18–49 y; avg. 26 y. 2. Same 18–34 y avg. 23.2 y all R. handed		GSM 902 MHz, Nokia 6110	217 Hz pulse width 577 $\mu$ s	0.25 W mean power	30–60 min left ear on and off skin temp. about 35°C	Koivisto et al. (2001)
Hypersensitivity symptoms associated with exposure to cellular telephones: no causal link	20: 7 m., 13 f.,		1. NMT 900 MHz. 2. GSM 900 MHz. 3. GSM 1800 MHz	1. CW, 2–3. GSM, 217 Hz pulse width 577 $\mu$ s	1. 1 W, 2. 2 W, avg. 0.25, 3. 1 W, avg. 0.125	30 min $\times$ 4 sessions over 1 d	Hietenan et al. (2002)
Headache was the most prevalent symptom among hand-held cellular telephone users in Singapore as compared to nonusers. Significant increase in headache with increasing duration of usage (minute/day)	808 m. and f., age 12–70 y; mobile users	GSM 900 or 1800 MHz				Self-report questionnaire	Chia et al. (2000)
Hypersensitivity symptoms associated with exposure to cellular telephone: No causal link for GSM. But UMTS-like exposure lead to significant number of feelings of unwellness on non-validated tests with only negative items. Replications underway	Sensitive 36: m. 11, f. 25; 31–74 y, avg. 55.7 y. Normal 36: m. 22, f. 14; 18–72 y; avg. 46.6 y	0.045 (900); 0.082 (1800); 0.64, 0.078 (2100)	GSM 900, 1800; UMTS 2100	GSM and UMTS-like modulations		? possibly 20 min a session during the Taskomat test?	Zwamborn (2003)
No influence on selected parameters of human visual perception of 1970 MHz UMTS-like exposure. Double blind	58: 29 m., 29 f.	High 0.37 W/kg 10 g. avg. range 0.16–0.84 W/kg Low 0.037 W/kg	1.97 GHz	UMTS 5 Hz wide UMTS-FDD signal 1500 Hz, 8–12 Hz components		4 tests $\times$ 3 exposure condition approximately 2 h left side	Schmid et al. (2005)
A survey found some subjective ocular symptoms and sensations experienced by LT (1–4 y) users of mobile phones	695: 193 f.; 157: 36 use–non. 502 m.; 392: 110 Use–nonElazigy, Turkey		MPs			LT users (1–4 y)	Balik et al. (2005)

continued



**TABLE 4.5 (continued)**

## Effects of MWs on Self-Reported Human Hypersensitivity Ideopathic Environmental Intolerance

A self-report pilot survey on symptoms of LT users (2–4 y) of mobile phones found no effect on dizziness, shaking in hands, speaking falteringly, and neuropsychological discomfort, but effects for headache, extreme irritation, increase in the carelessness, forgetfulness, decrease of the reflex, and clicking in the ears	695: 193 f.; 157: 36 use–non. 502 m; 392: 110 Use–nonElazigy, Turkey	MPs	LT users (2–4 y)	Balikci et al. (2005)
A review. Idiopathic environmental intolerance IEI is a belief characterized by an overvalued idea of toxic attribution of symptoms and disability, fulfilling criteria for a somatoform disorder, and a functional somatic syndrome				Staudenmayer et al. (2003)
Thirty-one experiments testing 725 electromagnetically hypersensitive (EHS) participants were identified. Metaanalyses found no evidence of an improved ability to detect EMF in hypersensitives	725 EHS persons	161 MP EHS		Rubin et al. (2005)
Thirteen EHS studies re MW of mobile phones were reviewed. They concluded no valid evidence for an association between impaired well-being and exposure to mobile phone radiation. The limited quantity and quality of research do not exclude long-term health effects	13 EHS studies	MPs		Seitz et al. (2005)

are sufficient to insure no adverse cognitive effects occur within the guideline limits. To date, all the literature reviews by expert panels have considered all the reported effects (ICNIRP, 1998; NRPB, 2004; IEEE, Std 95.1-2005) and concluded that none could be replicated and established as an adverse effect, except absolute temperature rise above the guideline limits. Present evidence does not indicate that modulation is biologically significant, apart from a few special cases such as intense pulses (ICNIRP, 1998; NRPB, 2004; IEEE, Std C95.1-2005); if the case were otherwise, the entire rationale for MW exposure guidelines would need revision (Foster and Repacholi, 2004).

There are two ways to proceed with future research and research is proceeding on both fronts. One way is to continue to investigate each modulation for biological effects without a known biophysical mechanism to support this research (Foster and Repacholi, 2004). It is paramount in this uncertain area below guideline limits that the quality of telecommunications research become the highest possible caliber using standardized protocols, double blind procedures, multicentered replications, well-validated psychometrics, and the proper statistics to rule out chance effects and experimental artifacts as we search for other as yet unknown biophysical mechanisms.

The other way to proceed with future research is to build on what we know, that is, to better define thermal effects of MWs. It is paramount that research proceeds to investigate the area where the known mechanism is heat, measured as absolute whole body temperature rise, to better define the effects of MW heat interactions with the body and better define the thresholds more clearly where heat effects becomes adverse to animal behavior and human cognition (Andersen and Moser, 1995; Adair et al., 1998, 2001a,b, 2003, 2005; Adair and Black, 2003; Allen et al., 2003, 2005; Hancock and Vasmatzidis, 2003; Hirata and Shiozawa, 2003; Kheifets et al., 2003; Foster and Adair, 2004; IEEE, Std C95.1-2005; Section C.2.2.2.1.2). Validated thermal models could be used to predict the cognitive effects of MW signals more accurately for new technologies. Due to changing MW exposures of new technologies (Andersen, 2005) and a large number of cognitive and behavioral factors that come into play during temperature changes that are just now beginning to be investigated (Hancock and Vasmatzidis, 2003), the thermal mechanism area remains open to further research.

#### **4.2.12 Microwave Effects: Overall Conclusion**

The studies of warmth detection by humans and responses of laboratory animals to similar exposures have provided confidence that behavioral changes in animal studies are indeed thermally motivated. D'Andrea (1999) postulated that performance of cognitively mediated tasks might be disrupted at levels of exposure lower than that required to cause performance disruption. The results from numerous performance and cognitive tasks of varying difficulty do not support this hypothesis.

Hancock and Vasmatzidis (2003), on reviewing the literature on the effects of heat stress on cognitive performance, found two trends: "First, heat stress affects cognitive performance differentially, depending on the type of cognitive task. Secondly, it appears that a relationship can be established between the effects of heat stress and deep body temperature." They concluded, "that much remains to be understood before a (temperature rise) limit becomes universally acceptable."

Several studies have shown that the disruption of ongoing behavior, including spatial memory in rats during acute MW exposure, is generally associated with a 1°C or greater increase of body temperature. The studies that examined human cognitive processes and mobile phone usage appear to show no established evidence of associated memory deficits. Replication studies with standardized protocols, larger samples (e.g., multicentered replications; Haarala et al., 2003b), better experimental controls, double blind

conditions, and Bonferroni or other statistical corrections for multiple comparisons have failed to replicate the initial positive results in this area. Further high-quality multicentered research in human cognitive studies including human spatial memory may be needed to strengthen these results.

Because the normal EEG appears to be too variable to be a reliable measure of possibly small MW effects, further EEG studies should not be considered until methodologies improve. The BBB research area remains open, while we are awaiting the publication of results of further BBB replications. The scientific consensus is that MW hypersensitivity is an idiopathic environmental intolerance with no demonstrated relation between the self-reported sensitivity to MWs and the exposures of these people. Further studies are underway to better characterize this intolerance.

The final conclusion must be that disruption of ongoing behavior during acute MW exposure is generally associated with 1°C or greater increase of body temperature. It seems counterintuitive to expect a chronic effect of MW exposure when no acute effect below 1°C is evident, however, there is limited cognitive information on chronic exposure of humans to low-level emissions.

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### 4.3 Extremely Low-Frequency Effects

Exposure to a time-varying electric field will induce current flow within the biological body relative to the external field strength. When exposed to a time-varying magnetic field, an electric field within the biological body will be induced leading to subsequent current flow. If the induced fields and currents exceed the stimulation thresholds of excitable tissues, then neural activity may be initiated (see Reilly, 1998). These basic effects are related to the interaction between the induced electric fields and voltage-gated ion channels of a variety of excitable tissues (see NRPB, 2004; Kandel et al., 2000).

Detection, perception, behavioral and cognitive effects from weak ELF EMFs have been studied in a variety of animal and human experiments, measuring many different biological end points. Animal and human studies have determined the thresholds at which EMFs are perceptible, disrupt behavioral performance, or produce effects on cognitive processes.

#### 4.3.1 Detection of Extremely Low-Frequency Electric and Magnetic Fields

Studies by Kalmijn (1971) have shown that some species, such as the dogfish, *Scyliorhinus canicula*, have an elegant apparatus, Ampullae of Lorenzini, for detection of ELF fields at extraordinarily low intensities around 5 nV/cm. Many studies have evaluated the use of earth magnetic fields by migratory bird species as a means of navigation (Keeton, 1971). These naturally occurring magnetic fields are of very low intensity and demonstrated to have effects on bacteria as well.

Mammals are not known to possess electrosensory receptors for detection of ELF fields at extraordinarily low intensities. Mammals perceive ELF fields by other mechanisms that stimulate other types of sensory receptors such as light, touch, mechanoreceptors and pain, which result in nervous stimulation at much higher ELF intensities than electrosensory fish with specialized and highly sensitive electroreceptors (Bullock, 1986).

In the ELF range, the effects of magnetic fields that penetrate the body and result in induced electric fields have been the main focus of behavioral research. Smith et al. (1994) evaluated the ability of rats to detect magnetic field levels using a conditioned suppression

**TABLE 4.6**

## Effects of ELF Fields on Cognition and Behavior

Effect	Species	Frequency (Hz)	Electric Field (V/m)	Magnetic Field (A/m)	Modulation	Duration	Ref.
Slowing of event-related potentials (ERP) recorded during behavioral performance	Humans, $n = 21$ and $18$ in two experiments	50		$100 \mu\text{T}_{\text{rms}}$	15 s on/off cycles	Three 30-min sessions	Crasson et al. (1999)
No effect of magnetic fields, bright light, or sham exposure on ERP or cognitive performance	Humans, $n = 18$	50		$100 \mu\text{T}_{\text{rms}}$	15 s on/off cycles	Three 30-min sessions	Crasson and Legros (2005)
High-voltage direct current static electric fields did not produce taste aversion learning	Male Long Evans rats, $n = 56$	DC	+75 to -75 kV/m		None		Creim et al. (1984)
Rats could not detect the MFs and were unable to acquire the two-choice discrimination	Male, specific pathogen-free (SPF) Sprague-Dawley rats	60 and DC		50 mT 60 Hz, 26 mT static fields	None		Creim et al. (2002)
Results of this study do not support the hypothesis that ELF MF affects human cognitive performance	Male humans 20-30 y old, $n = 32$	50		20 and 400 mT		65 min	Delhez et al. (2004)
Retarded learning in 12-arm radial maze test of spatial memory	Rats	60		0.75 mT		45 min	Lai (1996)
Magnetic field exposure causes a deficit in spatial "reference" memory in the rat	Male Sprague-Dawley rats, $n = 24$	60		1 mT		60 min	Lai et al. (1998)
Thresholds of phosphene induction for different background illuminations levels and wavelengths	Humans, $n = 11$ (exp 1) $n = 8$ (exp 2) $n = 6$ (exp 3) $n = 9$ (exp 4)	Threshold was lowest at 20-30 Hz		0-40 Hz tested Threshold lowest at 10-12 mT			Lövsund et al. (1980b)

*continued*

**TABLE 4.6 (continued)**

## Effects of ELF Fields on Cognition and Behavior

Effect	Species	Frequency (Hz)	Electric Field (V/m)	Magnetic Field (A/m)	Modulation	Duration	Ref.
Impairment of spatial memory observed in rats and decreased motivation by patterned magnetic fields	Male Wistar rats, $n = 28$			200 nT or 500 nT	Patterned magnetic fields with inter-stimulus intervals of 0, 2,000, 4,000, or 10,000 ms		McKay and Persinger (2000)
No work stoppage on a delayed match-to-sample task could be achieved	10 baboons ( <i>Papio cynocephalus</i> )	60	6 to 30 kV/m	50 mT and 100 mT	None		Orr et al. (1995a,b)
The average EF detection threshold was 12 kV/m; the range of means among subjects was 5–15 kV/m	6 baboons ( <i>Papio cynocephalus</i> )	60	5 to 15 kV/m				Orr et al. (1995a)
No effects on visual duration discrimination or recognition memory	30 male and 50 female human subjects	50		100 $\mu$ T		1 s on and 1 s off	Podd et al. (2002)
No effects on human reaction time	12 human subjects	Continuous	0.1 Hz, 0.2 Hz, or sham field	1.1 mT			Podd et al. (1995)
Removal of ambient magnetic field induces analgesia in mice. Response may be opioid related as morphine	Swiss CD-1 mice adult male, $n = 4$			Absence of ambient geophysical			Prato et al. (2005)

mimics effects and naloxone (antagonist) blocks effects	to $n = 48$ in different experiments			MF by Mu-metal enclosures		
Baboon response rates on a two component operant schedule showed work stoppage when initially exposed to strong electric fields	12 naive baboons ( <i>Papio cynocephalus</i> )	60	30 to 60 kV/m			Rogers et al. (1995)
Results magnetic fields affected spatial learning in rats by modifying rate of acquisition	Adult male C57BL/6J mice	60		0.75 mT	45 min	Sienkiewicz et al. (1998a)
Exposure to MF showed dose-dependent effects on spatial learning task in an eight-arm radial maze	Adult male C57BL/6J mice	60		7.5, 75, 0.75, or 7.5 mT	45 min	Sienkiewicz et al. (1998b)
Rats would suppress responding during MF exposure	Rats	7, 16, 30, 60, and 65.1		1900 $\mu$ T at 7 Hz to 200 $\mu$ T at 65.1 Hz	3 min	Smith et al. (1994)
Threshold of detection was between 4 and 10 kV/m. Probability of detection increased with increasing field strength	Rats	60	4 to 10 kV/m		3–4 s trials	Stern et al. (1983)
No effects on a performance, a two-alternative, forced-choice, duration-discrimination task	100 male and female human subjects ranging in age from 18 to 48 y of age	50		100 $\mu$ T	9 min exposures	Whittington et al. (1996)

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behavioral technique. They determined that rats were able to detect magnetic fields as low as 200  $\mu\text{T}$ , although this has been questioned by Stern and Justesen (1995) because adequate sham tests were not done.

Recently, Creim et al. (2002) used a two choice discrimination learning task in which the presence of magnetic fields was tested as a discriminative stimulus (SD) to cue food-deprived rats as to which of two directions food could be found. They reported that nine rats trained for 64 trials (eight trials per day) could acquire a two choice discrimination in a modified radial arm maze based on a specified SD of a change in ambient illumination ( $p < .001$ ), whereas nine rats similarly trained could not acquire a two choice discrimination based on the SD of a combination of sinusoidal and static magnetic field exposure (50  $\mu\text{T}$ , 60 Hz, 26  $\mu\text{T}$  static fields) and any cues attendant to energizing the coils that produced the MF exposure. The selection of the field (50  $\mu\text{T}$ , 60 Hz, 26  $\mu\text{T}$  static field) corresponded to the ion resonant conditions for calcium ions (Liboff et al., 1989), since spatial learning and memory tasks have been previously determined to be causally dependent upon the movement of free calcium in the animal cortex (Liboff et al., 1989; Colombo et al., 1997; Antoni et al., 1998; Gnegy, 2000). Recent research on calcium underlines the importance of intracellular calcium in the molecular mechanisms of learning (Kandel et al., 2000). In conclusion, detection thresholds for magnetic fields in animals are less clear and show greater variability than those for electric fields (ICNIRP 2003; NRPB, 2004).

In animal studies, perception refers to the behavioral response to the detection of an ELF electric field as a cue or discriminative stimulus. Orr et al. (1995a) used operant methods to train baboons (*Papio cynocephalus*) to perform a detection task for the presence of an ELF EMF. The animals responded for food reward and showed that the average ELF detection threshold for electric field was 12 kV/m with a range of detection for the different subjects from 5 to 15 kV/m. The ELF detection threshold of nonhuman primates is similar to thresholds reported for electric fields in rats in the range of 3–15 kV/m with probability of detection increasing as a function of electric field intensity (Stern et al., 1983; Creim et al., 1984; Stern and Laties, 1985). Animals may detect the presence of low-frequency electric fields, possibly as a result of surface charge effects, including hair stimulation (Weigel et al., 1987; Stell et al., 1993).

In humans, the CNS has generally been viewed as the chief target for EMF-induced effects because the CNS and peripheral nervous system consist of excitable tissues and are responsive to electric fields and the currents induced within the body by external exposure of ELF fields. This has implied that the CNS may be responsive to very low-intensity fields. One of the biological effects resulting from lower field strengths has been the generation of phosphenes, small sensations of light during exposure to electric and magnetic fields. Visual system phosphenes can be generated at the retina by magnetic fields of near 15 mT oscillating at 50–60 Hz, producing electric fields in tissue on the order of 1 V/m (Reilly, 1998). Although other estimates of electric fields needed to generate phosphenes in the retina vary around 10–60 mV/m at 20 Hz, there is large uncertainty in these values (NRPB, 2004). When a suprathreshold stimulus is recurrent, the force on innervated muscle increases in the case of motor neurons (McNeal and Bowman, 1985) and perceived sensory intensity increases in the case of sensory neurons. Reported adverse reactions to CNS electrostimulation above phosphene excitation thresholds consist of tiredness, headaches, after images (up to 10 min) and spasms of the eye muscles (Lövsund et al., 1980a,b; Silny, 1986), headaches (Aurora et al., 2003), and hyperpolarization (Aurora et al., 2003; Pollen, 2004). One question that has been recently addressed is the strength of the association between headaches and phosphene stimulation. Recently Aurora et al. (2003) have demonstrated that there is a direct neurophysiological correlate for clinical observations, which have inferred hyperexcitability of the occipital cortex in migraineurs. This has been confirmed electrophysiologically by Pollen (2004).

Another case for additional concern is the possible differences in sensitivity in spinal cord and autonomic nervous systems (ANS). However the ANS questions appear to be covered, for instance, by studies of the nervous sensitivity of the heart muscle and CNS responses related to phosphenes. The ANS is predominantly an efferent system transmitting impulses from CNS to peripheral organ systems. Simple autonomic reflexes are completed entirely within the organ concerned, whereas more complex reflexes are controlled by the higher autonomic centers in the CNS, principally the hypothalamus (Bakewell, 1995).

The effects are easily discernable at higher field intensity levels such as those found in magnetic resonance imaging (MRI), transcranial magnetic stimulation (TMS), and electroconvulsive therapy (ECT), which have been well-documented (see Shellock and Crues, 2004; Wasserman, 1998; Sackheim, 1991, respectively). For example, brain stimulation during TMS creates sites of focal neuronal activity and a functional lesion useful during studies of cognitive functions; it has a threshold of near 20 V/m (Reilly, 1998). A recent paper by Nadeem et al. (2003) calculated the electric fields near the head surface for ECT at 1.6–80 kV/m, while deep in the brain they are in the range of 100–500 mV/m for both TMS and ECT. The biological effects of ECT and TMS have been intensively studied and well-documented. At high power, magnetic fields can stimulate nerve tissue directly through the induction of circulating eddy currents (Ueno et al., 1986) but no effects on nerve function are seen within guideline limits. Electrophysiological studies (evoked potentials and ERP) carried out during exposure include the possibility that the electrodes used to record the responses can be the source of field-induced artifact (Angelone et al., 2004).

Recently Shupak et al. (2004b) have published results on perception of pulsed magnetic fields by humans; they confirmed that within their paradigm they did not appear to be perceived. They investigated magnetic field exposure on sensory and pain thresholds following experimentally induced warm and hot sensations, using a double blind design. The magnetic field was established by the presence of three nested orthogonal pairs of Helmholtz-like coils, 2 m square  $\times$  1 m separation, 1.751 m  $\times$  0.875 m separation, and 1.5 m  $\times$  0.75 m separation. A constant current amplifier excited via a digital-to-analog converter was used to drive each of the three coil pairs. The pulsed magnetic field had a frequency content between 0 and 500 Hz as determined by a Fourier analysis and a magnitude of  $\pm 200 \mu\text{T}$  peak in the vertical direction. The ambient static magnetic field (MF) present in both sham and exposed conditions was 11.6  $\mu\text{T}$  in the horizontal direction and 50  $\mu\text{T}$  in the vertical direction. The ambient 60 Hz field for both sham and magnetic field exposed groups was 0.2  $\mu\text{T}_{\text{rms}}$ . Their results indicate that within their experimental setup, the magnetic field exposure did not affect basic human perception, but can increase pain thresholds in a manner indicative of an analgesic response. But there is an apparent involvement of the placebo effect that is under investigation.

While strong fields in tissues, as described above, can produce biological effects, laboratory studies at low levels ( $<500 \mu\text{T}$ ) have produced only subtle and transient effects on neurobehavioral responses and conditions necessary to elicit these are not clearly defined (Cook et al., 2002). There are few laboratory data from human or laboratory animal experiments that would even suggest that ELF-EMF, at or below current safety standards, pose a health risk.

Human detection sensitivity for electric fields is evident in stimulation of the peripheral nervous system in the skin (Bailey and Nyenhuis, 2005). Reilly (1998, p. 354) described research on detection of electric fields by 122 men and 8 women volunteers. He pointed out one study in which men and women were tested at a high voltage facility with overhead transmission lines, and as they walked through the electric fields they rated their own response as no feeling, perception, or annoyance (Reilly, 1978). A few individuals rated



perception in remarkably low fields (<2 kV/m). For the women, the median threshold for perception with the hands raised was 17.5 kV/m, whereas median perception for men with hands raised was lower at 6.7 kV/m. Responses to electric fields depend on relative humidity, but these effects were reported to represent a wide range of environmental conditions.

Leitgeb and Schröttner (2003) investigated electrosensitivity characterized by perception threshold and its standard deviation in a sample of the general population of 708 adults, including 349 men and 359 women aged between 17 and 60 y. They used an isolated stimulator producing currents of less than 5  $\mu$ A on the lateral side of the forearms of volunteers. They reported that electrosensitivity and its range of variability change from day to day (see also ICNIRP, 2003).

Spark discharges from people to grounded objects may occur and can be annoying and sometimes painful (Reilly, 1998). The magnitude of the spark will depend on how well the person is insulated from ground, the charge voltage, and impedances involved. Spark discharge perception threshold is complicated by many factors which are involved such as "frequency, induced open circuit voltage and capacitance between the conducting object and exposed person, temperature, speed of making or breaking contact, bodily location where contact is made, and other variables" (IEEE, Std C95.1-1991/1999).

In conclusion, electrophysiological studies remain difficult to interpret, but none suggest that ELF effects within the guideline limits are hazardous (see ICNIRP, 2003; Foster, 2003). Despite the difficulties, research continues in this area but focused for the most part on medical applications, where therapeutic exposure levels are often well above the guideline limits.

#### 4.3.2 Extremely Low-Frequency Cognitive Effects: Animals

Adverse reactions to CNS electrostimulation below excitation thresholds ( $B = 0.75$  mT at 50 Hz) may result in transitory reduced acquisition of spatial learning in mice (Sienkiewicz et al., 1998a,b). Other laboratories have also reported effects on spatial memory of rats, mice, and voles at power frequencies at 100  $\mu$ T and above (Kavaliers et al., 1993, 1996; Lai, 1996). Other behavioral (Creim et al., 2002) effects tested and the various complexities of the signals (Thomas and Persinger, 1997; McKay and Persinger, 2000) make a conclusion difficult. Further replication and confirmation studies are required of the effects of 0.75 mT at 50 Hz field exposure on acquisition and retention of spatial memory in two species (rats and mice) before such an effect could be considered as established evidence.

The mechanism of an ELF effect of a 0.75 mT exposure at 50 Hz on memory has not been established. The possibility that it may be the result of electrostimulation in the CA1 region of the hippocampus needs to be further explored (Tattersall et al., 2001; Pakhomov et al., 2003) (for background neurobiology of the hippocampus, see Section 4.2.4.1). Hippocampal pyramidal cells fire in synchronized assemblies or networks (O'Keefe and Nadel, 1978). The possibility that electrostimulation from an outside ELF source such as 0.75 mT at 50 Hz could thus have a coordinated effect on the firing of an assembly of hippocampal cells above the endogenous biological noise in the system needs further investigation (Saunders, 2003).

Other animal researchers (Kavaliers et al., 1985; Lai, 1996; Thomas and Persinger, 1997; Lai and Carino, 1998), using various paradigms including the spatial memory paradigm of the radial arm maze, have suggested the ELF-associated learning deficits could result from changes in cholinergic system triggered by ELF interactions with the opioid system in the animal. However, as reviewed in Section 4.2.4.1 above, this result is not supported

by the established neurophysiological evidence and molecular mechanism for spatial memory (see Kandel et al., 2000).

Recently in support of the research on ELF modulation of the opioid system, Prato's group has investigated magnetic and electric fields' ability to modulate the opioid system in animals. Prato et al. (2005) reinvestigated magnetic and electric fields' ability to modulate the opioid system in CD-1 mice (Choleris et al., 2001; Shupak et al., 2004a). They extended the previous findings from studies performed independently in Pisa, Italy and London, Ontario, Canada (Choleris et al., 2001) to elucidate what effects the absence of the geomagnetic field may have on nociceptive sensitivity and pain sensation. Choleris et al. (2001) did a series of experiments which repeatedly showed that a 2 h stay in a Mu-metal box, shielding the animal from ambient magnetic and electric environment, resulted in a significant decrease in response latencies, which indicated a significant increase in nociceptive sensitivity and reduction in stress-induced analgesia (SIA) in C57 mice. The effect of the Mu-metal box was specific to the shielding of the ambient magnetic field, as a 2 h exposure within a copper box, which shields only the electric field, did not affect SIA. Prato et al. (2005) targeted several objectives. They tested whether 10 d of repeated 1 h acute exposures of mice to a near zero magnetic field environment (less than 1  $\mu$ T) produced by Mu-metal shielding would affect nociception. They validated the earlier finding that the effect is due to shielding of the magnetic field rather than only the ambient electric field by performing identical experimental procedures to the Mu-metal trial in a thinly clad copper box. They found that the opioid mediation only partly explains the interaction with repeated exposure to the shielded condition. Other variables in the experiment could be the source of variance such as light and moisture in the shielded environment. The authors noted that further research is required (Prato et al., 2005).

#### **4.3.3 Extremely Low-Frequency Animal Cognitive Studies: Conclusion**

The ELF exposure conditions and the associated effects on cognition are too variable to draw any conclusions (ICNIRP, 2003; NRPB, 2004; Sienkiewicz et al., 2005). There is no support for changes in the cholinergic system triggered by ELF interactions with the opioid system in the established evidence on the molecular mechanism of spatial memory. Indeed these differences in outcome may depend on other variables including the timing and arousal and motivation, lighting, and duration of exposure relative to learning (McKay and Persinger, 2000; NRPB, 2004; Prato et al., 2005). These results suggest that the neural processes underlying the performance of cognitive tasks may be vulnerable to the effects of magnetic fields. One possible mechanism for the vulnerability of cognitive tasks that remains to be investigated is CNS electrostimulation below excitation thresholds. Overall, what appear to be the transient small biological effects on memory do not suggest harmful disruption of memory (NRPB, 2004; Sienkiewicz et al., 2005).

#### **4.3.4 Extremely Low-Frequency Cognitive Studies: Humans**

Previously magnetic and electric field-induced effects on attention, vigilance, memory, and other information-processing functions have been reviewed by NIEHS (1998), Cook et al. (2002), IEEE (Std C95.6-2002), Crasson (2003), NRPB (2004), ICNIRP (2003), and Sienkiewicz et al. (2005). Few field-dependent changes have been observed within guideline limit values. Newer research reviewed for this paper, published 2002–2005, reinforces the conclusion previously made by expert bodies that this type of study seems particularly susceptible to environmental and individual noise factors that could decrease the power to detect small field-dependent effects (Foster, 2003; NRPB, 2004).

Podd et al. (2002) investigated both the direct and delayed effects of a 50 Hz, 100  $\mu$ T magnetic field on a visual duration discrimination task, with 40 subjects exposed to the field and 40 subjects sham-exposed. The delayed effects of this field were also examined in a recognition memory task that followed immediately upon completion of the discrimination task. Unlike their earlier studies, they were unable to find any effects of the field on reaction time and accuracy in the visual discrimination task. They concluded that after many years of experimentation, finding a set of magnetic field parameters and human performance measures that reliably yield magnetic field effects is proving elusive (Podd et al., 1995, 2002; Whittington and Podd, 1996; Whittington et al., 1996; Preece et al., 1998; Crasson et al., 1999). A recent review by Cook et al. (2002) evaluated cognitive effects and found incomplete and negative findings that ELF MF can alter human cognition or electrophysiology at  $<500 \mu$ T.

Crasson et al. (1999) performed two double blind studies to examine MF exposure effects and to determine the impact of temporal variation (continuous versus intermittent exposure) of 100  $\mu$ T<sub>rms</sub> 50 Hz magnetic field diurnal exposure on psychological and psychophysiological parameters in healthy humans. Three cephalic exposure sessions of 30 min, i.e., sham, continuous, and intermittent (15 s on-off cycles) magnetic field conditions were involved. They demonstrated event-related brain potential (ERP) latency and reaction time slowing in the oddball paradigm, a visual discrimination task, after real magnetic field exposure. These results also indicate that a low-level 50 Hz magnetic field may have a slight influence on ERP and reaction time under specific circumstances of sustained attention.

Crasson (2003) reviewed the effect of 50–60 Hz weak electric, magnetic, and combined electric and magnetic field exposure on cognitive functions such as memory, attention, information processing, and time perception. Studies overall are inconsistent and difficult to interpret with regard to possible health risks. Statistically significant differences between field and control exposures, when found, are small, subtle, transitory, without any clear dose–response relationship and difficult to confirm.

The Crasson group (Delhez et al., 2004) designed a study to carefully address several remaining concerns by exploring the possible effects of exposure at two field strengths usually encountered in everyday life through the use of domestic or industrial appliances. They examined whether a dose–response relationship between field strengths and cognitive effects might be established. They maximized the statistical power of their study by using a within-subject design and chose various measures of performance that might be sensitive to ELF magnetic field effects according to previous results. They investigated cognitive effects of a continuous, vertical ELF magnetic field of 20 and 400  $\mu$ T 50 Hz in healthy young men during performance on cognitive tests (divided attention, flexibility, memory updating, digit span, digit span with articulatory suppression, and time perception). Thirty-two volunteers (mean  $22.6 \pm 2.2$  y) participated in this double blind study. The total duration of the exposure was 65 min. No effect of MF exposure was observed on performance. Having addressed the critical mass of experimental concerns in this area and after a thorough review of the relevant literature (Crasson, 2003), the group concluded that their results do not support the hypothesis that ELF magnetic fields affect human cognitive performance at 20 and 400  $\mu$ T 50 Hz in healthy young men during performance on the cognitive tests administered.

#### **4.3.5 Extremely Low-Frequency Human Cognitive Studies: Conclusion**

New human and animal studies are continuing to explore the thresholds at which electric and magnetic ELF fields are perceptible, disrupt performance, or produce effects on cognitive performance (Saunders, 2003). Presently, there is no other known mechanism

to explain ELF effects on perception, disruption of performance, or effects on cognitive performance above threshold limits than electrostimulation based on nerve stimulation, muscle excitation, cardiac excitation (contractions and adverse), synaptic activity alteration including phosphenes, and visually evoked potentials (headaches). Within the ELF guideline limits as set out by expert groups such as the IEEE (Std C95.1-1999/1999, Std C95.6-2002) and ICNIRP (1998), no established adverse effects on perception, disruption of performance, or cognition occur (ICNIRP/WHO, 2003); however, research is continuing to investigate possible effects on cognition below guideline limits. New methods such as genomics, proteomics, and metabolomics and free radical research are used (Rushton and Elliott, 2003), for instance in research funded by the EMF Biological Trust Foundation in the U.K., looking for any mechanism that could explain any of the postulated effects (EMF National Grid, 2005), including altered learning, memory (for a review of established molecular mechanism, see Kandel et al., 2000) and attention, and pain perception (for a review of the opioid hypothesis, see Sienkiewicz et al., 2005). Though funded by industry, the Trust is fully independent in its funding decisions. The *in vitro* results when completed will be followed up by *in vivo* research to verify any positive findings.

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# 5

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## *Thermoregulation in the Presence of Radio Frequency Fields*

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David Black

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### 5.1 Introduction

Much of the research on thermoregulation in the presence of absorbed radio frequency (RF) energy has used commonly available laboratory animals. These, like all mammals, are endotherms, with a dependence for body temperature on controlled-heat production by metabolism, but there are important limitations. These arise from their size and consequent surface-to-volume ratio and also differences in thermoregulatory mechanisms, such as sweating, which are species dependant. In general, the function of most mammalian tissues and organs depends on the maintenance of temperature within a

range of some 5°C, although this will vary to some extent as a result of changes in activity, hormonal and psychological factors, as well as intake of food and physiologically active substances, such as pharmaceuticals and alcohol.

Most tissues are tolerant to low temperatures, at least insofar as potential for recovery, but since there is relatively low tolerance of overheating, organisms have evolved various sets of sophisticated control mechanisms to avoid this. The controls that are used in human physiological systems may be broadly categorized as behavioral and physiological. For behavioral control, the whole organism acts consciously or subconsciously to alter its environment with regard to radiated or convected heat energy or inhaled air. This provides gross control, which also serves to guard against entering or remaining in adverse thermal conditions, if they can be avoided. In humans this is connected to and mediated by much more complex behaviors, including anxiety, which attempts to predict an adverse environment, resulting in sophisticated avoidance behaviors. These mechanisms provide coarse control, and more primitive variants of such behaviors are also found in ectotherms such as reptiles. The added mechanism available to mammals, where body temperature is maintained to a set point, are the physiological automatic thermoregulatory systems, which are largely controlled by the autonomic nervous system, requiring a sophisticated network of sensors and a variety of control mechanisms that have to work in concert with other autonomic functions such as the metabolic demands of circulation.

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## 5.2 Maintenance of Heat Equilibrium

A generalized heat balance equation as used by thermal physiologists was originally proposed by Pennes [1] in 1948. In simpler terms, [2] a generalized heat balance equation using units of power density ( $W/m^2$ ) provides that

$$M \pm W = R \pm C \pm E \pm S [W/m^2]$$

where  $M$  is the rate of metabolic heat production,  $W$  is the rate of work expended (+) or received (-),  $R$  is the radiant exchange (+ for gain to the body),  $C$  is the convective exchange,  $E$  is the energy lost to latent heat of vaporization caused by surface evaporation, and  $S$  is the rate of heat storage (+ for net gain).

Essentially this is no more or less than an expansion of the first law of thermodynamics, which states that the change in internal energy equals the heat added to the system minus work done by the system,  $\Delta U = Q + W$ . (In chemistry, it is more common to write the law with a positive sign for work done on systems, whereas physics and engineering usually use a negative sign in the equation if the work done by the system is positive.)

A variation of this to deal with temperature rises associated with electromagnetic fields is described in detail in [Chapter 10](#) of *BBA*, where numerical methods are described which are of practical value in analyzing the ultimate fate of thermal energy lost to or gained from the environment by the living tissue of an endotherm.

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## 5.3 Endogenous Heat Production ( $M$ )

Basal metabolic rate (BMR) of a human is the term used to describe the heat production of a healthy human at rest in a thermal environment, which does not add or subtract energy

from the body. The standard rate for a man is about 84 W or 0.8 MET. MET is the traditional unit for measuring metabolism, equivalent to 58.15 W/m<sup>2</sup> of body surface. A normal adult has a surface area of 1.7 m<sup>2</sup> and so a person in thermal comfort with an activity level of 1 MET would have a heat loss of about 100 W. Human metabolism is at its lowest during sleep (0.8 MET) and at its highest during vigorous physical activity when increases of 10- to 15-fold are frequently reached. The basal metabolic rate can be altered by changes in the body mass, diet, or by variations in endocrine levels but it is probably not reset to accommodate living in a hot climate [2].

In the resting state, most human heat is generated in the central viscera and neurological tissues, particularly the brain and this is conducted away by the circulatory system to more peripheral tissues. Heat is eventually dissipated by the exchange mechanisms of convection, radiation or evaporative loss from the skin, which affords an autonomic mechanism for control of the rate. Under cold conditions, an increase in muscle tone and activation of skin hair (piloerection) can increase metabolic heat products (*M*) by about 35%. Active shivering increases this to several fold. However, this is temporary; constant heat production under cold conditions will no more than double *M* [3].

In order to lose excessive heat, after the opportunities for behavioral alterations in environment are exhausted, the autonomic changes in vasodilatation (the increase in caliber of superficial blood vessels) is both effective and relatively rapid. This is described as the vasomotor state. Sweating also provides a further and more efficient method of carrying away significant amounts of heat, lost to the latent heat of vaporization (sudomotor activity). Sweating is activated by both rising heat and by various physiological mechanisms during exercise. Sweating can be activated by ambient temperature rises above about 31°C or by a rise in core temperature above 37°C, providing an emergency mechanism to dump relatively large amount of heat into the environment. A core temperature rise cannot be allowed to go more than a few degrees before damage might occur.

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## 5.4 Thermoregulatory Control

The thermoregulatory system operates with a closed-loop feedback, which maintains a “set” temperature at a reference level determined in the hypothalamus. There are recognized disorders, which are compatible with continued life but have significant effects on the well-being and energy levels of the individual, in which this setting is abnormally low [4]. There are thermal sensors throughout the body in different tissue planes, which monitor the temperature of adjacent tissues and report back to the central control unit, which is in the preoptic/anterior hypothalamic area of the brain stem. The receptors are found elsewhere in the brain and spinal cord, in deep abdominal structures and most importantly in the skin. The autonomic control mechanisms are most sensitive and act pre-emptively to changes in the environment. These generally obviate the need for significant control from more central heating, although the latter can produce relatively powerful responses, particularly in response to hyperthermia (rising heat), which might be reasonably interpreted as a sign of the failure of the systems. Receptors at different levels would be expected to respond in different ways, as alterations in the environment have different implications in the maintenance of a steady state in the type of environment in which the organism has evolved. This must be borne in mind when considering the potential for effect of an agent, such as RF energy, which may not follow the same rules of deposition as sources of radiant and convected heat expected by the organism in the natural environment in which it has evolved.

### 5.4.1 Tolerance to Added Heat

Before taking into account the ability of various thermal sensors to initiate mechanisms of heat loss, the fundamental question during the gain of heat energy deposited by the dissipation of electrical currents deposited by RF energy is whether there are sufficient mechanisms (vasomotor, sudomotor in addition to convection and radiation) to carry away all of the unwanted heat. Without this, cooling cannot occur and the core body temperature will continue to rise. The extent of heat stress can be predicted by assessing the percentage of the skin surface that is wet with sweat. If this is less than 20% there is generally a state of thermal comfort, but higher values are indicative of erosion of available tolerance [5]. The same ratio is also known as the heat stress index (HSI) [6]. HSI values greater than 30% are uncomfortable whereas levels over 60% become intolerable to severe.

### 5.4.2 Heat Generated during Exercise

Additional heat is delivered to the circulatory system when muscles are used and under these conditions there are both autonomic changes and mechanical factors, which result in increased blood flow. The autonomic system activates vasodilatation in peripheral skin so that heat is lost from the body surface. Under conditions of equilibrium during constant exercise there may be a small increase in internal body temperature, resulting in a heat gradient into the environment and a stabilized rate of heat loss [7].

### 5.4.3 Exposure to Radio Frequency

If the body is exposed to RF energy, either by entering electric or magnetic fields or by intercepting an electromagnetic wave, the resultant currents that flow in conductive tissue may heat the various tissues at different rates, which depend on the frequency, amplitude, temporal and other characteristics of the signal and the electrical conductivity and dielectric properties of the absorbing tissue. This results in a markedly nonuniform distribution of heat, even from a collimated wave of constant density. This is not entirely different from the situation with endogenously generated heat, as heat-producing tissues are unevenly distributed throughout the body. However, the difference may be that the circulatory system is adapted to these intrinsic characteristics of the body, whereas the manner in which electromagnetic radiation or fields distribute energy is not anticipated by the adapted physiology. In 1965 Nielsen and Nielsen [8] described equivalence of thermophysical responses during exercise and passive heating using shortwave diathermy to deliver heat directly into deep tissues of the trunk of human subjects. Subsequently it has been argued that passive heating by RF and heat generated by active exercise produces similar thermal loads in the body as a whole, although this might not take into account the tissues that are physiologically protected from heating under normal circumstances. This has been clarified by more recent work by Adair et al. [9] from human studies at two frequencies, 450 and 2450 MHz. In general, these studies show that, despite the differences in depths of energy absorption of the two frequencies, overall absorption of energy varies as expected, and the thermoregulatory system was selectively mobilized to deal efficiently with comparable thermal burdens in the steady state.

### 5.4.4 Fever

In febrile states, which generally result from some pathology, there is a rise in the "set point," which is maintained by homeostatic mechanisms in a manner which parallels the

controls of normal temperature in an afebrile organism. There are important differences in the way the body behaves during fever under different conditions of ambient temperature. Under warm conditions, vasoconstriction will prevent heat loss. If this is inadequate then heat production is activated. Under cold conditions, vigorous shivering, pathognomonic of certain diseases, is induced. Adair et al. [10] in 1997 hypothesized that a febrile animal might use energy from RF exposure to generate a fever in response to an injection of pyrogen into the hypothalamus, thus sparing metabolic energy stores. Four squirrel monkeys were injected through Delrin injection cannulae and reentrant tubes in the medial hypothalamic area; it was found that during the chill and plateau phases of a fever cycle or energy delivered by RF absorption did substitute for metabolic energy in both the generation and the maintenance of fever. However, as the fever fell, as it would during resolution of a febrile illness, RF exposure tended to exacerbate the fever and interfere with mechanisms of heat loss.

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## 5.5 Human Experimental Data

*Homo sapiens* are very well equipped to deal with a wide range of environmental temperature conditions and to deal with relatively high levels of endogenously generated heat. Humans can tolerate rates of metabolic heat production ( $M$ ) of up to 10 to 15 times the resting rate by a combination of behavioral and physiological adaptations. Recent experiments using human volunteers exposed to moderate levels of RF energy have confirmed expected physiological responses, all of which have been shown to be nonharmful and reversible.

### 5.5.1 Overexposure

There is limited data on human overexposure. In China, Chiang and Shao [11] reported on experiments using “hundreds of male volunteers” who had their testes exposed to microwaves at 2450 and 450 MHz CW energy once a month for 30 min. It was found that if the testes were heated to 40 to 42°C, sperm counts were reduced significantly and did not recover. In 1991 a similar attempt to use RF energy to achieve sterility employed powers of 20 to 30 W, which raised the scrotal skin temperature by 10°C. Considerable damage was seen on biopsy samples of testicular tissue although this was more resistant than expected.

Mitchell in 1985 [12] indicated that over the preceding 10 y, 300 overexposure incidents were investigated, although only 1 in 5 was confirmed to be over the then-ruling permissible exposure power density limit of 10 mW/cm<sup>2</sup> for any 6 min period. Clinical findings of these subjects were inconsistent although few significant changes were observed.

In 1998 Adair et al. [13] reported the first in a series of studies of human volunteers exposed to plane wave RF fields at controlled power densities in a highly controlled environment. Transducers were mounted on the subjects to measure thermoregulatory responses of heat production and heat loss during 45 min dorsal exposures with 450 MHz continuous wave transmission. The boresight of the antenna was centered on the subjects’ back and peak power densities were measured to be 18 and 24 mW/cm<sup>2</sup>. The local normalized peak specific absorption rate (SAR) estimated at 0.32 W/kg. Subjects were tested in each of three ambient temperatures, 24, 28, and 31°C. No change in metabolic heat production was seen during any of these exposure conditions. Vigorous increases in local sweating rate on the back and the chest, which were influenced by both ambient temperature and power density, provided skin cooling and maintained regulation of core



temperature, which was measured with an oesophageal probe, to within 0.1°C of the normal level. At the highest local power density (24 mW/cm<sup>2</sup>) the normalized peak surface SAR was 7.7 W/kg. This level is in excess of the 20 mW/cm<sup>2</sup> allowed for partial body exposure by the IEEE/ANSI Standard [9,14] for a controlled environment, which is six times the level generally permitted for the general public. Under all conditions, the autonomic heat loss response mechanisms of the subjects, principally sweating, maintained thermal homeostasis.

In a later study, Adair et al. [9] compared these results with those collected from a second group of human volunteers at the same frequency. The protocol was identical, as were the ambient temperatures and response measures, but local skin blood flow was measured at three sites on the body. In this study, it was found that the increase in skin temperature was directly related to frequency but local sweating rates on the back and chest were related more to the ambient temperature and SAR. In a third study in the series Adair et al. [15] compared responses of human volunteers at 2540 MHz continuous and pulsed waves of equal average power density. No convincing difference was found between the two. Later studies extended the peak power density to 35 mW/cm<sup>2</sup>, with a normalized peak local SAR of 15.4 W/kg, well over current standards limits for partial body exposure, and with the whole body SAR over double the limit for a controlled environment in most standards (0.4 W/kg). Data from sites measured followed similar trends, as expected, with no change in oesophageal temperature or metabolic heat production; and reports of discomfort in the subjects indicated that there are strong warnings available to an over-exposed individual before homeostatic mechanisms for regulation are overcome.

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## 5.6 Magnetic Resonance Imaging

There is a considerable literature relating to the use of RF energy at 64 MHz coming from the alternating fields in the RF coils used in magnetic resonance imaging (MRI) procedures. It is accepted that these frequently exceed the current standards. Shellock in 1992 [16] reported that tissue temperature changes are small and well below the hazardous levels, and in a 1992 review Gordon [17] concluded that whole body SARs up to 2 W/kg may produce significant elevations in skin temperatures. These are still relatively slight and without evidence of adverse effect.

There have been some laboratory studies of humans exposed to RF in the course of MRI scans. In 1992 Adair and Berglund [18] reported tests on two normal male subjects who underwent a series of 20 minute MRI scans with a 1.5 Tesla static magnet of the trunk and were exposed to a whole body SAR of 1.2 W/kg. Reports of thermal sensation and discomfort were recorded throughout the tests. In both the subjects, skin temperatures of chest and thigh are increased by 1.1°C; however, oesophageal temperature was stable throughout nearly 3 h of testing, at the maximum excursion of 0.3°C.

---

## 5.7 Thermal Perception and Nociception of Radio Frequency Energy

It is generally accepted that it is the stimulation of nerve endings in the superficial 0.6 mm of human skin that provides the perception of temperature [19]. Whether a RF signal

**TABLE 5.1**

Dermal Warmth Detection Thresholds as a Function of Frequency

Frequency (GHz)	Threshold (mW/cm <sup>2</sup> ) (mean ± SEM)	Tissue Penetration <sup>a</sup> (l/e <sup>2</sup> Depth, mm)	Wavelength (m)
2.4	63.1 ± 6.7	32	122
7.5	19.5 ± 2.9	6.3	40
10	19.6 ± 2.9	3.9	30
35	8.8 ± 1.3	0.8	8.6
94	4.5 ± 0.6	0.4	3.2
1–3 × 10 <sup>5</sup>	5.34 ± 1.1	<0.1	about 0.002

<sup>a</sup>Skin depth ( $\delta$ ) is given by:  $\delta = [(67.52/f) \sqrt{(\epsilon')^2 + (\epsilon'')^2} - \epsilon']^{-1/2}$ , where  $f$  is frequency in GHz,  $\epsilon'$  and  $\epsilon''$  are the real and imaginary part of the relative permittivity, respectively. (Durney et al., 1986). Relative permittivity ( $\epsilon^*$ ) of skin is given (Hurt, 1996) by:

$$\epsilon^* = \epsilon' - i\epsilon'' = 4.3 - 3.19i/f + 105/(1 + if/0.05) + 32/(1 + if/20).$$

Source: Blick, D.W. et al., *Bioelectromagnetics*, 18 (6), 407, 1997. With permission.

produces a sensation of warmth depends on the intensity and duration of exposure, as well as the frequency, which affects the depth of deposition and absorption. Absolute thresholds for detection have been studied in the past by Schwan et al. in 1966 [20] and Eijkman and Vendrik in 1961 [21]. These studies generally involved brief exposures to restricted areas and have reported that shorter wavelengths generally require less energy to produce a threshold of detection, defined as the threshold perception of warmth. The greater the intensity, the shorter the time of exposure for which the effect is determined by intensity alone; this is called the “critical duration” [22]. In 1997 Blick et al. [23] studied the thresholds across a range of five frequencies from 2.54 to 94 GHz as well as for infrared at about 3000 GHz. (see Table 5.1)

The threshold at 2.54 GHz proved to be more than an order of magnitude larger than that at 94 GHz although this was not significantly different from the infrared threshold. In general, these thresholds were proportional to the skin depth at each frequency. The theoretical analysis presented by Riu et al. in 1997 [24] suggested that a constant temperature increase of 0.07°C at or near the surface of the skin would result in perception. Riu also suggests that the depth of the sensors is not in itself important, as long as they are within 0.3 mm of the skin surface. Pain sensation is proportional to temperature and occurs about 46°C [25]. Cook’s theoretical explanations were based on theoretical flow of heat energy, which was satisfactory for short exposure. However, that long exposures must also involve physiological responses in the skin and superficial tissues was recognized by Riu in 1997 [24].

## 5.8 Behavioral Responses to Excessive Heat Load

Both exotherms and endotherms exhibit voluntary activity, which affect the absorption of heat from their environment. There is a relative paucity of human data but substantial animal experimentation. Reptiles exhibit thermotropic reactions, seeking out and basking in sources of warmth including microwaves. Endothermic mammals exhibit

more sophisticated responses and experiments show that there is a threshold to initiate the behavior, which then continues until there is equilibrium or change in the environment [26]. Behavioral adaptation can be quite effective; at whole body SARs that are the equivalent of double the resting metabolic rate changes in thermoregulatory behavior can regulate body temperature to normal [27]. Typical values for these effects are given below in [Section 5.9](#) with respect to metabolic (5.9.1) and vasomotor (5.9.2) responses. If a part of the body is exposed to RF energy, behavioral adaptations are determined by the integrated effect of energy accumulation by the whole body [28]. The result of this research has been generalized to occupational exposure of sources of absorbed RF energy and are useful in providing workers and employers with the information about warning signs for early recognition of thermal overload.

---

## 5.9 Data from Animal Studies

A thermoregulatory response to an added head load from RF exposure can be reliably predicted under specified environmental and exposure conditions. By definition, there will be no effect below the lower threshold where the energy does not result in any perceptible or behavioral changes sensed by an experimental animal and noticed by an observer, or reported by an experimental human subject. At higher levels, various physiological responses will occur in a hierarchy of metabolic adjustment (5.9.1) and then vasomotor adjustment (5.9.2), followed by evaporative adjustment (5.9.3). Adair [29] provides a detailed discussion of these concepts and their implications for thermoregulation in the presence of RF/Electromagnetic (EM) fields.

### 5.9.1 Metabolic Responses

A threshold SAR must be exceeded before a reliable reduction in  $M$  occurs. In a nonhuman primate this has been shown to be between 0.5 and 1.5 W/kg [30–32], but it has not been systematically explored in other species. The maximum whole body absorption of RF energy occurs at the so-called resonant frequency, when the long axis of the body is parallel to the electric field vector and the length of the body is about 0.4 of the wavelength of the signal. However, exposure of nonhuman primates at their resonant frequency results in somewhat less efficient thermoregulation than exposure to other frequencies [33,34]. While the threshold for reduction of  $M$  may be lower at resonance [31], the response change may be less at a given SAR than it is at nonresonance and so the body temperature may rise. This means that, as would be expected, there is no physiologically important difference between exposure at resonance than at other frequencies for equivalent energy absorption. Recent experiments by Adair et al. [35] in which seated human adults undergo 45 min RF whole body exposures at frequencies near resonance (100 MHz) showed that no increase in core temperature occurred at power densities eight times higher than the reference level in the prevailing IEEE/ANSI C95 Standard 1991/2 [14]. It is also clear that if a part of the body is exposed to incident RF energy, the magnitude of the change in  $M$  reflects the total absorbed energy integrated over the whole body [28]. It follows that if an endotherm is exposed to RF energy at an SAR greater than that which will reduce  $M$  to the resting level, then thermoregulation will be accomplished by mobilization of the next phase in the hierarchy outlined in the preceding paragraph.

### 5.9.2 Vasomotor Responses

When RF energy absorption is sufficient to induce vasodilatation, this response progresses as a direct function of the total heat load. Conversely, when the field is removed there is rapid vasoconstriction. In nonhuman primates both the threshold and the degree of vasodilatation depend on the frequency, although there are differences in species. Experimental results suggest that vasomotor control results from a combination of central and peripheral neural inputs and that changes in the caliber of deep blood vessels increase dramatically when the temperature of heated tissue exceeds about 41°C. This phenomenon has been employed to good effect in the treatment of localized malignancies [36] but the possibility of unwanted effects cannot be ignored in predicting effects of unusually high localized exposure.

### 5.9.3 Evaporative Adjustments during Radio Frequency Exposure

Dry heat loss is at its greatest when vasodilatation is complete and further disposal of energy must be initiated by another mechanism. Evaporation is highly effective and therefore mechanisms for this become mobilized during the phase of the upper levels of peripheral vasodilatation. Depending on species, evaporative loss may be from panting, sweating, or both. Humans have a particularly large capacity to lose body heat by sweating [37].

### 5.9.4 Intense or Prolonged Exposure

Michaelson [38] showed the characteristic triphasic change in internal body temperature with whole body exposure of dogs at a power density of 1 kW/m<sup>2</sup> of a 2.8 GHz pulsed wave for periods of 2 to 3 h (SAR 6.1 W/kg) or 6 h (SAR 3.7 W/kg). The three phases were described as an initial increase in temperature, a hypothermic plateau, and thermoregulatory collapse. It was presumed that the mobilization of heat loss by panting counteracted the initial effects of RF energy absorption, but that this eventually failed. The ambient temperature was found to be particularly important. Adaptation was observed in the dogs through the series of experiments, resembling the acclimatization to a hot environment seen in humans [2].

Candas [39], experimenting with squirrel monkeys, investigated whether there might be a power density ceiling beyond which further changes in response did not occur. SARs of 5.3 W/kg were found necessary to reduce the elevated *M* to resting level within 10 min. At a lower SAR of 4.3 W/kg resting *M* was reached within 20 min. The resulting change in body temperature was held to about 1°C.

Walters [40] used microwaves to examine whether fatigue during exertional heat stress occurred at a critical body temperature independent of that at the start of the exercise. Rats were exposed to microwaves at 2.1 GHz at 100 mW/cm<sup>2</sup>. Using a treadmill, run time exhaustion was significantly reduced after preheating. These results support the idea that a critical temperature exists that limits exercise under hot conditions. The relevance of intrinsic core to skin thermal gradients has also been investigated by Ryan [41,42]. It was concluded that altered core to skin thermal gradient during RF exposure does provide enhanced potential for cardiovascular impairment.

Recently there has been interest in exposure to very high peak power nanosecond, (after nanosecond) pulses composed of an ultra wide band (UWB) of frequencies. It has been hypothesized that these may pose special health risks. Walters et al. [43] reported results from single 2 min exposures of 23 rats to UWB, but found nothing particularly referable to this exposure.

### 5.9.5 Thermal Hotspots

Thermographic studies on tissue equivalent models have repeatedly shown that wrists, ankles, and the neck are areas where high local absorption and consequent elevation of tissue temperature may be expected. This has been investigated experimentally [33,34] with variable results but somewhat lesser effects than theoretically predicted from a heat deposition model, probably because of the cooling effect of circulating blood.

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## 5.10 Summary

Thermoregulation of the human body in the presence of RF fields follows the laws of thermodynamics in which heat and work are balanced and conserved. More specialized adaptation of the relevant equations have been developed by thermal physiologists, and the non-SI unit MET is widely used for measuring metabolism and does not have an SI equivalent. Thermal comfort at rest equates to about 1 MET, but endogenously generated heat energy can be increased by physiologically generated heat by a factor of more than ten. The autonomic nervous system controls mechanisms for conserving and losing heat to maintain body temperature. These include shivering, vasodilatation, and facilitation of evaporative loss at the air interface. Thermoregulatory control is managed by the body's "central computer" which has a set temperature, maintained at a reference level in specialized tissue in the hypothalamus. The results of monitoring are fed back by thermal sensors in a number of tissues, both central and superficial. Absorption of RF energy, which results in internal fields, resolves into electric currents in conductive tissues, the ultimate fate of energy being heat deposition. Deposition is highly nonuniform, irrespective of the nature of the wave or field, because of variations in penetration, conduction, and current flow. Distribution of heat deposition inevitably affects the pattern of exposed thermoreceptors and thus the feedback to the hypothalamus used to form an impression of overall body temperature. Increases in the set point can result from naturally occurring fever and experimental comparisons have been made between this condition and heat gain caused by RF.

The effect of heat on tissue has been studied in animal experiments and to a limited and lesser extent on humans to the point of irreversible tissue damage. However, in recent experiments using human volunteers exposed to substantial levels of energy, heating to the point of discomfort has shown no elevation of core temperature. Thus, it is generally accepted that behavioral responses to excessive heat provide a generally reliable early warning.

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# 6

## *Epidemiologic Studies of Extremely Low-Frequency Electromagnetic Fields*

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## 6.1 Introduction

Given the ubiquitous nature of extremely low-frequency (ELF) electromagnetic fields (EMFs), there is concern regarding their potential to adversely affect the health. Numerous health effects have been studied in relation to the EMF exposure: cancer, reproductive disorders, as well as neurodegenerative and cardiovascular diseases. Cancer, especially childhood cancer, has received the most attention.

A number of reviews on the potential of EMF to cause damage to health have been published [1–4]. The general consensus is that cellular effects do not occur with exposures below 100  $\mu\text{T}$ . Also, except for a very few animal studies that suggest adverse effect of EMF, these studies have been largely negative.

The EMFs are imperceptible, ubiquitous, have multiple sources, and can vary greatly over time and short distances [5]. In the absence of a biological mechanism to implicate one or more specific field parameters, the exposure assessment of EMF has varied over the years. The epidemiologic studies in the last decade have employed improved exposure assessment methods. Most of the epidemiologic studies discussed below use the time-weighted average (TWA) measurements to characterize the exposure. Furthermore, with the technological advances and increased sample size studies, the higher exposures, i.e.,  $>0.4 \mu\text{T}$ , are being explored. Although the epidemiologic evidence is not conclusive, it is generally agreed that the possibility of a causal association between the EMF and the adverse health outcomes cannot be excluded and that the epidemiologic studies of childhood leukemia provide the strongest evidence of an association.

The epidemiologic evidence is a major contributor to the understanding of the potential effects of EMF on health. The International Agency for Research on Cancer (IARC) classified EMF as a Group 2B or possible human carcinogen [1]; this classification was mostly based on the consistent epidemiologic evidence of an association between exposure to these fields and childhood leukemia and of laboratory studies in animals and cells which were not supportive of exposure to EMF causing cancer. Although the body of evidence is always considered as a whole, on the basis of the weight of evidence approach

and incorporating different lines of scientific enquiry, epidemiologic evidence, as the most relevant, is given the maximum weight.

The epidemiologic data is routinely critically assessed to shed light on the potential of EMF to cause harm to health. This chapter provides an up-to-date review of the epidemiologic evidence and accompanying methodological concepts.

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## 6.2 Leukemia

### 6.2.1 Background

The leukemias are cancers of the blood and bone marrow. The classification of leukemias is conventionally based on the origin of the cell types (lymphocytes, myelocytes, monocytes) and rate at which the disease progresses (acute and chronic).

The age distribution of leukemias is bimodal, with a first peak occurring at 4 y, a decline at 15–29 y, and then a slow rise throughout the rest of life. Leukemia is the most common childhood malignancy, constituting more than half of all childhood cancers. Acute lymphocytic leukemias (ALLs) account for 75% of all the cases of childhood leukemia; the most common types in adults are acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL).

The age-standardized rate of leukemia for children younger than 15 y has been estimated to be 3.5 per 100,000 per year for females and 4.2 per 100,000 per year for males in the developed world and 2.2 per 100,000 per year for females and 2.9 per 100,000 per year for males in the developing world [6]. For adults, the rates for each type of leukemia have changed very little over the past 20 years. For children, however, incidence rates have risen slightly; similar or more pronounced increases have occurred for other childhood cancers [7]. Some of these increases might be attributable to the improved diagnosis.

The environmental, occupational, and genetic factors have been associated with one or more types of leukemia. Increased risks have been associated with radiation exposure (all types of leukemia except CLL), cigarette smoking (parental smoking for childhood leukemia), and exposure to the human T-cell leukemia virus type one (HTLV-1) (associated with adult T-cell leukemia and lymphoma). Occupational exposures associated with increased risk are exposure to benzene (AML), manufacturing processes of styrene and butadiene production (lymphoid leukemia), and petroleum refining (AML and to a lesser extent CLL). The genetic risk factors include the chromosomal and congenital abnormalities of Fanconi's anemia, along with Down's, Bloom's, Klinefelter's, and trisomy G syndromes [8].

In general, the relationship between socioeconomic status (SES) and childhood leukemia is weak and inconsistent [9]. The causes of leukemia, especially childhood leukemia, are not well understood. In addition, the presently known or suspected risk factors are likely to account for only a small proportion of all cases [10].

### 6.2.2 Childhood Leukemia

#### 6.2.2.1 Residential Exposures

##### 6.2.2.1.1 Wire Codes

In the first study by Wertheimer and Leeper [11] in 1979, each subject's exposure was categorized on the basis of (a) the type of electric utility wiring adjacent to the residence, i.e., transmission lines, primary and secondary distribution lines of various types, and (b) the distance from that wiring to the residence. This exposure is referred to as the "wire code" (see Table 6.1 for a list of the major epidemiologic studies using wire codes).

**TABLE 6.1**

## Epidemiologic Studies of the Association between EMF and Childhood Leukemia

Ref.	Study Population	Study Design	Exposure	RR (95%CI) High Category
Wertheimer and Leeper [11]	Cases: deaths between 1950 and 1973, <19 y old. Controls: birth certificates. Denver, Colorado	Case-control: 155 cases, 155 controls	Wire code	HCC 3.0 (1.8–5.0)
Fulton et al. [184]	Cases: <20 y old. Controls: birth certificates. Rhode Island	Case-control: 119 cases, 240 controls	Wire code	HCC 1.0 (—)
Tomenius [21]	Cases: <19 y old. Controls: birth certificates. Stockholm, Sweden	Case-control: 243 cases, 212 controls	Measured field (front door)	$\geq 0.3 \mu\text{T}$ 0.3(—)
Savitz et al. [13]	Cases: <15 y old. Controls: random digit dialling. Denver, Colorado.	Case-control: Wire code—97 cases, 259 controls	Wire Code	HCC 2.7 (0.9–8.0)
Myers et al. [185]	Cases: <15 y old. Controls: birth register. Yorkshire, England	Measured field—36 cases, 207 controls	Measured field (spot, child's bedroom, low power)	$\geq 0.25 \mu\text{T}$ 1.9 (0.7–5.6)
		Case-control: 243 cases, 212 controls (all cancers)	Distance (home to overhead line)	$\leq 25$ meters 1.1 (0.5–2.6)
London et al. [15]	Cases: <10 y old. Controls: friends and random digit dialling. Los Angeles, California	Case-control: Wire code—211 cases, 205 controls	Estimated field strength Wire Code	$\geq 0.1 \mu\text{T}$ 0.4 (0.04–4.3) VHCC 2.1 (1.1–4.3)
		Measured field—162 cases, 143 controls	Measured field (24 h bedroom)	$\geq 0.125 \mu\text{T}$ 1.2 (0.5–2.8)
Feychting and Ahlbom [16]	Residents within 300 m of power line. Cases: <15 y old. Controls: random from cohort. Sweden	Nested Case-control: 38 cases, 554 controls	Historically calculated fields	$\geq 0.3 \mu\text{T}$ 3.8 (1.4–9.3)
Olsen et al. [61]	Cases: <10 y old. Controls: Central Population Registry. Denmark	Case-control: 833 cases, 1666 controls	Historically calculated fields	$\geq 0.4 \mu\text{T}$ 6.0 (0.8–44)
Verkasalo et al. [18]	Residents within 500 m of power line. <17 y, 1974–1990. Finland	Cohort: 35 cases	Historically calculated fields	$\geq 0.2 \mu\text{T}$ 1.6 (0.3–4.5)
Tynes and Haldorsen [17]	Residents in ward with high-voltage power lines. Cases: <15 y old. Controls: random from cohort. Norway	Nested Case-control: 139 cases, 546 controls	Historically calculated fields	$\geq 0.14 \mu\text{T}$ 0.3 (0.0–2.1)

Linnet et al. [26]	Cases: <15 y old. Controls: random digit dialling. U.S. residents in 9 Midwestern and mid-Atlantic States	Case-control: Wire code—402 cases, 402 controls (acute lymphoblastic leukaemia)	Wire Code	VHCC 0.9 (0.5–1.6)
		Measured field—162 cases, 143 controls (acute lymphoblastic leukaemia)	Measured field (time-weighted average)	$\geq 0.3 \mu\text{T}$ 1.2 (0.9–1.8)
Michaelis et al. [28]	Cases: <15 y old. Controls: govt. office residents' registry. Residents of Northwest Germany and Berlin	Case-control: 176 cases, 414 controls	Measured field (24 h bedroom)	$\geq 0.2 \mu\text{T}$ 2.3 (0.8–6.7)
Dockerty et al. [33]	Cases: <15 y old. Controls: birth certificate. New Zealand	Case-control: 115 cases, 117 controls	Measured field (24 h bedroom)	$\geq 0.2 \mu\text{T}$ 15.5 (0.3–7.6)
McBride et al. [27]	Cases: <15 y old. Controls: health insurance records. Canada	Case-control: Wire code—303 cases, 309 controls	Wire code	VHCC 0.8 (0.4–1.6)
		Measured field (48 h personal)—293 cases, 339 controls	Measured field (48 h personal)	$\geq 0.2 \mu\text{T}$ 1.0 (0.7–1.6)
		Measured field (24 h bedroom)—272 cases, 304 controls	Measured field (24 h bedroom)	$\geq 0.2 \mu\text{T}$ 1.3 (0.7–2.3)
Green et al. [20]	Cases: <15 y old. Controls: telephone marketing lists. Canada	Case-control: Wire code—79 cases, 125 controls	Wire code	OHCC $\pm$ VHCC 1.5 (0.3–8.7)
		Measured field (48 h personal)—88 cases, 133 controls	Measured field (48 h personal)	$\geq 0.4 \mu\text{T}$ 1.1 (0.3–4.1)
		Measured field (spot)—21 cases, 46 controls	Measured field (spot)	$\geq 0.4 \mu\text{T}$ 4.5 (1.3–15.9)
UKCSS [30]	Cases: <15 y old. Controls: Family Health Services Authority. England, Wales, Scotland	Case-control: 1094 cases, 1096 controls	Measured field (Phase I: 90 min in family room + spot measurement in bedroom; Phase II: 48 h bedroom and spot measurement in school only for highest 10% in Phase I.	$\geq 0.4 \mu\text{T}$ 1.7 (0.4–7.1)
Bianchi et al. [19]	Cases: <15 y old and controls in Varese province identified by Lombardy Cancer Registry, Italy	Case-control: 101 cases, 412 controls	Historically calculated fields	$\geq 0.1 \mu\text{T}$ 4.5 (0.9–23.2)
Schuz et al. [186]	Cases: <15 y old German Childhood Cancer Registry. Controls: population registration files. West Germany	Case-control: 514 cases, 1301 controls	Measured field (24 h residential)	$\geq 0.4 \mu\text{T}$ 5.9 (0.8–44.1)

Wertheimer and Leeper [11] initially used a dichotomous exposure classification wherein subjects were classified as living in either high- or low-current configuration (HCC or LCC) homes. Later they introduced a finer degree of resolution into their wire code classification system: very low-current configuration (VLCC), ordinary low-current configuration (OLCC), ordinary high-current configuration (OHCC), and very high-current configuration (VHCC) [12]. Savitz et al. [13] and Kaune and Savitz [14] further refined the original wire code configuration classification scheme.

Wertheimer and Leeper [11] surmised that across an entire living space the outdoor electric lines and the associated grounding system were the dominant source of long-term magnetic field exposures; and unless the lines were modified over time or homes were demolished they provided an estimate of the past exposures. Wire coding did not require resident's participation; thus, the exposure assessment without intrusion was possible for cancer cases and controls (cancer-free comparison subjects) with verified addresses, and the study size was therefore maximized. On the basis of cancer cases between 1950 and 1973, they reported that childhood leukemia mortality was associated with residence in the HCC homes. However, methodological problems, such as nonblind exposure assessment, reliance on death certificates, and lack of evaluation of confounding, rendered this study difficult, if not impossible, to interpret. It, nevertheless, generated a hypothesis that has been followed up by several other studies.

A childhood cancer investigation by Savitz et al. [13], conducted in the same geographical area, was designed to replicate the Wertheimer and Leeper [11] study and to improve upon it through comprehensive ascertainment of incident cases, assessment of potential confounders, and blind exposure assessment. In addition to wire coding, they included point-in-time or spot magnetic field measurements in all available residences. In general, higher magnetic fields were registered in the OHCC and especially VHCC residences, but the wire code or the magnetic field correlation was weak. Savitz et al. [13] reported positive associations of wire code with childhood leukemia. The risk for spot measurements was lower and less precise, partially owing to the reduced number of homes available for measurement. The results for wire code and leukemia were basically consistent with those of Wertheimer and Leeper [11], with the additional evidence that wire code may serve as a weak indicator of contemporary measured magnetic fields.

In 1991, London et al. [15] conducted an incidence study of childhood leukemia similar in its basic design to the study by Savitz et al. [13], but with 24 h bedroom measurements added to the exposure assessment. Similar results emerged: (a) the residential magnetic field strength correlated weakly with wire code and (b) a trend of increased leukemia risk across wire code, with elevated leukemia risk among the VHCC sample relative to the referent.

#### 6.2.2.1.2 *Exposure Based on Distance and Calculated Fields*

There are a handful of studies on childhood leukemia using distance from residence to power lines as the exposure of interest. The results of these studies have not been wholly consistent. The studies using calculated field measurements seek to capture historical exposure levels. The exposure to EMF is most often assessed by using power line load data and specifications for power lines that are specific to the time period of interest. Both Feychting and Ahlbom [16] and Tynes and Haldorsen [17] used a nested case-control study approach to identify leukemia cases within defined cohorts during specific time periods and calculated the field measurements. Feychting and Ahlbom [16] showed that the risk of childhood leukemia was elevated for children exposed to 0.3  $\mu\text{T}$  or greater; however, Tynes and Haldorsen [17] showed that there was no elevated risk of leukemia among those exposed to 0.14  $\mu\text{T}$  and greater. No analysis for higher cut points was presented. A study from Finland using similar design showed a nonsignificant elevated

risk of childhood leukemia among those exposed to 0.2  $\mu\text{T}$  and greater [18]. A recent study by Bianchi et al. [19] demonstrated no increased risk at low exposures of 0.1  $\mu\text{T}$  and greater.

#### 6.2.2.1.3 *Household and Personal Exposures*

The majority of epidemiologic studies on EMF and childhood leukemia use residential EMF exposure measurements; they have been either spot measurements [13,20,21] or area (mostly bedroom) magnetic field measurements of 24 h and longer [20,22–30]. Although most of these studies observed elevated odds ratios (ORs), for many, the confidence intervals (CIs) around these estimates of effect are large due to small numbers of highly exposed subjects. Given the rarity of both the disease and the high exposure, securing enough highly exposed cases, needed for statistically stable estimates, is quite difficult.

Only two studies employed the use of personal EMF meters to measure exposure [20,27]. In both of these studies, there was no indication of elevated risk of leukemia due to high personal exposure. Although there is evidence that there is a close correlation between household exposure and personal measurements [30,31], use of personal measurements in case–control studies might be biased as disease status might effect personal exposure measurements. Personal measurements are even more problematic in case–control studies of children, as exposure at young age is quite age dependent, and measurements made few years after the etiologically relevant time period might be different than during the time period of interest.

#### 6.2.2.1.4 *Pooled Analyses*

Two pooled analyses represent the most powerful attempt so far to provide a cohesive assessment of the epidemiologic data of EMF and childhood leukemia [22,32]. These analyses, although focusing on a largely overlapping but distinct set of studies, come to similar conclusions.

In the pooled analysis by Greenland et al. [32], 12 studies using measured or calculated fields were identified; the study included a total of 2656 cases and 7084 controls. For this analysis, the metric of choice was the time-weighted average. The estimated OR for childhood leukemia was 1.68 (95% CI 1.23–2.31) for exposures greater than 0.3  $\mu\text{T}$  as compared with exposures less than 0.1  $\mu\text{T}$ , controlling for age, sex, and study.

Using more stringent inclusion criteria, Ahlbom et al. [22] included nine studies using measured and calculated fields. There were a total of 3203 cases and 10,338 controls in the pooled sample. Using the geometric mean as the metric of choice, the estimated OR for childhood leukemia was 2.00 (95% CI 1.27–3.13) for exposures greater than or equal to 0.4  $\mu\text{T}$  as compared with exposures less than 0.1  $\mu\text{T}$ , controlling for age, sex, SES (in measurement studies only), and East and West (in German study only).

#### 6.2.2.2 *Electric Appliance Exposure*

Studies that have evaluated the risks of childhood leukemia [15,33–35] associated with the use of electric appliances, used interviews of mothers to assess exposure information. Positive findings were observed in only a couple of these studies. There was an elevated risk of leukemia, but no evidence of dose–response relationship, among children watching black-and-white television [15] in one study; another study observed a rise in leukemia with increasing number of hours children watched television, regardless of the child’s distance from the television set [34]. Risks were increased for postnatal exposure to a few other appliances in one study [34], with no dose–response relationship. Postnatal use of electric blankets [33–35] and hair dryers [15,34] was linked with modestly elevated

risks in more than one study, but there was no evidence of dose–response relationships. Overall, the small number of studies, evaluation of one appliance at a time leading to a very large misclassification, and the absence of good measurement data within the studies preclude the straightforward interpretation of results.

### **6.2.2.3 Parental Exposure**

Of the studies that have examined parental occupation and childhood cancer [36] none have found any association with parental exposure to EMF. London et al. [15] reported an association between a mother’s exposure to nonionizing radiation during pregnancy and the risk of childhood leukemia. However, the exposure question did not focus on EMF and is likely to reflect exposures to higher than power frequencies.

Two studies [34,35] observed small increase in the risk of childhood leukemia with the prenatal use of electric blankets. In general, on the basis of these studies, there is little evidence of elevated risk of leukemia in offspring associated with mothers’ prenatal use of other types of electric appliances.

## **6.2.3 Adult Leukemia**

### **6.2.3.1 Residential Exposures**

The first study of residential exposures and adult cancer was also conducted by Wertheimer and Leeper [12]. Although the focus was on total cancers, leukemia did not contribute to the elevated risk found for all cancers combined.

Several other studies have attempted to examine the risk of adult leukemia in populations residing near power lines [37–40]. These studies are based on small numbers, low potential exposures, and very crude exposure estimation methods. The overall results are negative, with a few hints of a small, nonsignificant elevation of risk in some studies.

Since then, other studies on adult leukemia and residential EMF exposure have shed more light on the plausible relationship [41,42], with a follow-up study by Feychting et al. [43] and studies by Verkasalo and coworkers [44,45] and Li et al. [46]. A small increased risk for all leukemia was seen in some of these studies.

### **6.2.3.2 Electric Appliance Exposure**

In a case–control study of adult AML and CML in Los Angeles County that used cases and matched neighborhood controls [47], use of electric blankets was not related to leukemia risk. Lovely et al. [48] examined adult leukemia risk and personal appliance use, on the basis of the study of Severson et al. [42] mentioned above. Most noteworthy in this analysis were a small elevation in risk for ever/never use of electric razors (relative risk (RR) 1.3, 95% CI 0.8–2.2), and a larger increase in risk with duration of daily use (RR 2.4, 95% CI 1.1–5.5 for the highest relative to the lowest category). However, it appears that this finding is due to bias in the proxy-reported information and that there is in actuality no association between leukemia risk and either use versus no use or duration of use of electric razors [49].

### **6.2.3.3 Occupational Studies**

Studies on occupational exposure to EMF and adult leukemia have job titles mostly linked with cancer incidence or mortality. In a meta-analysis of this literature by Kheifets et al. [50], there was a small increased risk of leukemia associated with work in electric occupations. However, jobs thought to have higher exposure (welders, electricians,

linemen, and power plant operators) did not have higher risks than electric workers who generally have lower exposures (installers, engineers, and television or radio repairmen). Several occupational cohort and case-control studies have been published since this meta-analysis. Overall, the results are negative with excess risks reported only in some subgroups [51–56]. Of note is a small case-control study on electric workers from New Zealand [51], which constructed a job-exposure matrix based on measurements obtained in a different study in New Zealand. Based on the exposures for 0.50–1.0  $\mu\text{T}$  the OR was 2.9 (95% CI 0.7–11.4) and for  $>1.0 \mu\text{T}$  the OR was 3.2 (95% CI 1.2–8.3), both in reference to exposures less than 0.21  $\mu\text{T}$ .

#### 6.2.4 Summary

In summary, there is enough consistency in the results from the large body of high-quality research on childhood leukemia to reach a general agreement that postnatal exposures above 0.4  $\mu\text{T}$  are associated with an elevated risk of childhood leukemia as compared with exposures less than 0.1  $\mu\text{T}$ ; this RR has been estimated at around 2, on the basis of two large pooled analyses. This is unlikely to be due to chance but may be partly due to bias. For other exposure measures and for adult leukemia the evidence is considerably weaker.

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### 6.3 Brain Cancer

#### 6.3.1 Background

Of all nervous system cancers, 90% occur in or around the brain. Cancers of the brain and nervous system (brain cancer) are rapidly fatal but fairly rare, with an incidence rate of 6 per 100,000 people per year in the United States [57]. Glioblastoma and astrocytoma are two subtypes of brain cancer that have been examined in relation to EMF exposure. Brain cancers are more common among males than females. Malignant neoplasms of the brain and other parts of the nervous system are the second most common form of cancer in children aged 0–19 y, representing more than one-fifth of all their malignancies.

There are few clearly recognized risk factors for brain cancer. In addition to ionizing radiation, occupational exposures that have been linked to adult brain cancer risk in some studies including, organic solvents, and pesticides. Excess risks have been found among farmers and painters, but the specific agents responsible for these risks have not been identified. Brain cancers have also been linked to exposure to *N*-nitroso compounds and halomethanes. People with a family history of cancer, certain genetic diseases, or head injury may have an elevated risk of brain tumors. Tobacco and alcohol use have not been linked to brain cancer risk. Thus, the causes of brain cancer are not well understood. Presently known or suspected factors appear to account for only a small proportion of all the cases.

#### 6.3.2 Childhood Brain Cancer

##### 6.3.2.1 Residential Exposures

Several of the childhood cancer studies described earlier examined brain cancer in addition to leukemia and other childhood tumors (see Table 6.2 for a list of the major studies). Two studies based in Denver, Colorado [11,13] found an association between wire codes and brain tumors. In the study by Feychting and Ahlbom [16], however, none of the exposure metrics were significantly associated with brain cancer.



**TABLE 6.2**

## Epidemiologic Studies of the Association between EMF and Childhood Brain Tumors

Ref.	Study Population	Study Design	Exposure	RR (95%CI) High Category
Wertheimer and Leeper [11]	Cases: deaths between 1950 and 1973, <19 y old. Controls: birth certificates. Denver, Colorado	Case-control: 66 cases, 66 controls	Wire code	HCC 2.4 (1.0-5.4)
Tomenius [21]	Cases: <19 y old. Controls: birth certificates. Stockholm, Sweden	Case-control: 294 cases, 253 controls	Measured field (front door)	$\geq 0.3 \mu\text{T}$ 3.7 (—)
Savitz et al. [13]	Cases: <15 y old. Controls: random digit dialling. Denver, Colorado.	Case-control: Wire code—59 cases, 259 controls	Wire Code	HCC 1.9 (0.5-8.0)
Feychting and Ahlbom [16]	Residents within 300 m of power line. Cases: <15 y old. Controls: random from cohort. Sweden	Measured field—25 cases, 207 controls	Measured field (spot, child's bedroom, low power)	$\geq 0.25 \mu\text{T}$ 1.0 (0.2-4.8)
		Nested Case-control: 38 cases, 554 controls	Historically calculated fields	$\geq 0.3 \mu\text{T}$ 1.0 (0.2-3.9)
Verkasalo et al. [18]	Residents within 500 m of power line. <17 y, 1974-1990. Finland	Cohort: $N = 39$	Historically calculated fields	$\geq 0.2 \mu\text{T}$ 2.3 (0.8-5.4)
Preston-Martin et al. [64]	Cases: <20 y old. Controls: random digit dialling. Los Angeles, California	Case-control: 833 cases, 1666 controls	Wire Code	VHCC 1.2 (0.6-2.2)
Gurney et al. [59]	Cases: <20 y old. Controls: random digit dialling. Washington State	Case-control: 120 cases, 240 controls	Wire Code	VHCC 0.5 (0.2-1.6)
Tynes and Haldorsen [17]	Residents in ward with high-voltage power lines. Cases: <15 y old. Controls: random from cohort. Norway	Nested Case-control: 144 cases, 599 controls	Historically calculated fields	$\geq 0.14 \mu\text{T}$ 90.7 (0.2-2.1)
UKCSS [30]	Cases: <15 y old. Controls: Family Health Services Authority. England, Wales, Scotland	Case-control: 390 cases, 393 controls	Measured field (Phase I: 90 min in family room + spot measurement in bedroom; Phase II: 48 h bedroom and spot measurement in school only for highest 10% in Phase I.	$\geq 0.4 \mu\text{T}$ 0 cases and 2 controls

Wire Codes: VLCC, very low-current configuration; OLCC, ordinary low-current configuration; OHCC, ordinary high-current configuration; HCC, high-current configuration.

In the most comprehensive study to date of childhood brain tumors, Preston-Martin et al. [58] found that brain cancer risk was not related to measured fields or high (HCC or VHCC) wire codes. A companion study [59] also found no association between high wire code and occurrence of brain tumors. Other studies [21,60,61] have addressed the potential relationship between magnetic fields and childhood brain cancer with apparently inconsistent results.

In general, in the studies of childhood brain tumors and residential EMFs, no consistent relationship has been reported [62]. However, these studies have generally been small and of low quality; a formal pooled analysis for brain cancer has yet to be completed.

### **6.3.2.2 Electric Appliance Exposure**

Four studies examined brain tumor occurrence and use of appliances by children or their mother during pregnancy [35,59,63,64]. No consistent or remarkable associations were reported in any of the studies. Positive associations of certain appliances found in individual studies were generally not replicated in other studies.

### **6.3.2.3 Parental Exposure**

Six case-control studies have examined the occurrence of childhood brain cancer or neuroblastoma among the children of fathers whose work presumably exposed them to EMF [65-70]. As in most occupational studies, exposure assessment was based on job titles, a method that may not be very precise. Elevated RRs (above 2.0) reported in the first two studies were not confirmed in the later studies. Predominance of positive findings in earlier reports could be due to selective reporting.

Some additional information regarding parental occupations and childhood brain tumor is provided by analyses of the childhood brain cancer studies [64,71]. These studies further support the absence of any association.

## **6.3.3 Adult Brain Cancer**

### **6.3.3.1 Residential Exposures**

Few studies have examined the potential association between brain cancer and residential exposure in adults, and none have looked at appliance use. Cancer of the brain or nervous system has been examined as a subtype in several studies of adult cancers with unconvincing results [12,38,39,44,72]. Most notable is a study by Feychting and Ahlbom [41], which showed no evidence of an association between residential EMF exposure and adult brain cancer, regardless of the method of estimating exposure. In the Feychting et al. [43] study, the residential and occupational exposures were combined by incorporating the estimates of occupational magnetic field exposure into Feychting and Ahlbom's earlier residential study [41]; it found no association between occupational or residential exposure and central nervous system tumors in adults exposed to higher levels of magnetic fields both at home and at work. Although the study has limitations, particularly that the occupational magnetic field measurements were from a different study and hence their applicability is questionable, it is important in that it attempts to incorporate both residential and occupational exposures. Results from a large study [73] of adult glioma and residential EMF exposure assessed through spot measurements, wire codes, and distance from electric facilities did not support an association. For residences with measurements above 0.3  $\mu\text{T}$ , there was a suggestion of increased risk; however, the number of cases and controls in this category was small.

### **6.3.3.2 Occupational Studies**

Many epidemiologic studies have examined the possible association between occupational exposures to EMF and brain cancer. In 1995, Kheifets et al. [74] conducted a review of 52 occupational studies; of these, 29 represented original research published in English and provided enough information to be included in a meta-analysis. These studies examined populations in 12 different countries, although most were conducted in the United States and Scandinavia. Although most studies presented a small elevation in risk, there was considerable heterogeneity in the results. An inverse-variance weighted pooling of all the data resulted in a small but significant overall increase in risk among individuals employed in the broadly defined group of electric occupations. The relative risk was higher for some specific jobs and for gliomas. The findings of this meta-analysis were not affected by inclusion of unpublished data, influence of individual studies, weighting schemes, and model specification. Kheifets [75] also reviewed the studies published since the 1995 meta-analysis [43,76–87] and concluded that these studies mainly showed no risk.

Although positive associations have been reported in some studies with employment in electric occupations, these studies have often had methodological limitations, such as reliance on a single job title for exposure assessment in early studies. More rigorous studies on workers in the electricity supply industries, with a specific focus on occupations with high potential for EMF exposure workers [88–90], have had fewer of these limitations. In a comparative analysis by Kheifets et al. [91], conclusions based on these rigorous studies were compatible with a weak association between magnetic fields and brain cancer; on the basis of a combined analysis of data, the RR per 10  $\mu$ T-y was 1.12 (95% CI 0.98–1.28). However, results were also compatible with chance fluctuation. This analysis concluded that what previously appeared to be important differences in results across studies are small and not a result of analytic methods.

### **6.3.4 Summary**

Brain cancer studies have shown inconsistent results. A pooled analysis of childhood brain cancer studies may be very informative and can inexpensively provide insight into existing data, including possibility of a selection bias, and, if appropriate (i.e., if studies are sufficiently homogeneous), come up with the best estimate of risk.

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## **6.4 Breast Cancer**

### **6.4.1 Background**

Breast cancer is the most commonly occurring malignancy in women in the United States. A considerable body of epidemiologic research has identified numerous factors that affect the risk of developing breast cancer in females. The disease occurs most frequently among whites, women of upper social class, women without children or with few children, and those who had their first child at a late age. Other risk factors include early age of menarche (menses), late age of menopause, obesity for postmenopausal women, proliferative fibrocystic disease, and a first degree relative with breast cancer, especially if it was diagnosed at a young age. Considerably less is known about male breast cancer but indications are that genetic and environmental factors including obesity, familial history, and endocrine factors play a causative role. Occupational studies indicate elevated rates of breast cancer among men in such jobs as newspaper printing, soap and perfume manufacturing, and health care.

Breast cancer incidence rates are highest in North America and northern Europe and lowest in Asia and Africa. Until recently, the search for possible explanations of this pattern had focused on the differences in dietary and reproductive patterns of women in societies with different degrees of industrialization. However, the role of diet in the etiology of breast cancer remains uncertain and reproductive risk factors apparently account for only a fraction of the excess disease reported in the modernized societies.

It has been proposed that one factor contributing to the greater occurrence of breast cancer in industrialized compared with nonindustrialized societies is the use of electric power and higher exposures to light at night or to magnetic fields. Stevens [92] hypothesized that EMF and light at night can affect breast cancer through suppression of melatonin. Epidemiologic investigations addressing the potential link between breast cancer and exposure to magnetic fields include occupational exposures and residential studies that examine breast cancer risk in relation to proximity to electric installations and the use of electric blankets.

## **6.4.2 Female Breast Cancer**

### **6.4.2.1 Residential Exposures**

Several studies of residential exposures and other adult cancers examined the risk of breast cancer in populations residing near power lines. One of these studies found an association between pre- but not postmenopausal breast cancer and wiring configuration [12]. The other two studies [38,39] did not detect any associations. Three recently completed large studies on breast cancer found no association with exposure to electric or magnetic fields [93–95].

### **6.4.2.2 Electric Blankets**

Two early case–control studies [96,97] did not support the hypothesis that the use of electric blankets increases the risk of post- or premenopausal breast cancer. Vena et al. [96] combined these two studies and found elevated risk among women who reported some use of electric blankets throughout the night (RR 1.5, 95% CI 1.1–1.9) for the past 10 years; however, the risk was not the highest among the highest exposure group, that is, those who reported daily use of the blankets in season and continuously throughout the night for 10 years (RR 1.20, 95% CI 0.8–1.9).

### **6.4.2.3 Occupational Studies**

Few occupational studies of electric workers included sufficient numbers of females to address the potential association of occupational EMF exposure and development of breast cancer. Albeit based on small numbers, four studies found no elevation in risk of breast cancer among females working in occupations with potential exposure to EMF as compared with low-exposure occupations [98–101].

One large study [102] used computerized mortality files from the National Center for Health Statistics for the years 1985 to 1989. Death certificates included the occupation and industry in which the decedent usually worked, coded according to the 1980 U.S. Census. Excluded were women whose occupation was listed as homemaker and those whose death certificate provided no occupational data; these two groups made up more than half of the database. Seven electric occupations used in previous studies were included, along with seven other occupations, such as computer programmers and telephone operators, presumed to have a large number of female workers and some potential for above-background EMF exposure. All other occupations were considered unexposed. Among 27,882 breast cancer cases and 110,949 controls, 68 cases and 199 controls had been employed in traditional electric occupations. The RR for breast cancer among those employed in electric occupations was 1.4 (95% CI 1.0–1.8). In a more detailed analysis, the

association was the strongest for the managerial and professional class and for those of 45–54 years of age. The RRs for the other occupations with potential exposure were around 1.0 or lower. In a separate analysis of the same data, but with a different approach to exposure grouping, Cantor et al. [103] did not find an association of female breast cancer and potential workplace exposure to EMF.

Epidemiologic studies based on death certificates alone have many limitations and are considered to be of a preliminary nature. In such studies, the population at risk is unknown. Thus, an apparent increase in the proportion of deaths from breast cancer among electric workers may be due to an increase in the proportions of deaths from other causes in the control group. The validity of the study results depends upon the accuracy of the occupational information reported on death certificates and the extent to which job titles alone reflect exposure to magnetic fields. Also, the authors did not take into account many risk factors known to be associated with breast cancer, including reproductive and family histories. Working in male-dominated jobs may have favored nulliparity, delayed childbearing, or other characteristics related to risk of breast cancer. The authors attempted to control for some of these characteristics by adjusting for social class. However, it is not clear how effectively social class was determined, and at best it can serve only as a partial control for known risk factors of breast cancer.

Although some earlier registry-based studies provided some support for a possible association between EMF exposure and female breast cancer [104], the most recent very large study, which incorporated exposure measurements in female workers, did not find an association [105].

### **6.4.3 Male Breast Cancer**

#### **6.4.3.1 Occupational Studies**

As described earlier, many studies have examined cancer among workers in electric occupations. As part of that examination, many considered breast cancer as one of the outcomes. Male breast cancer is so rare and most of the studies are not based on sufficiently large populations, so estimates of risk for male breast cancer often are not included in tables of results unless an excess risk has been observed [100,101,106–111]. This makes it difficult to evaluate the potential for the excess risk of male breast cancer. Nevertheless, several reports [98,112–115] are suggestive of a positive association, although negative results were reported by Loomis [116] and Cammarano [117]. The large studies of electric workers [88–90] did not identify any excess of male breast cancer.

#### **6.4.4 Summary**

For male and female breast cancer, the research began with a biological hypothesis confirmed by some early studies. More rigorous epidemiologic studies that followed showed no effect, thus it appears that EMF fields are not involved in the development of breast cancer.

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## **6.5 Lung Cancer**

Of the many studies [89,90,100,106,108–111,117] that have examined the possible association between electric occupations or power frequency magnetic field exposure and lung cancer, only two [101,107] report some elevation in risk.

Armstrong et al. [76] reported an association between lung cancer and cumulative high-frequency electromagnetic transients, or pulsed electromagnetic fields (PEMFs). In this study of EMF exposure and lung cancer it is possible that some residual confounding from smoking remains, because (a) smoking is a strong risk factor for lung cancer and (b) the length of employment is likely to be strongly correlated with both cumulative PEMF exposure and the amount and length of smoking rather than just current smoking status.

In summary, presently available evidence does not suggest an association between power frequency EMF exposure and lung cancer. The study of lung cancer and PEMFs needs replication.

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## 6.6 Other Cancers

Sporadic reports of elevated risks for other cancers have appeared in the literature, most notably for malignant melanoma, but none have been sufficiently suggestive to warrant presentation here. Finally, cancers of the prostate and pituitary gland could be of interest because of their roles in the production of hormones. Patterns of risk for these cancers in relation to jobs with exposure to magnetic fields remain largely unexplored.

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## 6.7 Reproductive Outcomes

### 6.7.1 Maternal Exposure

#### 6.7.1.1 Video Display Terminals

Video display terminals emit both extremely low frequency fields as well as higher frequencies of up to 100 kHz. The health risks associated with use of such devices, particularly adverse birth outcomes during pregnancy, have been examined in several studies [118–123]. For the most part, studies have not shown increased risks for spontaneous abortion, low birth weight, preterm delivery, intrauterine growth retardation, or congenital abnormalities.

#### 6.7.1.2 Electric Blankets and Heated Beds

Electric blankets and heated beds can be a major source of ELF EMF; it is estimated that exposure from water beds can be up to 0.5  $\mu\text{T}$  and electric blankets produce fields up to 2.2  $\mu\text{T}$  [124–126].

The findings of the studies on electric blankets or heated beds and reproductive health effects have been mostly negative (see Table 6.3). Furthermore, a related outcome, urinary tract anomalies, has also been shown to be unrelated to EMF exposure from such electric devices [127].

#### 6.7.1.3 Residential and Occupational Exposure

Residential EMF exposures have also been studied for possible association with reproductive outcomes (see Table 6.4). Studies using wire codes or proximity to power lines as EMF exposure proxy-measurements have suggested no association. Studies that have

**TABLE 6.3**

## Epidemiologic Studies on Electric Blanket and Heated Bed Exposure and Adverse Reproductive Outcomes

Ref.	Study Population	Design	Exposure	RR (95%CI) High Category
<i>Miscarriage</i>				
Belanger et al. [187]	Women receiving care at 11 private obstetric practices and 2 health maintenance organizations in New Haven, Connecticut area	Prospective: N = 2967	Electric blanket use at: Conception Conception, high setting Interview Interview, high setting Heated bed use at: Conception Conception, high setting Interview Interview, high setting	1.74 (1.0–3.2) 1.65 (0.6–4.9) 1.61 (0.8–3.2) 2.05 (0.7–4.7) 0.59 (0.3–1.1) 0.59 (0.3–1.1) 0.63 (0.4–1.1) 0.49 (0.2–1.1)
Lee et al. [188]	Women whose first prenatal appointment at one of three California Kaiser Permanente Medical Care Program facilities, 1990–1991	Prospective: N = 5144	Electric blanket Electric blanket, high setting Heated bed Heated bed, high setting	0.8 (0.6–1.2) 1.6 (0.6–3.3) 1.0 (0.7–1.3) 1.0 (0.7–1.5)
<i>Birth defects</i>				
Dlugosz et al. [189]	Cases from New York State Congenital Malformations Registry, born in 1983–1986. Controls selected at random from birth registrations	Case–control: 535 cases, 535 controls	Neural tube defect: Electric blanket Heated bed Oral cleft defect Electric blanket Heated bed	0.9 (0.5–1.6) 1.1 (0.6–1.9) 0.7 (0.4–1.2) 0.7 (0.4–1.1)
Milunsky et al. [190]	Women attending obstetric practices	Cohort: N = 23,491	Neural tube defect: electric blanket	1.2 (0.5–2.6)
Shaw et al. [123]	Cases from liveborn infants and fetuses, 1989–1991. Controls randomly selected from each area hospital	Study 1 case–control: 538 cases, 539 controls Study 2 case–control: 265 cases, 481 controls Study 3 Case–control: 662 cases, 734 controls	Neural tube defect: Electric blanket Heated bed Neural tube defect: electric blanket Heated bed Oral cleft defect (multiple): Electric blanket Heated bed	1.8 (1.2–2.6) 1.2 (0.8–1.8) 1.2 (0.6–2.3) 1.2 (0.8–1.9) 1.3 (0.5–3.4) 1.8 (1.0–3.2)

*Low birth weight and growth retardation*

Bracken et al. [124]

Women receiving care at 11 private obstetric practices and 2 health maintenance organizations in New Haven, Connecticut area

Prospective:  
N = 2967

Low birth weight—Electric blanket or heated bed:	1.2 (0.5–2.8)
Low setting, 3 <sup>rd</sup> trimester	1.2 (0.6–2.5)
High setting, 3 <sup>rd</sup> trimester	
Intrauterine growth retardation—	0.8 (0.4–1.7)
Electric blanket or heated bed:	1.6 (1.0–2.6)
Low setting, 3rd trimester	
High setting, 3rd trimester	

*Urinary tract anomalies*

Li et al. [127]

Cases identified from the Washington Birth Defects Registry. Controls randomly selected from among infants born in five large hospitals

All women: 118 cases, 369 controls  
Subfertile women: 37 cases, 85 controls

Electric blanket	1.1 (0.5–2.3)
Heated bed	0.8 (0.3–2.7)
Electric blanket	4.4 (0.9–23)

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TABLE 6.4

## Epidemiologic Studies on Residential EMF Exposure and Adverse Reproductive Outcomes

Ref.	Study Population	Design	Exposure <sup>a</sup>	RR (95%CI) High Category
<i>Miscarriage</i>				
Savitz and Ananth [191]	Residents Denver, Colorado Standard Metropolitan Statistical area	Case-control: <i>N</i> = 257	Home spot measurement $\geq 0.2 \mu\text{T}$	0.8 (0.3-2.3)
Belanger et al. [187]	Women receiving care at 11 private obstetric practices and 2 health maintenance organizations in New Haven, Connecticut area	Prospective: <i>N</i> = 2967	Very high wire code	0.4 (0.2-1.1)
Li et al. [129]	Pregnant women within northern California Kaiser Permanente medical care system	Prospective: <i>N</i> = 969	Personal TWA $\geq 0.3 \mu\text{T}$ Personal 24 h maximum $\geq 1.6 \mu\text{T}$	1.2 (0.7-2.2) 1.8 (1.2-2.7)
Lee et al. [130]	Members of the northern California Kaiser Permanente medical care system	Nested case-control: 155 cases, 509 controls	Very high wire code Home spot measurement $\geq 0.2 \mu\text{T}$ Personal TWA $\geq 0.128$ Personal 24 h maximum $\geq 3.51 \mu\text{T}$ Personal rate-of-change $\geq 0.094 \mu\text{T}$ Home spot measurement $\geq 0.2 \mu\text{T}$ Personal TWA $\geq 0.2 \mu\text{T}$	1.2 (0.7-2.1) 1.1 (0.5-2.2) 1.7 (0.9-3.2) 2.3 (1.2-4.4) 3.1 (1.6-6.0) 3.1 (1.0-9.7) 1.9 (0.6-6.1)
		Prospective: <i>N</i> = 219	Personal 24 h maximum $\geq 2.69 \mu\text{T}$ Personal rate-of-change $\geq 0.069 \mu\text{T}$ Early pregnancy loss Home spot measurement $\geq 0.25 \mu\text{T}$ Home spot measurement $\geq 0.63 \mu\text{T}$	2.6 (0.9-7.6) 2.4 (0.9-6.6) 1.1 (0.6-2.3) 5.1 (1.0-26)
Juutilainen et al. [120]	Work and fertility study of women attempting to become pregnant	Case-control: 89 cases, 102 controls		
<i>Low birth weight and growth retardation</i>				
Bracken et al. [124]	Women receiving care at 11 private obstetric practices and 2 health maintenance organizations in New Haven, Connecticut area	Prospective: <i>N</i> = 2967	Low birth weight: Very high wire code Personal TWA $\geq 0.2 \mu\text{T}$	0.8 (0.3-2.1) 1.3 (0.3-6.1)
			Intrauterine growth retardation: Very high wire code Personal TWA $\geq 0.2 \mu\text{T}$	0.7 (0.4-1.6) 1.2 (0.4-3.1)
<i>All anomalies</i>				
Robert et al. [192]	Cases from population-based registry in Central-East France, 1988-1991. Controls, matched for birth year and municipality.	11 cases	Distance to power line $\leq 100 \text{ m}$	0.9 (0.5-3.2)

<sup>a</sup>Table reports highest exposure category for measured fields.

used measurement-based exposure assessment have shown mixed results. One study using spot measurement at the front door suggested an association between early pregnancy loss and front door fields of above 0.63  $\mu\text{T}$  [120]. Although spot measurements have been shown to be correlated with personal exposure, there is concern that they result in large misclassification [128].

Using personal measurements, there was no association between exposure to time-weighted fields above 0.2  $\mu\text{T}$  and low birth weight or intrauterine growth retardation [124]. Most recently, a similar prospective study found no association between TWA personal measures and spontaneous abortion [129]. There was, however, a significantly increased risk (OR 1.8, 95% CI 1.2–2.7) when exposure above 1.6  $\mu\text{T}$  was used as the exposure metric of interest. In the same year, a nested case–control study using personal exposure measurements [130] showed that TWA magnetic fields were not associated with miscarriage, but there were in fact statistically significant dose–response trends for two different exposure metrics, maximum exposure and magnetic field rate-of-change.

Thus, there is some evidence for increased miscarriage risk associated with high residential EMF exposure; however methodological problems make interpretation of these studies difficult due to possible information bias. There is little evidence, of increased risks of adverse reproductive outcomes other than miscarriage.

### 6.7.2 Paternal Exposure

Paternal magnetic field exposure has also been studied in relation to adverse reproductive outcomes. The earliest study was by Buiatti et al. [131], in which persons with infertility were more likely to report radioelectric work than controls. However, Lundsberg et al. [132] observed no association between job titles with high EMF exposure and semen abnormalities. Similarly, there was no raised risk of abnormal birth outcome among offspring of power-industry workers [133].

An excess of birth defects was observed among children of fathers who were electronic equipment operators [134]. Similarly, an increased frequency of congenital malformations and fertility difficulties was observed among the offspring of high-voltage switchyard workers [135]. It has also been suggested that there is an association between EMF exposure and decreased male to female ratio in the offspring [136,137]. However, the results of the studies on paternal exposure and adverse reproductive outcomes are unconvincing.

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## 6.8 Cardiovascular Disease

Concerns about cardiovascular changes resulting from exposure to EMFs originated from descriptions in the 1960s and early 1970s of the symptoms among Russian high-voltage switchyard operators and workers [138]. Although these reports remain unconfirmed [139], more recent investigations have focused on direct cardiac effects of EMF exposure, mostly related to heart rate variability and subsequent acute cardiovascular events.

On basis of the idea put forth by Sastre et al. [140], Savitz et al. [141] hypothesized an association between exposure to EMF and acute cardiovascular disease. This hypothesis was based on two independent lines of evidence: The first was experimental data in which intermittent 60 Hz magnetic fields were found to reduce the normal heart rate variability [140]. The second came from several prospective cohort studies, which have indicated that reductions in some components of the variation in heart rate increase the

risk for (a) heart disease [142–144], (b) overall mortality rate in survivors of myocardial infarction [145–147], and (c) risk for sudden cardiovascular death [148]. Thus, Savitz et al. [141] postulated that occupational exposure to EMFs will increase the risk for cardiac arrhythmia-related conditions and acute myocardial infarction (AMI), but not for chronic cardiovascular disease. As postulated, they observed an increased risk of AMI and arrhythmia-related death with high exposure, but not for chronic cardiovascular disease. Other studies were not able to replicate their findings [149,150]. Similarly, using morbidity as the outcome measure Johansen et al. [151] and Ahlbom et al. [152] did not observe any association (see Table 6.5 for a summary of the major studies).

Hakansson et al. [153] observed an increased risk of AMI with high exposure, though nonsignificant. Only mortality studies of the association between occupational exposure to EMF and cardiovascular diseases have suggested an association. However, studies that use mortality records are questionable due to possible inaccuracies of the diagnosis of AMI on the death certificates. In general, it can be concluded that the evidence does not support a plausible etiologic relation between EMF exposure and cardiovascular disease.

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## 6.9 Neurodegenerative Disease

### 6.9.1 Alzheimer's Disease

Alzheimer's disease (AD) is characterized clinically by progressive loss of memory and other cognitive abilities (e.g., language, attention). Its onset is thought to be heralded by a phase of mild cognitive impairment in which cognition is not normal but not severe enough to warrant a diagnosis of dementia. The exact duration of mild cognitive impairment is unclear, but is likely to last at least a few years. Many persons with Alzheimer's disease also develop motor, behavioral, and affective disturbances. In particular, parkinsonian signs, hallucinations, delusions, and depressive symptoms are present in half or more of persons with the disease.

When evaluated across all the studies, there appears to be an association between estimated EMF exposure and disease risk (see Table 6.6). However, this result is mainly confined to the first two studies [154,155] and it is not clearly confirmed by the later studies [156–160]. The exception might be a study by Hakansson et al. [161]. The two studies [154,155] that show excess risk may have been affected by selection bias. Because the study populations are undefined, there is no way to determine the extent to which the controls are representative with respect to exposure of the population from which the cases originated. On the other hand, many of the negative studies are based on death certificates, which is particularly problematic because this diagnosis is often not reported as an underlying cause and is underrepresented as a contributing cause as well. Better studies of Alzheimer's are warranted.

### 6.9.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized clinically by progressive motor dysfunction, including painless muscle wasting and spasticity. Persons with ALS may develop cognitive and autonomic dysfunction. In particular, a frontal lobe dementia and hypotension may develop in persons with the disease. As the disease progresses, pulmonary function and dysphagia result in the need for artificial respiratory support and the insertion of feeding devices to maintain life.

**TABLE 6.5**

## Epidemiologic Studies of General Cardiovascular Events in Relation to EMF

Ref.	Study Population	Study Design	Exposure	Category
<i>Circulatory disease mortality</i>				
Baris et al. [179]	Workers employed in electric company between 1970 and 1988	Cohort: $N = 21,744$	Job-exposure matrix	Highest exposure category; SMR: 0.63 (0.53–0.74); RR: 0.91 (0.73–1.14)
<i>CVD</i>				
Kelsh and Sahl [83]	Utility workers, employed between 1960 and 1991, followed to 1992	Cohort: $N = 40,335$	Occupational categories	SMR: 0.62 (0.59–0.65); Linemen, RR: 1.42 (1.18–1.71)
Johansen and Olsen [164]	Male utility workers, employed between 1990 and 1993, followed 1974 and 1993	Cohort: $N = 21,236$	Classification of workplaces based on measurements	AMI—High exposure; SMR: 1.0 ( $O = 160$ )
Savitz et al. [141]	Male utility workers, employed between 1950 and 1986, followed to 1988	Cohort: $N = 138,903$	Duration of work in jobs with elevated EMF	AMI—Highest $\mu$ T-y group; RR: 1.62 (1.45–1.82)
Sahl et al. [149]	Utility workers, employed between 1960 and 1991, followed to 1992 (same as Kelsh and Sahl, 1997)	Cohort: $N = 35,391$	Duration of work in jobs with elevated EMF	Chronic CHD—RR: 1.0 (0.86–1.77) AMI—Highest $\mu$ T-y group; RR: 0.99 (0.65–1.51)
Hakansson et al. [153]	Swedish twins responding to job questionnaire in 1967 or 1973	Cohort: $N = 27,790$	Job-exposure matrix	Chronic CHD—RR: 1.19 (0.79–1.77) AMI—Highest exposure group; RR: 1.3 (0.9–1.9)
Sorahan et al. [150]	Utility workers, employed between 1973 and 1982, followed to 1997	Cohort: $N = 79,972$	Duration of work in jobs and locations with elevated EMF	AMI—Highest $\mu$ T-year category; RR: 1.03 (0.88–1.21) Chronic CHD—RR: 0.92 (0.73–1.16)
<i>Pacemaker implantation</i>				
Johansen et al. [151]	Male utility workers, employed between 1990 and 1993, followed 1974 and 1993 (same as Johansen and Olsen, 1998)	Cohort: $N = 24,056$	Classification of workplaces based on measurements	Highest exposure category; SMR: 1.00 (0.6–1.5)
<i>AMI morbidity</i>				
Ahlbom et al. [152]	Male population of Stockholm 1992–1993	Population based case-control: 695 cases, 1133 controls	Job titles, job exposure matrix	Highest exposure category; RR: 0.57 (0.36–0.89)

AMI, acute myocardial infarction; CHD, coronary heart disease.

**TABLE 6.6**

## Epidemiologic Studies of the Association between Alzheimer's Disease and Dementia and EMF

Ref.	Study Population	Study Design	Exposure	RR <sup>a</sup> (95%CI) High Category
<i>Alzheimer's Disease</i>				
Sobel et al. [155]	Patients at University of Helsinki, Finland, 1982–1985; Koskela Hospital, Helsinki, 1977–1978; and University of Southern California, 1984–1993	Case-control: all sites 387 cases, 465 controls	Work in occupations with exposure	0.2–1 $\mu$ T or > 1 $\mu$ T Intermittent to high > 1 $\mu$ T or > 10 $\mu$ T Intermittent exposure: 3.0 (1.6–5.4)
Sobel et al. [154]	Patients from an Alzheimer's disease treatment and diagnostic center	Case-control: 326 cases, 152 controls	Occupational exposure: medium and high EMF	3.9 (1.4–10.6)
Savitz et al. [156]	U.S. death certificates of males with occupational coding	Case-control: 256 cases, 768 controls	Electric occupation	1.2 (1.0–1.4)
Feychting et al. [158]	Population-based Swedish twin register	Case-control: 55 cases, 2 control groups: (i) 228 controls; (ii) 238 controls	Primary occupation Last occupation	$\geq 0.2 \mu$ T (i) 0.9 (0.3–2.8) (ii) 0.8 (0.3–2.3) $\geq 0.2 \mu$ T: (i) 2.4 (0.8–6.9) (ii) 2.7 (0.9–7.8)
Savitz et al. [157]	Cohort of electric utility workers	Cohort: $N = 139,905$	Occupational exposure	Cumulative career—2.1–4.7 $\mu$ T: 2.0 (0.6–7.0)
Feychting et al. [159]	Economically active individuals in the Swedish 1980 census followed from 1981 through 1995	Cohort: $N = 4,812,646$	Job-exposure matrix	$\geq 0.5 \mu$ T: 1.3 (1.0–1.7)
Håkansson et al. [161]	Cohort of Swedish engineering industry workers 1985–1996	Cohort: $N = 718,221$	Job-exposure matrix	$\geq 0.53 \mu$ T: 4.0 (1.4–11.7)
Qiu et al. [160]	Cohort of age 75 y and older in Stockholm, Sweden followed 1987–1989 until 1994–1996	Cohort: $N = 931$	Job-exposure matrix, measurement on historical equipment, and expert estimation high exposure	$\geq 0.18 \mu$ T: 1.1 (0.7–1.5)
<i>Dementia</i>				
Feychting et al. 1998 [158]	Population-based Swedish twin register	Case-control: 77 cases, 228 and 238 controls	Primary occupation Last occupation	$\geq 0.2 \mu$ T: 1.5 (0.6–4.0) and 1.2 (0.5–3.2) $\geq 0.2 \mu$ T: 3.3 (1.3–8.6) and 3.8 (1.4–10.2)
Johansen and Olsen [164]	Men employed in utility companies between 1900 and 1993 in Denmark	Standardized mortality study: $N = 21,236$	Job-exposure matrix, employment records	$\geq 0.1 \mu$ T: 0.6 (nonsignificant) senile dementia

<sup>a</sup>Relative risk for  $\mu$ T-y cumulative exposure.

The earlier studies on ALS examined the possible association of electric shocks and ALS. After the first study in 1964 suggested a plausible relationship [162], two subsequent studies from Japan, where the prevalence of electric work (as recorded in the medical history) and of electric shock was low, failed to provide any support for the hypothesis [163]. Although some of the later studies did report analyses that linked electric shocks to ALS [164–166], most of these studies do not allow examination of confounding of magnetic field effects from electric shock. It is conceivable that exposure to electric shocks increases ALS risk; and, clearly work in the utility industry carries a risk of experiencing electric shocks.

Numerous studies of the relationship between electric work and the experience of electric shocks have been published since the first reports (see Table 6.7 for a review of these studies). In sum, there is modest epidemiologic evidence to suggest that employment in electric occupations may increase the risk of ALS, however, separating the increased risk due to receiving an electric shock from the increased exposure to EMFs is difficult. Studies of ALS that can distinguish between electric shocks and magnetic fields would be of value.

### 6.9.3 Parkinson's Disease and Other Neurodegenerative Diseases

Parkinson's disease is characterized clinically by progressive motor dysfunction, including bradykinesia, gait disturbance, rigidity, and tremor. Many persons with Parkinson's disease develop cognitive, behavioral, and autonomic signs.

Of the three neurodegenerative diseases that have been considered, Parkinson's disease has received the least attention in epidemiology. Occupation has been considered as a possible cause of Parkinson's disease in several studies. The study by Wechsler et al. [167] included jobs likely to involve relatively high exposures to EMF and reported 3 of 19 affected men were welders against 0 out of 9 controls and that 2 other affected men had worked as electricians or electrical engineers. However, Savitz et al. [157] found very little evidence of an increased risk in electric workers. Overall the OR derived from the occupations of 168 men dying from Parkinson's disease and 1614 controls was 1.1 (95% CI 0.9–1.2).

In the Danish cohort study, the standardized mortality ratio (SMR) for parkinsonism was 0.8 based on 14 deaths and even lower for the more heavily exposed men (0.5). In the study of Savitz et al. [156], positive relationships were observed with both cumulative cancer exposure and exposure more than 20 years before death, neither of which were, however, statistically significant (RR 1.03 per  $\mu\text{T}\cdot\text{y}$  (95% CI 0.90–1.18), and 1.07 per  $\mu\text{T}\cdot\text{y}$ , (95% CI 0.91–1.26). Noonan et al. [168] reported a positive association with an OR of 1.50 (95% CI 1.02–2.19) for the highest exposure category for Parkinson's disease and magnetic field exposure in electric workers.

Feychting et al. [159] found no increased risk for vascular dementia, senile dementia, presenile dementia, Parkinson's disease, multiple sclerosis, or epilepsy for either men or women. Hakansson et al. [161] and colleagues also found no increased risk for Parkinson's disease or multiple sclerosis and they observed a decreased RR for epilepsy.

In sum, there is no evidence linking EMF and Parkinson's disease and only very weak evidence to suggest that they affect Alzheimer's disease. The evidence that people employed in electric occupations have an increased risk of developing ALS is substantially strong, but this could be because such employees have an increased risk of experiencing an electric shock rather than any long-term exposure to the fields *per se*.

**TABLE 6.7**

## Epidemiologic Studies of the Association between Amyotrophic Lateral Sclerosis and EMF

Ref.	Study Population	Study Design	Exposure	RR <sup>a</sup> (95%CI) High Category
Gunnarsson et al. [166]	Male population of Sweden 1970–1983	Case–control: 1067 cases (32 exposed), 1005 controls	Job title in census 1960: electric worker	1.5 (0.9–2.6)
Gunnarsson et al. [193]	Male population of central and southern Sweden in 1990	Case–control: 58 cases, 189 controls	Questionnaire: electric work and exposure to MF	0.6 (0.2–2.0)
Davanipour et al. [194]	Patients at outpatient clinic	Case–control: 28 cases, 32 controls	75th percentile of case distribution average exposure	2.3 (0.8–6.6)
Savitz et al. [156]	U.S. death certificates of males with occupational coding	Case–control: 114 cases	Electric occupation	1.3 (1.1–1.6)
Savitz et al. [157]	Cohort of electric utility workers	Cohort: <i>N</i> = 139,905	Occupational exposure	Cumulative career— 1.1–15.4 $\mu$ T: 1.2 (0.4–3.3) $\geq$ 0.3 $\mu$ T: 2.5 (1.1–4.8)
Johansen and Olsen [164]	Men employed in utility companies between 1900 and 1993 in Denmark	Standardized mortality study: <i>N</i> = 21,236	Job-exposure matrix, employment records	$\geq$ 0.5 $\mu$ T: 0.8 (0.6–1.0)
Feychting et al. [159]	Economically active individuals in the Swedish 1980 census followed from 1981 through 1995	Cohort: <i>N</i> = 4,812,646	Job-exposure matrix	$\geq$ 0.5 $\mu$ T: 2.1 (1.0–4.7)
Håkansson et al. 2003 [161]	Cohort of Swedish engineering industry workers 1985–1996	Cohort: <i>N</i> = 718,221	Job-exposure matrix	$\geq$ 0.53 $\mu$ T: 2.1 (1.0–4.7)

<sup>a</sup>Relative risk for  $\mu$ T-y cumulative exposure.

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## 6.10 Depression and Suicide

### 6.10.1 Depression

There have been few of studies on depression and EMF (see Table 6.8). The early studies on the effects of EMF on depression did not use validated scales for identification of depressive symptoms [169,170], hence limiting the inferences based on the results [171]. A number of recent studies, however, have used validated depression scales. Although one of these studies demonstrated an association between proximity to power lines and depression [172], others [173,174] have not provided support for such an association. Similarly, Savitz et al. [175] did not find an association in their study of U.S. veterans. In sum, the findings on depressive symptoms and EMF are largely inconsistent.

### 6.10.2 Suicide

The earliest studies relating EMF exposure and suicide showed that cases came from homes with significantly higher exposure levels than controls [176,177]. Studies since then have improved exposure assessment techniques; they have used different approaches, such as distance between home and power lines, measurements, and job titles [38,164,178–181]. The most recent occupational study, based on job titles recorded on death certificates, suggested a weak association with suicide [182] (see Table 6.9 for details on these studies). In sum, the recent studies on workers, particularly the study by van Wijngaarden et al. (2000) [181], suggests an excess risk of suicide in electric workers, but the data is sparse.

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## 6.11 Discussion

### 6.11.1 Challenges

As mentioned above, consistent epidemiologic evidence carries a good deal of weight when considering the potential health effects of EMF. Consistency in results is key because epidemiologic studies of EMF are difficult to design, conduct, and interpret for a number of methodological reasons. In addition to the usual methodological difficulties of designing and carrying out almost any good epidemiologic investigation, studying effects of EMF exposure poses unique and substantial difficulties. The difficulties that arise are in connection with three attributes of epidemiologic studies: outcome (disease), assessment of exposure, and interpretation of findings.

### 6.11.2 Outcome

Leukemias and malignant brain tumors have received the most attention in both residential and occupational EMF studies. While they are the most common childhood malignancies, in absolute terms they still are quite rare, and even rarer in adults. Thus, a major challenge in EMF epidemiology is the small number of cases available in any given study and the necessity for retrospective study designs. Furthermore, the etiologies of these diseases are poorly understood, making a search for confounding as an explanation for any observed association difficult.



**TABLE 6.8**

## Epidemiologic Studies on Depression and EMF

Ref.	Study Population	Study Design	Exposure	RR (95%CI) High Category
Dowson et al. [169]	Persons living near 132 kV line and persons who lived 3 miles away.	Cross-sectional: $N = 226$	Distance from overhead line	Association between depression and proximity to overhead power line
Perry et al. [170]	Persons discharged with depression from hospital and controls from electoral list	Case-control: $N = 359$	Measurements at front door	Average measurement: Cases = $0.23 \mu\text{T}$ Controls = $0.21 \mu\text{T}$
Poole et al. [172]	Residents along a transmission line right-of-way. Center for Epidemiologic Studies—Depression (CES-D) scale used	Cross-sectional: $N = 382$	Distance from power line	Near versus Far: 2.8 (1.6–5.1)
Savitz et al. [175]	Male veterans who served in the U.S. army. The Diagnostic Interview Schedule and the Minnesota Personality Inventory were used	Cross-sectional: $N = 4044$	Present job and duration	Electric worker: 1.0 (0.5–1.7)
McMahon et al. [173]	Neighborhood near a transmission line; sample of homes near a power line and one block away from the power line. CES-D scale used.	Cross-sectional: $N = 152$	Measurements at front door	Average for homes on easement, $0.486 \mu\text{T}$ and one block away, $0.068 \mu\text{T}$ : 0.9 (0.5–1.9)
Verkasalo et al. [174]	Persons from Finnish Twin Cohort Study who answered the Beck Depression Inventory	Cross-sectional: $N = 12,063$	Calculated fields	$\geq 0.1 \mu\text{T}$ : 0.5 (0.1–2.3)—mild depression 1.3 (0.2–9.4)—moderate depression 15.3 (3.5–66.5)—severe depression

**TABLE 6.9**

## Epidemiologic Studies on Suicide and EMF

Ref.	Study Population	Study Design	Exposure	RR (95%CI) High Category
Reichmanis et al. [177] and Perry et al. [176]	Suicide cases and controls in England	Case-control: $N = 589$	Distance from power lines Measurements at the homes of subjects	Higher estimated and measured fields at case homes
McDowall [38]	Persons resident in the vicinity of transmission facilities	Standardized mortality study: $N = 8$	Distance from power lines	Home within 50 m from substation or 30 m from overhead line 0.75 (nonsignificant)
Baris and Armstrong [178]	Deaths in England and Wales during 1970–1972 and 1979–1983	Proportional mortality study: $N = 495$	Job titles on death certificates	No increase for electric workers
Baris et al. [179,180]	Male utility workers. Cases: deaths from suicide in mortality registry. Controls: 1% random sample from the cohort	Case-control: 49 cases, 215 controls	Job-exposure matrix	Very high exposure ( $\geq 1.56 \mu\text{T}$ ): 1.38 (0.38–5.0) Blue collar electric workers only
Johansen and Olsen [164]	Male employees in utility companies observed during 1974–1993. Cases: deaths from suicide in mortality registry	Standardized mortality study: $N = 21,236$	Employment records and job-exposure matrix	$\geq 0.1 \mu\text{T}$ : 1.4 (nonsignificant)
van Wijngaarden [181]	Male electric utility workers	Case-control: 36 cases, 5348 controls	Jobs and indices of cumulative exposure based on measurement survey	Electrician: 2.18 (1.25–3.80) Line worker: 1.59 (1.18–2.14)
van Wijngaarden [182]	United States death certificate files for the years 1991 and 1992	Case-control: 11,707 cases, 132,771 controls	Occupation code; usual occupation and industry on the death certificates	1.3 (1.2–1.4)

EMF does not appear to be genotoxic, but biological evidence suggests that it might influence cellular function and proliferation. Therefore, it could act as a promoter or growth enhancer in carcinogenesis. Epidemiologic studies that attempt to consider such secondary events are rare and methodological developments in this area are needed.

### 6.11.3 Exposure

That the assessment of exposure is a major weakness of epidemiologic studies of EMF is not surprising, because several factors make assessment of EMF exposure more difficult than assessment of many other environmental exposures. Magnetic fields are variable in time and space, and our understanding of the contributions of the multitude of different sources to total exposure is limited. EMF exposure is ubiquitous, but neither detectable nor memorable in most circumstances. The difficulties in exposure assessment are further exacerbated by the retrospective nature of most EMF epidemiologic research, as many of diseases have long latency periods. To quantify past exposure that was unnoticed and unmeasured, epidemiologists rely on surrogate measurements or indicators of exposure. The surrogates used to study EMF have included wire codes, occupational job titles, questions regarding appliance use, and present day measurements. Further, some studies must rely on information provided by proxy respondents, especially for cancer and Alzheimer's disease, if the study subject has died or has been incapacitated.

Although occupational exposures are generally much higher than exposures encountered elsewhere, they are usually fleeting. The highly exposed worker generally encounters high fields not for hours but for seconds or minutes at a time while working. When we consider EMF exposure integrated over time, the brevity of high exposures in most work places and the large amount of time the individual spends in nonoccupational environments combine to wash out the distinctions between the supposedly highly exposed occupational groups and the general population. Thus, often we might not have enough separation between high and low-exposure groups to detect an effect of EMF exposure if we rely on TWAs.

Because EMF exposures are complex, numerous parameters have been used to characterize them, including transients, harmonic content, resonance conditions, peak values, as well as average levels. It is not known which of these parameters or what combinations of parameters, if any, are biologically relevant. If there were a known biological mechanism of interaction for carcinogenesis, it might be possible to identify critical parameters of exposure, including the relevant period or timing of exposure. Furthermore, environmental EMF is not detectable by the exposed person, nor is it memorable. Because it is ubiquitous, exposure assessment has to separate the more exposed from the less exposed, a much more difficult task than simply delineating the exposed from the nonexposed. There is also a considerable degree of variability in exposure in both the short- and long-terms, both of which are influenced by the variability in exposure over space, for example occupational versus household exposure.

All of these difficulties with EMF exposure assessment are likely to have led to substantial exposure misclassification, which is likely, in turn, to interfere with detection of an association between exposure and disease, if indeed such an association exists. In particular, if the true association is small or moderate, it will be difficult to detect with this amount of measurement error.

### 6.11.4 Interpretation

Three circumstances make it difficult to judge whether EMF poses a cancer risk: (a) difficulties in the appraisal of small risks (b) inconsistencies among and within studies,

and (c) lack of knowledge of other risk factors for some diseases that should be considered as potential confounding variables.

Small risks are notoriously hard to evaluate, both because it is difficult to achieve enough precision to distinguish a small risk from no risk and because small risks are more vulnerable to subtle confounding and biases that can go undetected. Study quality remains an important consideration in evaluating epidemiologic evidence of EMF. As noted earlier, the quality of recent studies has improved in a number of important ways. However, earlier studies, many of which were fraught with methodological difficulties, still represent a substantial proportion of available evidence. Any attempt to summarize the available evidence, whether narrative or meta-analytic in its approach, must explicitly consider study date and study quality to arrive at a summary risk estimate that is not unduly influenced by less well-done studies. Given the difficulties with EMF exposure assessment and the other methodological difficulties outlined above, it is not surprising that many results of EMF studies are inconsistent. Nevertheless, an assessment of the internal and external consistency of studies is paramount to our understanding of the underlying risks.

Another impediment to a clear interpretation of these epidemiologic studies is the absence of a dose–response relationship. Distortion of the underlying dose–response relationship by substantial exposure misclassification [183] or by a nonmonotonic relationship between exposure and disease, has been offered as potential explanation. However, both possibilities remain speculative. A well-characterized and reproducible relationship between the most relevant aspect of exposure and disease is needed for the association to be scientifically acceptable.

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## 6.12 Conclusions

Overall, with over two decades of epidemiologic investigation on the relation of EMF to the risk of various diseases, we have learned a great deal; however there remain a number of uncertainties. Among all the outcomes evaluated in epidemiologic studies of EMF, childhood leukemia in relation to postnatal exposures above 0.3–0.4  $\mu\text{T}$  is the one for which there is most evidence of an association. Further study is warranted only if investigations are of high methodological quality, of sufficient size and with sufficient numbers of highly exposed subjects, and studies must include appropriate exposure groups and sophisticated exposure assessment. Particularly for childhood leukemia, little can be gained from further repetition of investigation of risks at moderate and low exposure levels, unless such studies can be designed to test specific hypotheses, such as selection bias or aspects of exposure not previously captured. Investigations of major diseases, such as breast cancer and cardiovascular disease, although initially biologically driven, did not confirm biological hypotheses or early positive studies. There is good evidence that these diseases are not associated with EMF exposure. Further work on neurodegenerative diseases is warranted as current evidence indicates a potential association, but is based on studies with numerous methodological limitations.

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## *Epidemiological Studies of Radio Frequency Fields*

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**Maria Feychting**

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### 7.1 Introduction

Historically, exposures to more than minimal radio frequency (RF) fields have been rare and mostly limited to occupational settings. In the general population the exposure sources were distant radio and television transmitters, with fields far below exposure guidelines. With the introduction and widespread use of mobile phone technology, the number of people in the general population exposed to low level RF fields has increased tremendously, not only through the use of the phone itself but also through the exposure from base stations, although fields from base stations are considerably lower than from the phones. The rapid increase in mobile phone use has led to a growing concern among the public about potential harmful effects of these exposures.

Current exposure guidelines are based on acute effects from heating of tissue. For exposure levels below these guidelines there is no known mechanism by which extremely low frequency (ELF) or RF fields might cause health effects such as cancer or different types of symptoms. This chapter will review the epidemiological evidence regarding potential health effects of exposure to RF fields below current guidelines that cannot cause substantial heating of tissue.

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## 7.2 Epidemiology

Epidemiological studies are important in the process of health risk assessment; they study the effects of exposures under real circumstances and in the relevant host (humans). However, epidemiological studies also have some limitations; the most frequently discussed are the possibility of confounding, exposure misclassification, and selection bias. These problems can be handled more or less successfully, depending on how the study is designed and conducted. As with all types of research, we must evaluate the scientific evidence carefully to assess the quality of all the available studies to determine if any, and in what way, potential sources of error might have influenced the results.

The two most commonly used designs of epidemiological studies are cohort studies and case-control studies. In a cohort study, assessment of the studied exposure and potential confounding factors must be made for all persons included in the study population. The study population is followed over a period of time, and all cases of the studied disease are identified. It is essential that the same methods to identify the cases be used in both the exposed and unexposed part of the population. In a case-control study, all cases of the disease are identified, just like in a cohort study, and assessment of the exposure and potential confounding factors is made. If population-based disease registers are not available, cases are often identified directly from one or several hospitals. The study population is then defined as the individuals that would seek care at these hospitals had they contacted the studied disease; often the catchment areas of the hospitals that are included. For the part of the population that did not have the disease, information about the exposure is obtained for a representative sample of the population from which the cases came (controls). If the controls truly are a random sample of the study population, they will provide valid information about the exposure distribution in the population. Sometimes when population registers are not available for identification of controls, other methods are used such as selection of controls from patients with other diseases that are not related to the studied exposure (hospital controls) or among friends or persons living in the same neighborhood as the cases.

Selection bias can occur if the controls in a case-control study are not selected randomly from the study population and if the probability of getting selected as a control is related to the studied exposure, either directly or indirectly, e.g., through socioeconomic status. Selection bias might also occur if not all cases are participating in the study and if the probability of participation is related to the exposure.

Exposure misclassification occurs to a varying degree in all studies. A very crude exposure estimate is, for example, the use of job title information from censuses to estimate occupational exposure; obtaining detailed information about a person's job history, including specific job tasks and substances used, reduces but does not remove the possibility of exposure misclassification. If misclassification of the exposure is unrelated to the disease, i.e., degree of exposure misclassification is similar for cases and others (nondifferential exposure misclassification), the effect on the risk estimate will be a dilution toward unity. If the probability of exposure misclassification is related to the disease (differential exposure misclassification) the risk estimate can be affected in any direction. If exposure information can be collected independently of the disease, e.g., before disease onset or from historical records, there is no risk for differential exposure misclassification. In case-control studies, however, exposure information is often collected through a questionnaire or interview after occurrence of disease. If cases and controls differ in their ability to remember their exposures, differential exposure misclassification may occur, i.e., recall bias.

Confounding can occur when a risk factor other than the studied exposure is related to both the exposure and the disease. A classic example is a finding of an association

between yellow fingers and lung cancer. Here it is obvious that smoking is a confounder and if controlled in the analysis, the association between yellow fingers and lung cancer would disappear. To completely explain an observed association, in the sense that the confounder rather than the exposure can be considered as the cause of the association, a confounder needs to be strongly related to the exposure and at the same time have an association with the disease that is considerably stronger than the observed association between the studied exposure and the disease. As long as information has been collected about exposure to potential confounding variables, adjustments can be made in the analyses to eliminate the effect of the confounders.

Generally, a cohort study is considered to be the most valid study design, because the potential for bias is greater in the case-control studies. This is, however, a somewhat too simplistic assumption. In cohort studies of rare diseases the study population needs to be very large (several hundred thousand individuals), and it is often not possible to do a very detailed exposure assessment for such a large number of individuals. Therefore, many cohort studies use quite crude exposure assessment methods, e.g., register-based data on occupational titles, which hampers the possibility to detect modestly increased risks because of dilution of the effect estimates caused by nondifferential exposure misclassification. A case-control study can use more sophisticated exposure assessment methods; and sometimes it may be possible to combine information obtained independently of cases and controls, e.g., from registries or historical records, with information collected through questionnaires to limit the potential for recall bias. If population registries are available, controls can always be selected randomly from the studied population to avoid potential selection bias.

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### 7.3 Exposure Sources

People may be exposed to different sources of low-level RF exposure, but the most common source today is exposures related to mobile telephones. Currently this technology uses the frequencies from 450 to 2500 MHz, although this is a technology in constant development and therefore the frequency range may change in the future. Radio and television transmitters, which are the other major sources of exposure to the general population, operate at frequencies between 200 kHz and 900 MHz. The third main exposure source is occupational, e.g., RF PVC welding machines, plasma etching, and military and civil radar systems, all operating at different frequencies.

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## 7.4 Cancer

### 7.4.1 Brain Tumors and Mobile Telephony

For RF exposure during mobile phone use, although below current guidelines, the exposure levels are of the same order of magnitude as the exposure standards. The exposure is concentrated in the part of the head closest to the handset and the antenna, and it declines rapidly with distance to the antenna. Therefore, the use of hands-free equipment while talking on the phone reduces the exposure of the head considerably while usually increasing the exposure of another part of the body that is near the phone's antenna.



Epidemiological studies of health effects related to RF exposure from mobile telephony is a relatively new research area, and only a limited number of studies are available. These have primarily focused on brain tumors, although a few of the studies included other types of tumors. Handheld mobile phones have only been available since the later part of the 1980s and have become common in the general population only during recent years. Today, a vast majority of the population in many countries use mobile phones; in Sweden, for example, over 80% of the population were mobile phone users in 2003, whereas it was less than 10% in the beginning of the 1990s. Considering the relatively short period of exposure it is currently possible to evaluate only the short-term effects of mobile phone exposure.

Seven studies of mobile phone use and brain tumors (excluding acoustic neuroma) have been published so far [1–7]. The majority of these have found no effects on brain tumor risk, but there are a few exceptions. A Finnish case–control study found an increased risk of glioma related to use of analogue phones [1]. Information about the exposure was collected from registries kept by the mobile phone operators, and therefore there was no risk of recall bias. The exposure misclassification is likely to be considerable, as the investigators had no access to information about corporate mobile phone users, who are likely to be among the heaviest mobile phone users. This type of error cannot, however, explain the increased glioma risk as there is no reason to believe that the exposure misclassification would differ between cases and controls. The risk increase was found after only 1 to 2 years of subscription to an analogue phone. This very short time between exposure and effect seems unlikely to be real; it usually takes a longer time for a tumor to develop and be diagnosed. Furthermore, if mobile phone use truly has an effect on glioma risk after such a short duration, the incidence of glioma should have increased during recent years in countries where there has been a rapid increase in mobile phone use in the general population. There is, however, no evidence of such an increase in a study of the glioma incidence in the Nordic countries, where mobile phone use in the general population started relatively early [8].

A Danish study used exposure assessment methods similar to the Finnish one, but was designed as a cohort study [5]. No indications were found of increased risks for any tumor types. The Danish study has the same limitation as the Finnish study in terms of exposure misclassification, and a modest risk increase would probably remain undetected. A more severe limitation in both the Danish and Finnish studies is the very short duration of exposure in the population when the studies were performed, e.g., only 8% of the cohort in the Danish study had used a mobile phone for at least six years.

Two case–control studies from the United States reported no association between brain tumor risk and mobile phone use [4,6]. Specific analyses of the side on which the mobile phone usually was held did not change these findings. These studies are so-called hospital based studies; cases are identified at certain hospitals and controls are selected from other patients at the same hospitals. This type of design assumes that patients with other diseases can be viewed as a representative sample of the population from which the brain tumor cases came and correctly reflects the exposure distribution, i.e., mobile phone use, in this population. The strength of these studies is the rapid case ascertainment, which is important in a study of malignant brain tumors where the poor prognosis of the disease may prevent many cases from participating in the study. A limitation is the short period of mobile phone use in both studies; very few subjects had used a mobile phone more than five years. Therefore, no conclusions can be drawn about potential effects of long-term exposure.

A Swedish case–control study reported no overall increase in brain tumor risk related to mobile phone use [3], but found an increased risk on the side of the head where the mobile phone was usually held [9]. On the opposite side of the head the risk was reduced. Recall bias is a likely explanation for this finding; it seems biologically implausible that

mobile phone use would protect against brain tumors on the opposite side of the head. The same group reported similar results in a subsequent larger study [2,10]. In a reanalysis of the larger study using a different method, the risk reduction on the opposite side of the head was not as evident [11]. The studies by Hardell et al. have been criticized for limitations in methods, analysis, and presentation of the study [12].

#### **7.4.2 Acoustic Neuroma and Cancers Outside the Brain**

Acoustic neuroma is a slow-growing benign tumor situated in the vestibular portion of the eighth cranial nerve, a part of the head where the RF exposure from the mobile phone is high relative to other sites, though still below exposure guidelines. Currently, eight studies have investigated the association between mobile phone use and acoustic neuroma [2–5,13–16]. The Danish cohort study described above had too few cases with long-term exposure to provide any meaningful information [5], and the study by Warren et al. had a design that was not adapted for studying acoustic neuroma [16] and thus will not be further mentioned here.

The two case–control studies from the United States mentioned in [Section 7.4.1](#) about brain tumors reported no increased risk of acoustic neuroma, regardless of which side of the head the mobile phone was used [4,15]. The problem with the short exposure duration in these studies is even more pronounced for acoustic neuroma because of the slow-growing nature of the tumor, resulting in a long latency period before the tumor is detected. Both studies reported a slightly increased risk of acoustic neuroma in the category of users with the longest duration of use, but the risks were not statistically significant.

A Swedish case–control study that is part of the Interphone study, a large international collaboration coordinated by the International Agency for Research on Cancer (IARC), found an increased risk of acoustic neuroma among subjects who started to use a mobile phone at least 10 years before diagnosis [14]. The risk increase was confined to tumors on the same side of the head as the phone was usually held, and risk estimates on the opposite side were close to unity. There was no indication of increased risks for mobile phone use with less than 10 years' duration, which is consistent with the findings in the U.S. studies. A Danish study that is part of the same international collaboration and used the same study protocol found no risk increase [13], but the number of subjects with long-term mobile phone use in the Danish study was very small. Thus, the power to detect an effect after long-term mobile phone use was severely limited.

In the latest study by Hardell et al. described above, a more than threefold risk increase of acoustic neuroma was found among users of analogue mobile phones, but with no relation to induction period [2], i.e., an increased risk was found also after a short duration of exposure. It seems highly unlikely that a higher incidence of acoustic neuroma would be detected within a few years of mobile phone use, considering that the tumor can be present more than five years before it is diagnosed [17].

Other types of tumors that have been studied are salivary gland tumors and uveal melanoma. Three studies are available on salivary gland tumors, and none of them reported any effects on disease risk [1,5,18]. They are however limited by poor statistical precision especially for analyses of long-term mobile phone use. Two studies have investigated the risk of uveal melanoma but with conflicting results [19,20].

#### **7.4.3 RF Exposure from Transmitters**

The levels of RF exposure from mobile phone base stations and radio and television transmitters are several orders of magnitude lower than current guidelines and are only a

fraction of the exposure emitted during use of a mobile phone. An important difference compared to mobile phone use is, however, that transmitters expose the whole body and exposure duration is usually considerably longer.

To date, all studies on exposure from transmitters and cancer risk are based on populations living in the vicinity of radio and TV antennas; no studies around mobile phone base stations have been published as yet. Previous reviews have noted major limitations in the available studies, which weaken the conclusions that can be drawn [12,21,22]. One of the most pronounced problems is related to the exposure assessment. All studies have used distance from a broadcasting tower as a proxy for exposure, and no measurements have been taken. There is evidence that the correlation between distance from a transmitter and RF exposure is poor. The broadcast beam is aimed distantly, and the areas closest to the transmitter may have low-exposure levels, whereas more distant areas are those with the highest exposure. Furthermore, no account is taken of ground reflections and signal reductions from buildings, vegetation, and the surrounding landscape. Furthermore, all studies have had an ecological design; exposure has not been determined for each individual, but for the entire population living within certain distance bands from the transmitter. Therefore, no information has been available on potential confounding variables. Yet another problem is that several of the studies were conducted as a response to public concern about an excess of cancer cases in the vicinity, which has influenced the design of the studies. Several of the studies are also based on a small number of exposed cases.

Some studies have suggested an increased risk of leukemia in people living in the vicinity of radio and television transmitters [23,24], whereas another considerably larger study found no risk increase [25]. The limitations discussed above prevent any conclusions from these studies.

The research on exposures to RF fields from transmitters and cancer risk is at a very early stage of development. A prerequisite for an informative study of high quality is that the exposure assessment is improved considerably, and that study designs using individual exposure assessments is applied, including the ability to adjust for potential confounding factors. Development of a meter that can be used in large-scale epidemiological studies is underway and will be of great value.

#### **7.4.4 Occupational Studies**

Occupational exposure to RF fields has been studied over more than 20 y, and most investigations have focused on the risk of hematological malignancies, e.g., leukemia, and brain tumors. A variety of occupations have been investigated, including a range of different exposure frequencies, but exposure assessment methods have generally been poor. None of the studies have made measurements of the RF exposure for the subjects included in the study, and often the exposure classification has been based on the job title alone with no actual knowledge about the exposure levels for the workers in the particular occupations. No or only limited control of confounding has been made, and several of the studies have a poor statistical power.

In a study of Norwegian electrical workers [26], based on a categorization of occupational titles as probably exposed to RF fields, an increased risk of leukemia and a risk estimate below unity for brain tumors was reported. It is not clear which occupations these were and if other potentially harmful exposures could be present in the occupations that might possibly explain the findings.

A study of Polish military personnel reported increased risks for both leukemia and brain tumors, as well as for other cancer types [27,28]. This study has severe

methodological limitations and an unconventional study design. The authors put much more effort into finding exposures among the cancer cases than among the personnel that had not been diagnosed with cancer, using multiple sources of information for the cases, but not for healthy persons. The bias introduced by this kind of procedure will inevitably lead to findings of increased cancer risks, even if no such associations exist; and no weight can be given these studies in an evaluation of the scientific evidence regarding this question.

A cohort study of radar technicians in the US navy reported no increased leukemia mortality and a risk reduction for brain tumor mortality when compared to the general population [29]. An reduced mortality overall and for many specific diagnostic categories is noticeable in the comparisons with the general population. Internal comparisons within the cohort showed an increased risk of leukemia in the highest exposed group, primarily for the group of aviation electronics technicians. No potential confounding factors were controlled in the analyses, and there were indications that smoking was less common in the cohort compared to the general population. Another US study found a decreased leukemia risk among aviation electronics technicians [30], but based on small numbers.

A cohort study of workers employed in the design, manufacture and testing of wireless devices such as mobile phones reported reduced risks of both leukemia and brain tumor mortality when compared to the general population [31]. A considerably reduced overall mortality was noted, as well as reduced overall cancer mortality. Exposure was assessed on the basis of expert opinion; no personal measurements were available. Internal comparisons within the cohort did not indicate any associations between the exposure and disease risk.

Although some increased cancer risks have been found in certain studies, there is no consistent evidence of risk increases for any cancer sites. The available studies have, however, not convincingly demonstrated that RF exposure has no effect on cancer risk, mainly because of limitations in the exposure assessment which could lead to a dilution of risk estimates, should there be an increased risk. There is also evidence of the so-called healthy worker effect in several of the studies, demonstrating considerably reduced risk estimates in comparisons with the general population. A working population is overall healthier than the general population, and certain occupations have specific demands on good health, e.g., policemen, making comparisons with the general population invalid. The use of mortality as the outcome of interest is also a limitation in several of the studies. A thorough review of all the occupational studies can be found in the Stewart report [21] and the more recent AGNIR report [12].

#### **7.4.5 RF Exposure and Cancer—Conclusions**

For brain tumors other than acoustic neuroma, the current evidence does not indicate any increased risks related to mobile phone use. It is important to note, however, that the available evidence only relates to mobile phone use over a short period of time and that conclusions about long-term exposure cannot be drawn based on the data available today. Several of the studies have limitations, especially regarding exposure assessment, which may hamper the possibility to detect a modestly increased risk, should one exist.

For acoustic neuroma there are some findings that indicate that there might be a risk increase associated with mobile phone use over a longer time period. It is, however, not possible to exclude other explanations to the findings, and additional well-designed studies are needed. Several studies included in the international Interphone study are currently under way and will provide additional information. A way to reduce the potential for recall bias would be to combine information from questionnaires or interviews with register-based data about mobile phone subscriptions from the mobile phone operators.

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## 7.5 Subjective Symptoms

Very few epidemiological studies are currently available of subjective or self-reported symptoms, such as headache, dizziness, loss of appetite, fatigue, local or generalized feelings of heat, related to mobile telephony. In addition, all studies available on mobile phone use [32,33] or on exposures from mobile phone base stations [34,35] are cross-sectional, which makes them of limited value for health risk assessment. The subjects themselves reported the occurrence of various subjective symptoms and estimated their own exposure, e.g., distance to the nearest base station or amount of mobile phone use, and no attempt was made to verify the exposure or disease. The studies of base stations have not adequately described how subjects were selected to be participants in the study, and there is a strong possibility that both selection and reporting bias have affected the results. To date, the available epidemiological studies on symptoms do not provide information of sufficient quality for an evaluation of the effect of mobile phones or base stations on the occurrence of different types of symptoms. It is sometimes discussed that there might be a subgroup of the population that are particularly sensitive to electromagnetic field exposure, "electrically hypersensitive" subjects, and that studies of symptoms in the general population would be unable to detect effects in this small subgroup. In studies trying to identify persons with "electrical hypersensitivity," a severe problem is that there are no clearly defined diagnostic criteria and the exposure is part of the disease definition.

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## 7.6 Pregnancy Outcomes

Various pregnancy outcomes have been studied in relation to RF exposure: spontaneous abortions, birth weight, gender ratio, and congenital malformations. Epidemiological studies have focused on occupational exposures, primarily among physiotherapists. A detailed summary of the available studies can be found in Ref. [12].

Findings for spontaneous abortions are mostly negative; an increased risk was reported in association with use of microwave diathermy in one study [36], but three other studies did not support this finding [37–39].

Several studies of female physiotherapists exposed to high-frequency electromagnetic fields have found no effect on the risk of congenital malformations in their offspring [39–41]. An increased risk was, however, reported in a Swedish study [42], although based on small numbers. Work with deep heat therapy was found to be associated with an increased risk of congenital malformations in a Finnish study [37], but with no dose response pattern.

Two studies are available on the gender distribution in offspring to female physiotherapists. A Danish study found a lower proportion of male offspring [38], but this finding was driven by the higher prevalence of female offspring in the unexposed comparison group. A study based on members of the Swiss Federation of Physiotherapists reported no effects on gender ratio in offspring to exposed mothers [43]. This study also analyzed the prevalence of low birth weight ( $\leq 2500$  g) in offspring born to physiotherapists and found no effects. In contrast, an increased risk of low birth weight was reported in a study of physiotherapists in Israel [40].

The evidence on potential effects of RF exposure on pregnancy outcomes is virtually limited to occupational exposures among physiotherapists; and although some positive findings have been reported, these findings have not been consistent with regard to specific

type of malformation or other adverse outcome. Several of the studies have limited statistical power, especially for rare outcomes such as malformations, and there is a potential for recall bias. The available data does not allow conclusions in any direction.

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## 7.7 Discussion and Conclusions

Compared to ELF epidemiology, studies of RF exposure, particularly from mobile phones, form a relatively new and undeveloped research field, especially regarding exposure assessment. Considerable improvements of the exposure assessment methods are to be expected in future studies, with consideration taken of factors related to the adaptive power control used in mobile telephony that makes the mobile phones down-regulate the output power to lower levels when the user is close to a base station, thereby decreasing the exposure up to 1000 times [44]. Relevant variables are, for example, mobile phone use in urban or rural areas, use of the phone inside or outside, or when being stationary or moving. There is also a need to be able to utilize sources of exposure information that is independent of the subjects included in the study, to avoid potential recall bias in studies performed retrospectively, such as case-control studies. This could for example be information from registries kept by the operators, combined with questionnaire data. An alternative would be to perform a prospective cohort study of mobile phone users, where information about mobile phone use is collected from both the operators and the users, but before the occurrence of the disease. A cohort study has the additional advantage that several different outcomes can be studied at the same time.

Occupational studies have been performed over the last 20 years, but these studies are limited to certain occupational groups, generally with no information about the actual exposure in the included occupations. Currently, efforts are being made to improve the knowledge about RF exposure levels in different occupations through actual measurements, but these efforts are not yet available for use in epidemiological studies. A meter suitable for use in large-scale epidemiological studies has been developed and is likely to be available in the near future [45]. This will greatly improve exposure assessment in occupational studies and thereby also the possibility to detect modestly increased risks, should they exist.

There is a public concern about potential health effects of exposure from mobile phone base stations, even if the exposure levels are orders of magnitude lower than the exposure from the phone itself. The feasibility of epidemiological studies of base station exposure is currently being investigated [46], and the newly developed RF field meter may make it possible to perform studies also on base station exposures.

Currently, there is no or only very limited evidence from epidemiological studies suggesting health effects of exposure to RF fields at levels below exposure standards. The findings for acoustic neuroma may suggest an increased risk, but are still highly uncertain [47], and additional studies are needed before any conclusions can be drawn.

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## *EMF Standards for Human Health*

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### 8.1 Introduction

The strength of electromagnetic fields (EMF) to which humans are exposed has been increasing gradually with the growth of electric power generation and transmission, the development of new telecommunication systems, and advances in medical and industrial applications. Although the health effects of EMF on humans have been of research interest for several decades, development of standards that incorporate the limits of

human exposure to nonionizing radiation (NIR) has occurred more recently for some parts of the electromagnetic frequency spectrum.

Historically, emerging technologies have triggered the development of EMF standards in different parts of the spectrum. In the radio frequency (RF) fields range, limits to protect human health were introduced in the 1950s, soon after the introduction or proliferation of radar and radio and television broadcasting. The need for exposure standards was highlighted by the observation of acute thermal injury in people exposed to high-level RF fields, especially in the microwave range. The United States and the Soviet Union were the first countries to develop RF standards, but their limits were based on different scientific evidence. With the introduction of industrial equipment, such as induction heaters and heat sealers, later standards recognized the vastly expanded use of the electromagnetic spectrum, especially at lower-frequency RF. Early RF standards were aimed at restricting thermal effects seen at high levels of exposure [1]. However, scientists in both the East and the West continue to postulate that nonthermal effects could also be important, and this has been the driving force for RF research for some decades.

In the extremely low frequency (ELF) region, an epidemiological study by Wertheimer and Leeper [2] was the key publication suggesting that there could be a health concern with exposure to ELF magnetic fields. Their studies suggested that exposure to ELF fields from high current configurations in power lines seemed to be associated with an increased incidence of childhood leukemia. This was supported by a few of the subsequent studies, including those by Savitz and coworkers [3] and more recently by two pooled analyzes by Ahlbom et al. [4] and Greenland et al. [5].

Research results drove legislation for both RF and ELF fields. More recently, the well-publicized putative link between ELF magnetic fields and childhood leukemia and the growing exposure to new technologies at home and in the workplace, e.g., computer monitors and televisions, microwave ovens and mobile phones, have triggered public concern and calls for control and regulation limiting public and occupational EMF exposure.

This chapter describes the available types of EMF standards, and includes an overview of the standard-setting bodies involved in NIR at the international and the national level. It also provides a discussion of the methodology for developing standards, which entails both a scientific approach and a political process. Finally, a snapshot on trends in worldwide NIR exposure standards is given.

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## 8.2 Types of Standards

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 a group of people.

The ultimate goal of health-based EMF standards is to protect human health. However, there is often confusion about the various types of standards that exist to limit human exposure to EMF. Standards can specify either limits of emissions from a device or limits of human exposure from all devices that emit EMF into a living or working environment. These limits can be developed for the general public or for specific populations such as workers, medical patients, military personnel, children, or the elderly. Also, EMF standards can be classified as to whether they are voluntary measures or legally binding.

Many forms of EMF find application in medical practice, often at exposure levels that are much greater than those subjected to the general population. EMF exposures that are part of medical treatment or diagnosis are usually considered to lie outside the scope of

exposure guidelines as they involve different risk-benefit considerations. This chapter discusses various types of EMF standards for emission and exposure limitation applied for nonmedical purposes.

### 8.2.1 Emission versus Exposure Standards

Emission standards set various specifications for electrical devices and are generally based on engineering considerations, e.g., to minimize electromagnetic interference with other equipment and to optimize the efficiency of the device. They also provide internationally recognized measurement protocols for devices, e.g., phantom measurement for specific absorption rate (SAR) values for mobile phones. Emission standards are usually developed by the International Electrotechnical Commission (IEC), the Institute of Electrical and Electronic Engineers (IEEE), the International Telecommunications Union (ITU), the Comité Européen de Normalization Electrotechnique/European Committee for Electrotechnical Standardization (CENELEC), as well as other independent organizations and national standardization authorities.

Although emission limits are aimed at ensuring, *inter alia*, compliance with exposure limits, they are not explicitly based on health considerations. In general, emission standards aim at ensuring that the aggregate exposure to the emission from a device will be sufficiently low that its use, even in proximity to other EMF emitting devices, will not cause exposure limits to be exceeded (Table 8.1).

Exposure standards that limit human EMF exposure are based on studies that provide information on biological effects from EMF, as well as the physical characteristics and the sources in use, the resulting levels of exposure, and the people at risk. Exposure standards generally refer to maximum levels to which whole or partial body exposure is permitted from any number of sources. This type of standard normally incorporates safety factors and provides the basic guidelines for limiting personal exposure. Such standards have been developed by the International Commission on Nonionizing Radiation Protection (ICNIRP), the IEEE, and many national authorities.

### 8.2.2 Voluntary versus Mandatory Standards

At present, there are no internationally mandated standards for EMF, such as exists for ionizing radiation (International Basic Safety Standards, IAEA [6]), in large part because

**TABLE 8.1**

Microwave Ovens and Mobile Phone Base Stations Operate in the Same Microwave Frequency Range, but the Rationale for Their Emission Standards Is Different

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*Microwave ovens* are designed to heat food in an enclosed cavity, so spurious emissions should be limited as much as possible outside the cavity. The internationally agreed power density limit of  $5 \text{ mW/cm}^2$  at 5 cm from the oven surface was set as an exposure limit to avoid ocular cataract formation from long-term exposure to a person watching the food being cooked. While this upper limit was subsequently found to be very conservative, the limits have remained the same.

*Base station antennas* for mobile telecommunications are designed to radiate RF energy and are located to provide coverage over a defined area or cell. The emission (or radiated power) from each base station depends on such factors as the size of the cell, the amount of information being transmitted, and the number of calls made. The power emitted from the base station must be optimized, i.e., enough to provide full coverage over the cell while not causing interference with neighboring cells. Thus, emission limits are not necessary because the signal strength is orders of magnitude below health limits and the signal strength is self-regulated by the network.

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of the relatively large scientific uncertainty about adverse health effects and the lack of agreement on exposure limits by national authorities. Rather, there are international guidelines, such as those developed by ICNIRP, IEEE, and others, which provide guidance to national agencies but are not legally binding. Guidelines would only become legally binding if a country incorporates them into their own legislation. At the national level, exposure EMF regulations can be broadly categorized as either voluntary or mandatory instruments.

Voluntary instruments include guidelines, instructions, and recommendations that are not legally mandated. However, it is worth checking the national definitions of these terms, as for example, the term “Guideline Document” in Canada, refers to a mandatory, rather than voluntary, instrument.

Mandatory, compulsory, or legally binding instruments include laws, acts, regulations, ordinances, decisions, and decrees, and require a legislative framework. Because many countries do not possess legislation regarding protection standards for EMF, WHO’s International EMF Project has developed model legislation on limiting exposure to NIR ([http://www.who.int/emf/standards/emf\\_model/en/](http://www.who.int/emf/standards/emf_model/en/)).

Procedures should exist to ensure compliance of mandatory standards. For EMF exposure standards, an agency is normally mandated to check compliance through calculations and measurements made in the workplace and other areas. For emission standards, compliance for devices is usually certified by the manufacturer.

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### 8.3 Relevant Authorities

This section provides an overview of the relevant authorities involved in setting EMF standards. A number of international organizations have the mandate to establish EMF exposure and emission standards and have formulated limits for occupational and residential EMF exposure. National governments may regulate NIR protection through specialized legislative and regulatory agencies or ministries. At the regional and local levels, states and municipalities may have jurisdiction over certain aspects of public EMF protection.

#### 8.3.1 Nongovernmental and International Organizations

The International Commission on Non Ionizing Radiation Protection (ICNIRP, <http://www.icnirp.org>)—ICNIRP is an independent nongovernmental organization (NGO), established in 1992 to evaluate the state of knowledge about the effects of NIR on human health and well being and to provide scientifically based advice on exposure limits and other methods of protection against harmful effects of NIR. It functions as an international scientific advisory body that does not include political, social, and economic considerations. Members of ICNIRP are experts who are not affiliated with a commercial or industrial enterprise. ICNIRP has developed guidelines on limiting exposure to nonionizing radiation from 0 to 300 GHz [7]. These guidelines have been adopted by the majority of countries that regulate NIR in Europe, Asia, and elsewhere.

The International Committee on Electromagnetic Safety (IEEE ICES, <http://grouper.ieee.org/groups/scc28>)—ICES is a committee established in 2001 by the Institute of Electrical and Electronic Engineers (IEEE). ICES and its predecessor committees have,

since 1988, developed standards for safety levels with respect to human exposure to NIR in the frequency range of 0 Hz to 300 GHz. ICES sponsors and oversees the work of five subcommittees, with representatives of all five continents drawn from industry, government, the military, academia, and medicine. Participation in the meetings and deliberations of ICES is open to all. ELF limits were developed in IEEE Std C95.6 [8] and RF limits in IEEE Std C95.1 [9]. ICES guidelines have been adopted as mandatory standards by US governmental agencies as well as by Canada.

The International Electrotechnical Commission (IEC, <http://www.iec.ch>)—The IEC is an international organization, which consists of IEC National Committee members. IEC publishes standards for all electrical, electronic, and related technologies. IEC standards are developed to ensure interoperability between devices, product and service quality, and better production and delivery efficiency. IEC/TC106 is a technical body that prepares international standards in the field of characterization of the electromagnetic environments, measurement methods, instrumentation and procedures, calculation methods, assessment methods for the exposure produced by specific sources, and assessment of uncertainties with regard to human exposure.

Comité Européen de Normalisation Electrotechnique/European Committee for Electrotechnical Standardization (CENELEC, <http://www.cenelec.org>)—CENELEC is a European organization, which consists of the European national committees of IEC. It produces voluntary electrotechnical standards relating to measurement techniques for electrical and electronic goods and services, which are harmonized at the European level.

The International Telecommunications Union (ITU, <http://www.itu.int>)—The ITU is an international organization within which governments and the private sector work together to coordinate the operation of telecommunication networks and services. The activities in the three sectors of the Union—radiocommunication, telecommunication standardization, and telecommunication development—cover all aspects of telecommunication, from setting standards that facilitate interworking of equipment and systems on a global basis to adopting operational procedures for the vast and growing array of wireless services and designing programs to improve telecommunication infrastructure in the developing world. All ITU recommendations are nonbinding, voluntary agreements.

The World Health Organization (WHO, <http://www.who.int/emf>)—Through the International EMF Project, WHO (i) provides information on worldwide EMF standards in a web-accessible database; (ii) advocates harmonization of standards and facilitates the development of health-based exposure standards through a framework describing the scientific process required to develop them; and (iii) recognizing that some national authorities may want to provide greater protection than the current international guidelines provided in the presence of scientific uncertainty, has published a practical framework for developing such measures. WHO is in formal relation with ICNIRP and formally endorses and promotes ICNIRP's international exposure guidelines.

### 8.3.2 Multinational and National Bodies

In the past few decades, a number of multinational or regional agreements have been signed to deal with economic and trade issues, e.g., the North American Free Trade Agreement (NAFTA), MERCOSUL in South America, and the European Union (EU). So far, the latter is the only one to provide regulatory guidance on EMF exposure limits to its 25 member states (for more details, see Section 8.5). At the national level, regulations and legislation relating to EMF standards fall within the purview of specialized bodies. Certain governments have set up independent agencies to provide recommendations on EMF. Details on EMF standards are posted on the WHO web site (<http://www.who.int/>

[docstore/peh-emf/EMFStandards/who-0102/Worldmap5.htm](http://docstore/peh-emf/EMFStandards/who-0102/Worldmap5.htm)) where country-specific information can be found for over 50 countries.

One of the challenges faced at the national level stems from the fact that systems and devices emitting EMF are used for vastly different applications and may cover overlapping parts of the nonionizing radiation spectrum. This often poses confusion about final responsibility regarding protection from EMF exposure. For example, extremely low frequency (ELF) fields from generation, transmission, and distribution of electricity may come under the Ministry of Industry or of Energy, while radio frequency (RF) fields may be regulated by the ministries of Telecommunications (e.g., mobile phones), Transport (radar equipment for air navigation), or Environment. In situations where multiple authorities, sometimes with conflicting interests, are involved, communication and coordination, perhaps through the establishment of interagency committees, is key to evolve commonly accepted measures to protect the population.

The development of technology policy and its regulation must take into account many considerations in addition to health. In some countries, exposure standards are drafted by committees that represent several stakeholders, and the resulting national standards often reflect the compromises needed to satisfy the diverse points of view. Also, regulators need to facilitate the provision of varied and competitive utility services, from electric power to telecommunications, to most of the population. They have to create an environment that supports innovation and technical development and that best utilizes limited resources, whether it is land in the case of power lines or the frequency spectrum allocation for telecommunications devices.

### 8.3.3 Local Government

In many federated countries, the state or province has had to deal with the issue of EMF regulation. In California for example, an independent EMF research, evaluation and protection program was established in 1994 under the auspices of the state Department of Health ([www.dhs.ca.gov](http://www.dhs.ca.gov)). This resulted in state-level recommendations on how to manage EMF exposure to the public, especially in sensitive areas such as schools and kindergartens.

At the local level, municipalities often have authority over land use and building, including installation permits for power lines and mobile telephone base stations. As such, they are often confronted directly by public anxiety and discontent. It is therefore important that local authorities have at least a minimum knowledge of the EMF issue to answer questions from the public or be ready to direct requests to appropriate sources of information. An example of a successful information campaign was the initiative by the Elettra 2000 consortium in Italy to send a brochure on EMF risk management to every mayor in the country in 2003 (<http://www.eletretta2000.it>).

Some countries have opted for a structured approach to communication on EMF emitting sources. In France, for example, a charter between mayors and the association of mobile operators was developed in 2004 to provide information and dialogue regarding the placement of mobile phone base stations (<http://www.afom.fr>). This allows for a more transparent and fairer process over the whole territory.

Municipalities sometimes override national regulations and introduce further conservative measures based on political considerations rather than science. The case of Austria is well known, whereby federal legislature adheres to ICNIRP limits, but the city council of Salzburg, backed by its Public Health Department, agreed with mobile telecommunications operators to a precautionary limit value for radiation around 100 times lower than the ICNIRP's exposure limit [10]. This example has brought up criticisms regarding the technical feasibility of setting such limits in GSM and UMTS networks, along with the precedent that such local decisions establish.

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## 8.4 Basis for Exposure Standards

Exposure to EMF may cause different biological effects, with a variety of physiological responses for a human being. Some effects may have beneficial consequences for a person, whereas others result in pathological conditions. Annoyance or discomforts caused by EMF exposure may not be pathological *per se* but, if substantiated, can affect the physical and mental well being of a person and the resultant effect may be considered as a health hazard. A health hazard is thus defined as a biological effect that has health consequences outside the compensatory mechanisms of the human body and is detrimental to health or well-being.

The development of guidelines or standards that limit EMF exposure should ideally be based on established scientific evidence; otherwise the limit values may be either non-protective or unduly restrictive. Once the evidence is properly evaluated, usually through a health risk assessment of all scientific studies, it can then be used to develop and implement policy and standards. However, policy makers must also take public opinion into account and ultimately policy development should include both a scientific approach and a political process to produce accepted and effective outcomes.

### 8.4.1 Scientific Methodology

Science-based evaluations of any hazards from EMF exposure are an essential part of an appropriate public policy response, and form the basis of international guidelines on exposure limits. Criteria and procedures for determining the exposure limit values are outlined in WHO's Framework for Developing Health-Based EMF Standards (<http://www.who.int/emf>). A summary of the procedure to derive numerical exposure limits is given in Table 8.2.

**TABLE 8.2**

Steps in the Development of EMF Exposure Standards

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1. Conducting a detailed review of the scientific literature, using those studies having sufficient quality and information to be useful for health risk assessments and a determination of whether the biological effects found in these studies could lead to any adverse health consequence. This normally involves
    - (a) using studies published in the peer-reviewed literature that have good dosimetry and experimental methodology, and
    - (b) conducting a weight-of-evidence analysis to determine from the many studies showing effects and those not showing effects, whether an adverse health outcome is really likely to occur, at what level of exposure, and whether the effect is biologically plausible.
  2. Identifying the lowest EMF exposure level, called threshold level, at which the first established health effect is seen and assessing all aspects of the uncertainty at this threshold level.
  3. Applying a safety factor to the threshold level that accounts for all foreseen uncertainties in the threshold level to derive the exposure limits for the populations being protected. Normally an additional safety factor is applied to the exposure limits for more vulnerable populations, such as children, aged, and sick people that will be found in the general population.
  4. Using the predominant mechanism(s) of interaction and results from studies conducted at other frequencies to extrapolate the exposure limits over the full frequency range of the population standard. It is important that the same or similar margin of safety in the exposure limits be applied to the whole frequency range for the standard, thus providing the same level of health protection for all frequencies considered.
  5. Including an explanatory text with a full scientific rationale to accompany the standard so that subsequent studies, that may challenge the basis for the limits, can be properly assessed with respect to the original scientific justification for the limit values.
  6. Performing a periodic review of the scientific literature to ensure that the basis for the standards, and especially the limit values, continue to be scientifically valid.
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Some countries have the human and economic resources to undertake their own scientific evaluation of EMF health related effects through a formal health risk assessment process (e.g., the EMF RAPID program in the United States, NIEHS [11]), through an independent advisory committee (e.g., the independent Advisory Group on Non-Ionizing Radiation report in the United Kingdom, AGNIR 2001 [12]), or through a parliamentary report (e.g., the *Téléphonie mobile et santé* Senate report in France, 2002 [13]). Other countries may go through a less formal process to develop science-based guidelines or a variation thereof. Whereas some countries have adopted the ICNIRP guidelines, others use them as the *de facto* standard without giving them a legal basis. This may provide the local manufacturers and equipment users all the guidance they need to set their exposure limits within an installation.

Exposure guidelines in most countries have been based on acute, well-established effects of relatively high fields. Epidemiological evidence for long-term effects has so far been judged insufficient for the formulation of limits. Actual exposures are typically are considerably lower than guideline limits, and research at present mostly focuses on the possibility of long-term effects at low levels of exposure.

#### **8.4.1.1 Types of Studies**

The development of exposure standards requires an in-depth evaluation of the established scientific literature related to health effects. Research in this field rests on the conventional approaches used in toxicology, in particular the scientific studies undertaken in the laboratory on human volunteers, animals or cells and those undertaken on human populations by means of epidemiology. All these studies provide useful and valid information, but no single study or type of study can give a definitive answer. The relevance of different studies varies when considering health risks in people. Epidemiological studies of the distribution of disease in populations and the factors that influence this distribution provide direct information on the health of people exposed to an agent and are given the highest “weighting.” However, they may be affected by unintentionally biased samples and confounding factors, and their observational nature makes it difficult to infer causal relationships, except when the evidence is strong. Experimental studies using volunteers can give valuable insight into the transient, physiological effects of acute exposure, although for ethical reasons these studies are normally restricted to healthy people. For obvious reasons, they cannot provide information on possible long-term effects.

Studies on animals, tissues, and cell cultures are also important but are given less weight. Animal studies can often be expected to provide qualitative information regarding potential health outcomes, but the data may not be extrapolated to provide quantitative estimates of risk, largely because of differences between species. However, IARC [14] considers that exposure to any biological, chemical, or physical agent is likely to cause cancer in humans if such a risk has been identified in at least two different animal species. Studies carried out at the cellular level are normally used to investigate mechanisms of interaction, but are not generally taken alone as evidence of effects *in vivo* until the effects are also demonstrated *in vivo*. Nevertheless, all types of studies have a role to play in determining the scientific plausibility of any notional health risk.

Both the published and unpublished reports are considered but preference is always given to published data in peer-reviewed scientific journals. Research that is reported to the press before going through the peer-review process poses a problem on two levels. First, when research results are publicized, they may be presented to maximize public interest. This may introduce distortions that raise unfounded public fears. Second, more dangerously, without first attempting to replicate the results, research results may also be used by policy makers to change regulations or policies. Such decisions could have

important economic and free trade repercussions and most importantly, could undermine the scientific basis of human exposure guidelines.

#### **8.4.1.2 Multiple Tiers**

An important part of the rationale for any exposure standard is the definition of the population to be protected. Exposure standards can have multiple tiers to protect different populations. The ICNIRP guidelines [7] and, more recently, the IEEE standards [8,9] have adopted a two-tier set of limits; one for the general public and one for workers. Occupational standards are intended to protect healthy adults, exposed as a necessary part of their work, who are aware of the occupational risk and who are likely to be subjected to medical surveillance. By contrast, general population standards are based on broader considerations, including people with a wide range of health sensitivities, age, and illness. The general public will not necessarily have any knowledge of their EMF exposure or be able to minimize it. Thus, an additional safety factor is incorporated into the public exposure limits, which also takes into account the possibility of a 24 h exposure compared with occupational exposure of 8 h or whatever the duration of the workday.

#### **8.4.1.3 Safety Factors**

Safety factors in health protection standards represent an attempt to compensate for unknowns and uncertainties in the science. Once the threshold exposure level that produces an adverse health effect at the lowest exposure level has been identified, exposure limits may be derived by reducing this threshold level by a safety factor. Examples of sources of uncertainty about threshold levels include the extrapolation of animal data to effects in people, differences in the susceptibility of different groups or individuals, statistical uncertainties in the dose–response function, and the possibility of combined effects of exposures at different frequencies and other environmental factors.

Identification and quantification of various adverse effects of EMF exposure on health are difficult at best, and such judgments require extensive experience and expertise. Generally, acute effects can be quantified with reasonable precision and so derivation of limits to prevent these effects will not require a substantial safety factor below the observed threshold levels. When the uncertainty of the relationship between exposure and adverse outcome is greater, a larger safety factor may be warranted. There is no rigorous basis for determining precise safety factors. The magnitude of the reduction that results in the limit values is a matter of expert judgment, which explains the small differences in exposure limits derived independently by ICNIRP (<http://www.icnirp.org>) and IEEE (<http://grouper.ieee.org/groups/scc28>) (see below).

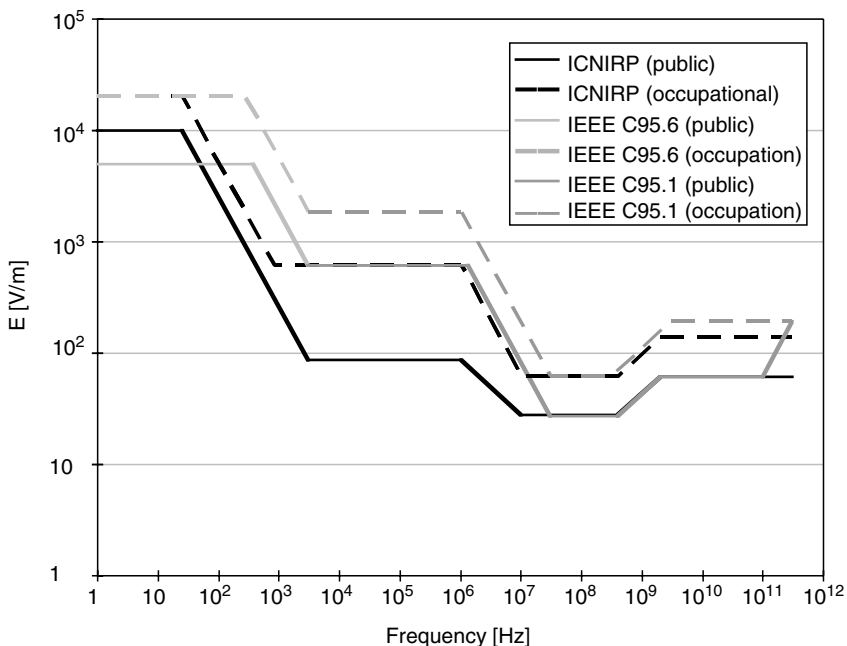
#### **8.4.1.4 Exposure Limits**

Limits on EMF exposure are termed basic restrictions and are based directly on established health effects and biological considerations. The physical quantities used in the guidelines reflect the different concepts of “dose” relevant to the lowest threshold for a health effect at different frequencies. In the low-frequency range (between 1 Hz and 10 MHz) the current ICNIRP and ICES basic restriction is the current density ( $J$ , in  $A/m^2$ ) for preventing effects in excitable tissues such as nerve and muscle cells; and in the high-frequency range (between 100 kHz and 10 GHz), the basic restriction is the specific absorption rate (SAR) in W/kg, for prevention of whole-body heat stress and local heating. In the intermediate frequency range (between 100 kHz and 10 MHz), restrictions are on both the current density and SAR, whereas in the very high-frequency range (between 10 and 300 GHz) the basic restriction is the power density ( $S$ , in  $W/m^2$ )

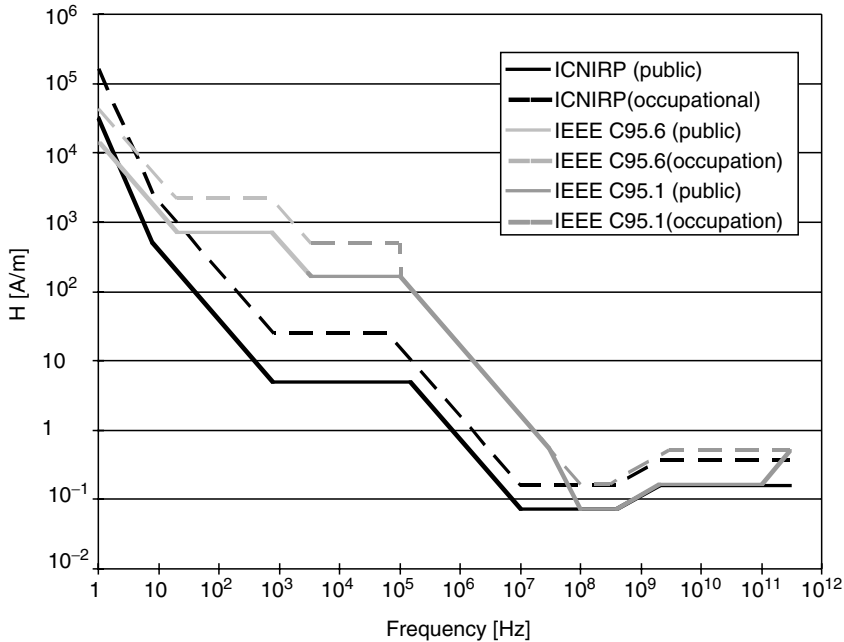
for excessive tissue heating near or at the body surface. Protection against known acute adverse health effects is assured if these basic restrictions are not exceeded. Numerical values of the basic restrictions over the frequency range up to 300 GHz can be found on the websites of ICNIRP and IEEE.

Because basic restrictions are often specified as quantities that may be impractical to measure, other quantities are introduced for practical exposure assessment purposes to determine whether the basic restrictions are likely to be exceeded or not. These reference levels (ICNIRP) or maximum permissible exposure levels (IEEE ICES) correspond to basic restrictions under worst case exposure conditions for one or more of the following physical quantities: electric field strength ( $E$ ), magnetic field strength ( $H$ ), magnetic flux density ( $B$ ), power density ( $S$ ), limb current ( $I_L$ ), contact current ( $I_C$ ), and, for pulsed fields, specific energy absorption ( $SA$ ). Exceeding the reference levels does not necessarily imply that the basic restrictions are exceeded. However, in this case it is necessary to test compliance with the relevant basic restrictions and to determine whether additional protective measures are necessary. Reference levels (ICNIRP) and maximum exposure levels (IEEE ICES) are compared in Figure 8.1 and Figure 8.2 for general public and occupational exposures up to 300 GHz. As can be seen from Figure 8.1 and Figure 8.2, ICNIRP generally provides more conservative limits than IEEE, especially below 1 MHz. For the RF range, the peak SAR values are now essentially harmonized. Whereas little data is available between 100 and 300 GHz, the ramp for the general public IEEE limits reflects harmonization with the IEEE infrared standard at 300 GHz.

It is worth noting that several large countries have adopted standards based on different quantities. EMF standards in the Russian Federation are based on the energy loading, a concept that is different from SAR (W/kg). Energy loading is defined either as the square of the electric (or magnetic) field multiplied by the duration of exposure, with units expressed in  $(V/m)^2 \cdot h$  or  $(A/m)^2 \cdot h$ , respectively, or as the product of power density



**FIGURE 8.1**  
ICNIRP and IEEE ICES exposure limits for time varying electric fields.



**FIGURE 8.2**  
ICNIRP and IEEE ICES exposure limits for time varying magnetic fields.

and time ( $W/cm^2$ )·h. The reference level quantities are the electric and magnetic fields, magnetic flux density, and power density. The People’s Republic of China has until now followed the former Soviet Union standard, but is in the process of revising its national RF standard, especially relating to SAR limit for mobile phones.

#### 8.4.1.5 Other Quantitative Measures

EMF standards include mostly field strengths limits, but now many authorities also have guidance on other measures. Examples of such measures for ELF fields from power lines include minimum height of electrical conductors, necessary clearance between a transmission line and buildings (more specifically schools), and the electrical field strength on the ground surface under the line or at the edge of the right-of-way (corridor of clear land along a transmission line, originally used to allow line maintenance). For RF fields, guidance on the location of base stations has been provided by different authorities, including the widely quoted expert committee in the United Kingdom [12].

#### 8.4.1.6 Scientific Uncertainty

There is an increasing awareness of the need to account for uncertainty in the science database. This is traditionally addressed by further research, and the WHO International EMF project addresses these needs through the development of a “research agenda” (<http://www.who.int/peh-emf/research/en>). However, research programs may take several years to complete, and the long latency associated with diseases such as cancer in people may also preclude a rapid outcome in some studies. Some countries have been addressing the issue of current uncertainty through the development of precautionary measures as discussed below.

### 8.4.2 Political Process and Precaution

As protecting the health of the population is part of the political process, it is expected that different countries may choose to provide different levels of protection against environmental hazards, responding to their national health policy. Various approaches to protection have been suggested to deal with scientific uncertainty. In recent years, increased reference has been made to cautionary policies and in particular the Precautionary Principle.

The Precautionary Principle is a risk management tool applied in situations of scientific uncertainty where there may be some need to act before there is strong proof of harm. It is intended to justify drafting provisional responses to potentially serious health threats until adequate data are available to develop more scientifically based responses. The Precautionary Principle is mentioned in international law [15,16] and is the basis for European environmental legislation [17]. It has also been referred to in some national legislation, for example in Canada [18], and recommendations of certain EMF exposure situations, for example in Switzerland [19]. The Precautionary Principle and its relationship to science and the development of standards have been discussed by Foster et al. [20].

With regard to the EMF issue, governmental and industry authorities have responded by implementing a wide variety of mandatory and voluntary precautionary approaches, based on cultural, social, and legal considerations. These include the importance given to avoiding a disease that affects mostly children, the acceptability of involuntary, as opposed to voluntary, exposures, and the varying importance given to uncertainties in the decision-making process. Several examples are presented below (Table 8.3).

*Prudent avoidance:* Prudent avoidance, a version of the Precautionary Principle (although not specifically identified as such), was developed for power-frequency EMF. It is defined as taking steps to lower human exposure to EMF by redirecting facilities and redesigning electrical systems and appliances at low to modest costs [21]. This policy has generally been applied only to new facilities, where minor modifications in design can reduce levels of public exposure at a low cost. Prudent avoidance has been adopted as part of policy in many countries, including Australia, New Zealand, and Sweden. Measures that can be taken at “modest cost” include routing power lines away from schools, phasing and configuring power line conductors to reduce magnetic fields near their rights of way, and optimizing the location of mobile phone base stations within a given area.

*Passive regulatory action:* This recommendation, introduced in the United States for the ELF issue [11], advocates educating the public and the regulated community on means aimed at reducing exposure, rather than setting up actual measures to reduce exposure.

**TABLE 8.3**

Examples of Precautionary Approaches

Precautionary Approach	Country	Measures
Prudent avoidance	New Zealand, Australia, and Sweden	Adopt ICNIRP guideline values and add low-cost voluntary measures to reduce exposure
Passive regulatory action	USA	Educate the public on measures to reduce exposure
Precautionary emission control	Switzerland	Adopt ICNIRP guideline values and set lower emission limits if feasible
Precautionary quantitative exposure limits	Italy and Greece	Decrease exposure limits using arbitrary reduction factors

*Precautionary emission control:* This policy is used in Switzerland to reduce EMF exposure by keeping emission levels “as low as technically and operationally feasible,” implying that they have been successfully tested on comparable installations or in trials. Measures to control emissions should be “financially viable” (<http://www.umwelt-schweiz.ch/buwal/eng/index.html>). Therefore, it is the emission levels from a device or class of devices that is controlled whereas the international exposure limits (ICNIRP) are adopted as the maximum level of exposure to people from all sources of EMF. Such a measure has been taken up by Switzerland regarding base stations emission levels.

*Precautionary exposure limits:* Some countries have taken precautionary approaches leading to limits on exposure that do not consistently draw on the scientific evidence for adverse health effects\*. For example, in 2003, Italy adopted ICNIRP standards but introduced two further limits for EMF exposure: (i) “attention values” of one-tenth of the ICNIRP field reference levels in specific locations, such as children’s playgrounds, residential dwellings, and school premises and (ii) further restrictive frequency-dependent “quality goals” that only apply to new sources and new homes.

This diversity of approaches by national authorities led WHO’s International EMF Project to develop a “Policy Framework” for rational and cost-effective guidance of policy options in areas of scientific uncertainty (<http://www.who.int/emf>). Briefly, this aims to develop a set of measures for protecting public health according to the degree of scientific uncertainty and the anticipated severity of the harm that might ensue, taking into account the size of the affected population and the cost. Effective risk communication and consultation between stakeholders are seen as integral parts of this process.

A principal recommendation by WHO is that these types of policies be adopted in such a way that scientific assessments of risk and science-based exposure limits are not ignored. WHO specifically recommends not to reduce the limit values in international standards to some arbitrary level in the name of “precaution” as this undermines the science base on which the limits were based and can introduce an additional cost of compliance for no known health benefit. Also, from a sociological standpoint, there is increasing evidence that making arbitrary reductions in exposure limits lead to increasing public concern, rather than reducing it [24]. Furthermore, arbitrary additional safety factors could lead to limit values so low as to exclude highly beneficial technologies.

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## 8.5 Discussion

Over the past decade, several factors have caused countries to develop or modify their standards for protection against EMF exposure. One of these factors is the technological evolution of telecommunication devices and the overhaul of the electrical grid in many countries. Political changes have also had an impact, notably the break-up of the former

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\*The European Union (EU) has published Council Recommendation (1999/519/EC) [22] regarding EMF protection for the general public and EC Directive (2004/40/EC) [23] establishing minimum requirements for the protection of workers. Both are based on ICNIRP guidelines. Member States of the EU are required to adopt and incorporate the EC Directives into their legislation. However, in accordance with the Maastricht Treaty, they may provide a higher level of protection than that set out in EC Recommendation or Directive, a prerogative that has been taken up by several European countries on precautionary grounds.

Soviet Union and the enlargement of the EU to include a number of eastern European states, which previously had EMF standards based on Soviet legislation. Furthermore, from a social view point, there has been a rise in public awareness and concern regarding new technologies, from vaccines to genetically engineered plants and mobile phones, and a loss of trust in national responses to public health threats, such as the tainted blood or bovine spongiform encephalopathy (BSE) crises [25].

### **8.5.1 Technology Leading Health Research**

The recent development of new EMF-emitting devices, from novel wireless communication systems to superconducting magnets used in magnetic resonance imaging (MRI), has resulted in a significant increase in human exposure to electromagnetic fields. Given the rapidly expanded use of such devices, any potential health impacts need to be properly assessed, ideally prior to widespread human exposure. Unfortunately, this technological development and its applications usually outpace epidemiological and biological research and the development of proper health risk assessments and consequent standards. For example, a recent review by WHO (in press) indicates that research on exposures to static magnetic fields has been conducted up to about 2 T, but medical and occupational exposures now exceed 3–8 T where no effective studies to determine their safety have been conducted.

### **8.5.2 Unregulated Portions of the Spectrum**

Many countries have now established health protection standards or guidelines. However, in a number of them, only certain parts of the spectrum are regulated. To date, few regulatory exposure standards have been promulgated limiting human exposure to static fields. The greatest interest has been in regulations and guidelines for ELF fields at power frequencies and RF fields at mobile phone frequencies.

### **8.5.3 Disparities in Standards**

Globalization of trade and the rapid expansion in the use of devices emitting EMF have focused attention on differences existing in exposure guidelines or standards limiting EMF exposure. In some cases, these differences are large. Some of the disparities in EMF standards around the world have arisen from the use of only national databases, different criteria for accepting or assessing individual studies, varying interpretations of the scientific data, or different philosophies for public health standards development. Such differences in EMF exposure guidelines might reflect, in part, deficiencies in communications among scientists in different regions as well as certain social differences, at least as far as precautionary measures are concerned. Large disparities between national limits and international guidelines can foster confusion for regulators and policy makers, provide a challenge to manufacturers and operators of communications systems, who need to tailor their products to each market, and increase public anxiety.

### **8.5.4 Risk Perception and Communication**

The lack of worldwide policy harmonization is one of many factors that may exacerbate public anxiety. People's perceptions of a risk depend on personal factors, external factors as well as the nature of the risk [26]. Personal factors include age, sex, and cultural or educational backgrounds while external factors comprise the media and other forms of

information dissemination, the current political and economic situation, and opinion movements, as well as the structure of the regulatory process and political decision making in the community. The nature of the risk can also lead to different perceptions, depending on how much control the public has over a situation (e.g., mobile phones versus base stations), fairness and equity aspects in locating EMF sources, and dread of specific diseases (e.g., cancer versus headache). The greater the number of factors adding to the public's perception of risk, the greater is the potential for public concern. Public concern can be reduced through information and communication between the public, scientists, governments, and industry. Effective risk communication is not only a presentation of the scientific calculation of risk, but also a forum for discussion on broader issues of ethical and moral concern [27].

### 8.5.5 Need for Periodic Evaluation

As new scientific information becomes available, standards should be updated. Certain studies, because of their strength of evidence or because of the severity of the health outcome under study, may be more likely to prompt a reevaluation of the science informing standards than others. Changes to standards or policies should only be made after a proper assessment of the science base as a whole to ensure that the conclusions of the research in a given area are consistent.

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# 9

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## *Electroporation*

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James C. Weaver and Yuri Chizmadzhev

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### 9.1 Background

Cells and tissues contain multiple, spatially distributed barriers that compartmentalize charged and large molecules. These barriers are largely constructed out of lipids, usually phospholipids. For this reason, only very small molecules with effective high lipid solubility spontaneously penetrate the single or double phospholipid bilayer-based membranes of cells and their organelles [1]. Of course, these membranes have a large variety of channels and transporters that facilitate transport of particular ions and molecules. Other significant barriers consist of one or more layers of cells connected by tight junctions around bladders and ducts that help retain specialized fluids, and the tough, flexible stratum corneum of mammalian skin that prevents water loss, entry of toxic molecules and infectious agents. Electroporation results in an essentially universal physical reduction of such barriers by creating membrane-spanning aqueous pathways. Aqueous pathways (large dielectric constant  $\epsilon_w \approx 80$ ) across lipid-containing (small dielectric constant  $\epsilon_1 \approx 2$ ) barriers greatly favor transport of even small, monovalent ions [2,3].

Applied electric field pulses with dominant frequency content below  $\sim 300$  MHz cause concentration of voltage across membranes of isolated cells [4,6], and groups of cells spaced close together in a tissue [7]. If the time-dependent transmembrane voltage,  $U_m(t)$ ,

becomes sufficiently large, then stochastic rearrangements of membrane phospholipid molecules are hypothesized to occur at a high rate, such that water-containing defects (“pores”) measurably alter the membrane’s transport properties. This is electroporation.

The simplest statement is that electroporation “creates new aqueous pathways” through lipid-based barriers. Almost all electroporation studies to date have focused on bilayer membranes (BLMs), both artificial planar BLMs and cell membranes. In BLMs electroporation occurs under biochemically mild conditions, usually with a small temperature rise. For most easily observable phenomena such as cell transfection and molecular uptake, electroporation-related phenomena are believed to depend non-linearly on the transmembrane voltage,  $U_m(t)$ . An exposure time-dependent onset within the range  $\sim 0.2 < U_m < \sim 1$  V is usually found for single pulses with  $t_{\text{pulse}} > \tau_{\text{mv}}$ , the membrane charging time. For conventional mammalian cell electroporation this is readily satisfied for widely used pulses that have  $t_{\text{pulse}}$  in the range 1  $\mu$ s to 50 ms.

Both dramatic electrical behavior (“reversible electrical breakdown” = REB for cells) and significant molecular transport occur. Most interest to date has focused on the electropermeabilization that coincides with REB conditions, which involves a large increase in membrane permeability for ions and molecules, particularly for longer pulses. Other consequences of electroporation are electrofusion of cells to other cells or tissue and electro-insertion of membrane proteins into cell membranes.

The ability of a lipid-containing sheet to exclude ions and charged molecules is fundamental and is a result of the change in “Born energy” associated with moving a charge from a medium with a large permittivity, e.g., water, to a region with a low permittivity, e.g., the interior of a phospholipid BLM. Here the Born energy is the electrostatic energy of an ionic charge embedded in a medium with permittivity  $\epsilon$ , written here in terms of the electric field,  $E$ , and with  $\epsilon = K\epsilon_0$  where  $K$  is the dielectric constant and  $\epsilon_0 = 8.85 \times 10^{-12}$  F/m [8]:

$$W_{\text{Born}} = \int_{\text{all space but ion}} \frac{1}{2} \epsilon E^2 dV \quad (9.1)$$

The essential barrier function of cell membranes can be represented by a thin sheet of lipid. This allows the magnitude of the Born energy barrier,  $\Delta W_{\text{Born}}$ , to be computed by the following process: a charged sphere, representing the ion or molecule, is initially located in water far from the lipid sheet ( $W_{\text{Born},i}$ ) and then moved to the center of the sheet ( $W_{\text{Born},f}$ ) which requires the expenditure of energy. The corresponding barrier height,  $\Delta W_{\text{Born}} = W_{\text{Born},f} - W_{\text{Born},i}$ , is large even for small monovalent ions, e.g.,  $\text{Na}^+$  and  $\text{Cl}^-$ , is still larger for multivalent charged molecules, and also depends slightly on the membrane thickness, sphere radius, and the amount and distribution of charge within the sphere.

For a single, isolated charge such as a small ion, e.g.,  $\text{Na}^+$ , the largest contribution to  $\Delta W_{\text{Born}}$  arises from the region close to the ion. The small diameter of an ion (solute) of type “s” (typically  $2r_s \approx 0.4$  nm) is significantly smaller than a typical membrane thickness of  $d_{\text{m},l} \approx 4$  nm) for the lipid hydrocarbon chains. The corresponding full thickness is  $d_{\text{m}} \approx 5$  nm, which includes the phospholipid’s headgroups. As noted above  $\Delta W_{\text{Born}}$  can be estimated by calculating the change in energy to move the ion from bulk water to bulk lipid. This allows  $\Delta W_{\text{Born}}$  to be estimated by neglecting the membrane thickness and instead considering bulk lipid. This is justified because the greatest contribution to the electric field is in the volume near the ion. This estimate yields

$$\Delta W_{\text{Born}} \approx \frac{e^2}{8\pi\epsilon_0 r_s} \left[ \frac{1}{K_{\text{m}}} - \frac{1}{K_{\text{w}}} \right] \approx 100 kT \quad (9.2)$$

where the relevant temperature is  $T = 37^\circ\text{C} = 310\text{ K}$ . Numerical solutions to the electrostatic problem for a thin, low dielectric constant sheet immersed in water yields relatively small corrections. A barrier of this size is surmounted at a negligible rate by thermal fluctuations (spontaneous ion movement). Moreover, a transmembrane voltage,  $U_{m,\text{direct}}$  which is much larger than physiological values, would be needed to provide this energy. Uncharged molecules can partition into the membrane and then cross the membrane by diffusion; these species are not significantly affected by  $\Delta W_{\text{Born}}$ . Instead, their transport is governed by a passive permeability due to the combined effect of dissolution and diffusion.

Spontaneous barrier crossing is therefore negligible. An early calculation considered not only the “intact sheet” case, but also the case of a fixed cylindrical pore [2,3]. Both aqueous configurations lowered  $\Delta W_{\text{Born}}$ , but the greater reduction was achieved by the pore. The basic pore structure, penetration of the lipid membrane by an aqueous pathway, is of course the essence of channels based on proteins. It is also the basis of the “transient aqueous pore” theory of electroporation [9–19], but with the significant difference that the fluctuating and expandable electroporation aqueous pathways can be created rapidly (time scale of nanoseconds), but are metastable, with inferred pore lifetimes ranging from milliseconds to minutes.

To our knowledge, the first experimental report of electroporation-related phenomena were the irreversible [20] and reversible [21] observations of “breakdown” of the excitable membrane of the node of Ranvier. Almost a decade later nonthermal killing of microorganisms by electric field pulses was reported [22–24], followed a few years later by the observation of a large, field-induced molecular permeability increase in natural vesicles [25]. Increasing numbers of experimental reports involving electrical behavior of field-pulsed cell membranes came in the next few years [26–28]. Artificial planar BLMs [29,30] exhibited dramatic electromechanical behavior, and the first pore-based theory was advanced to explain the fate of pulsed planar membranes [9–15] and then extended in a series of further experimental [31–33] and additional theoretical studies [16,17,34]. Other reports confirmed and extended observation of cell membrane transport due to field-induced permeability increases [35–39]. This included introduction of “inactive” DNA into red blood cells [40], followed by the critically important demonstration of transformation of cells by electrically mediated DNA uptake [41].

An important feature of artificial and cell membranes is that they concentrate electric field pulses with slow rise times (relative to the membrane charging time) because of the large membrane resistance relative to that of the extra- and intracellular media [4–6,42,43]. This form of field amplification can be regarded as voltage concentration due to a spatially distributed voltage divider effect within a single or multicellular system. For the case of current injection (current clamp), field amplification can also arise from current density concentration, arising from multiple cells in close proximity or nearby insulating objects. In the case of cell and organelle membranes providing the predominant barriers and fast pulses with rise times smaller than  $\sim 3\text{ ns}$  (significant frequency content above  $\sim 300\text{ MHz}$ ), spatially distributed dielectric voltage division emerges, electric fields tend toward approximate uniformity, and voltage concentration at membranes is much smaller [7].

However, most electroporation studies and applications have utilized pulses with rise times that exceed a typical cell charging time. In the case of artificial planar BLMs the membrane completely blocks the current pathway, so that the entire voltage across the experimental apparatus electrodes appears, after a characteristic charging time,  $\tau_m \approx 10^{-6}\text{ s}$ , across the membrane. In the case of cells, the situation is more complicated, but an approximate guide is obtained for the case of spherical cell for which  $U_{m,\text{max}} \approx 1.5E_{\text{app}}r_{\text{cell}}$ , which shows that the change in transmembrane voltage is given approximately by the product of the electric field and the cell size.

Conventional pulse conditions involve large electric field magnitudes and relatively long rise times (relatively low frequencies). Field pulses applied in aqueous extracellular media typically have magnitudes of 0.1 to 1 kV/cm (mammalian cells) and  $\sim 10$  kV/cm (bacteria). As discussed below, most cells have characteristic linear dimensions of  $L_{\text{cell}} \approx 1$  to 10  $\mu\text{m}$ , although skeletal muscle and nerve cells are typically much larger. Such pulses result in  $U_m(t)$  reaching 0.2 to 1.5 V in times of order 1  $\mu\text{s}$  to 10 ms. The widely used “exponential pulse” is often specified only by its decay ( $E = E_0 \exp - t/\tau_{\text{pulse}}$ ), with the rise time probably longer than  $\tau_m$  but usually not reported. Square (rectangular; actually trapezoidal in the approximation that rise and fall times are linear) pulses ( $E = E_0$  or 0) are less frequently employed, but usually have pulse widths (durations) of 1 to 100  $\mu\text{s}$ , and sometimes longer. Other waveforms, such as gated RF pulses, bipolar pulses and multiple pulses, have received relatively little attention.

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## 9.2 Pore Models of Planar Lipid Bilayer Electromechanical Behavior

Artificial planar (BLMs are widely used to investigate basic aspects of the bilayer portion of cell membranes [29,30]. Laboratory conditions provide an electric field amplification by the factor  $A_{\text{BLM}} = L_{\text{elec}}/d_m$  where  $L_{\text{elec}}$  is the electrode separation and  $d_m$  is the membrane thickness. Within a charging transient characterized by a time constant  $\tau_m = R_e C_m$  (resistance of the electrolyte on both sides and the membrane capacitance) the electric field within a passive (fixed) membrane reaches  $E_m = U_m/d_m$ . For example, if one-half volt is applied to the electrodes,  $U_m$  reaches 0.5 V within about a  $\tau_m$  of several microseconds. An advantage of such membranes for mechanism studies is that several measurements can be made on the same preparation. These attributes allow short pulse studies to be carried out in which the pulse duration is the same order of magnitude as  $\tau_m$  or even faster. During the charging transient, the full electrode voltage does not appear across the membrane, and the resistance of the charging pathway should be included. For pulses with rise times shorter than about 3 ns, the dielectric properties of the bathing electrolytes should be included by assigning a capacitance with the dielectric constant of the electrolyte [7] in parallel with  $R_e$ .

Creation of artificial planar membranes originally involved use of relatively large amounts of organic solvent [29,30]. Significant improvement was subsequently achieved by using two (different, if desired) previously formed monolayers on aqueous solutions [44]. Unlike their cellular counterparts, these membranes are essentially macroscopic, typically spanning a circular aperture ( $D_{\text{ap}} \approx 1$  mm; corresponding membrane area is  $A_m \approx 7 \times 10^{-7}$  m<sup>2</sup>) in an electrically insulating septum. The convenient access to the chambers on both sides of the septum allows electrical measurements to be readily made, and the macroscopic chamber size results in a small electrolyte electrical resistance (sum of resistances on both sides,  $R_e \approx 100 \Omega$ ) between the membrane and macroscopic electrodes located about 1 cm away on each side. BLMs have a capacitance per area of about  $C_{\text{area}} \approx 1 \mu\text{F}/\text{cm}^2$ , and therefore a typical BLM has a capacitance of order  $C_{\text{BLM}} \approx 3 \times 10^{-8}$  F. As a result  $\tau_m \approx 3 \mu\text{s}$ .

The bulk charge relaxation time of an aqueous electrolyte is  $\tau_e = \epsilon_e/\sigma_e = K_e \epsilon_0 \rho_e$ . For physiological saline this basic relaxation time is small compared to the characteristic times of conventional electroporation pulses. Using a resistivity  $\rho_{\text{sal}} = 0.83 \Omega \text{ m}$  and a dielectric constant  $K_{\text{sal}} = 72$  the relaxation time is  $\tau_e \approx 0.5$  ns. This is much less than the charging times of typical BLM (and of cell membranes). For this reason, BLM experiments often have excellent time resolution for electrical measurements.

The measurement of a BLM transmembrane current,  $I(t)$ , following a step change in the applied electrode voltage,  $V_0$ , allows the induced membrane conductance to be followed. In such experiments it is found that during its lifetime a membrane can pass through up to four distinguishable stages. The first stage is a simple charging of the membrane, governed by  $\tau_m$  given above. The second stage is characterized by a constant voltage and associated (small) current. The third stage has a fluctuating current, but sometimes in this stage the membrane reverts to a quiet, steady current state. If a large voltage is applied to the membrane for a short time, then the intact membrane often makes a transition into a peculiar long-lived (tens of minutes) excited state that is characterized by a large conductance and pronounced current fluctuations, even at low  $U_m$ . This peculiar state was termed a "stress state" [45]. A fourth stage is often reached in which  $I(t)$  exhibits a drastic irreversible increase to saturation. Visual inspection shows that upon saturation the membrane has ruptured; the membrane material no longer spans the aperture and instead has collected on the aperture rim.

If a new BLM is created and an experiment repeated, then the next  $I(t)$  is qualitatively reproduced, but the membrane lifetime,  $\tau_m$ , the time from step voltage application to onset of a drastic current rise, is usually different. Both the character of the fluctuations and their duration are different. In short, the lifetime exhibits stochastic behavior. This characteristic feature of the membrane response can be explained by the hypothesis of transient aqueous pores.

The dependence of the mean membrane lifetime,  $\bar{\tau}_m$ , on various factors was studied to clarify the mechanism of membrane rupture [9,31–33]. The effect of varying  $U_m$  was most revealing. Over the range 300 to 600 mV the dependence of  $\log_{10} \bar{\tau}_m$  on  $U_m$  is usually almost linear, e.g., an increase in  $U_m$  by 100 mV causes a tenfold decrease in  $\bar{\tau}_m$ . At larger  $U_m$  the decrease in  $\bar{\tau}_m$  becomes less pronounced. If, for example,  $U_m$  is increased from 200 mV to 1.4 V the mean lifetime changes by more than six orders of magnitude, decreasing to about 10  $\mu$ s. This highly nonlinear dependence on  $U_m$  is not expected for electrostrictive mechanisms and provides key support for the transient aqueous pore hypothesis. Rupture (irreversible breakdown) was also studied by the charge injection technique [31]. In this method, a short (e.g., 400 ns) square pulse of moderate amplitude is applied to the membrane, with the result that  $U_m(t)$  rises quickly to about 0.4 V. After 300 to 400  $\mu$ s,  $U_m$  drops to zero over about 50  $\mu$ s because of rupture. Many features of rupture following charge injection can be described quantitatively by a transient aqueous pore model [9,46,47].

One of the most dramatic aspects of electroporation is "reversible electrical breakdown" (REB), which is actually a high conductance state that acts to protect the membrane through a rapid discharge [46]. Planar membranes made of oxidized cholesterol have been studied using the charge injection method. If such membranes are charged to about 1 V in about 400 ns the membrane resistance reversibly decreases by nearly nine orders of magnitude [31]. In typical experiments  $U_m$  reached 0.9 to 1.2 V. Significantly, the value  $U_m \approx 1.2$  V cannot be exceeded by further increasing the pulse amplitude. This includes experiments on cell membranes [48–50]. After a rapid discharge, the membrane survives, remains mechanically stable, and can be recharged. This was the first observation of REB in planar BLMs. Later REB was also studied using a voltage-clamp method.

Significantly, REB of cell membranes is similar to the behavior of oxidized cholesterol membranes [31,32] or  $\text{UO}_2^{2+}$  planar BLMs [51]. An important experimental observation is that such planar membranes can exhibit either rupture or REB, depending only on the charging procedure. A moderate pulse of injected charge leads to destruction, with  $U_m(t)$  decreasing in a two-stage, sigmoidal curve: following an initial post pulse decay,  $U_m(t)$  begins to level off, but then decays further as the membrane ruptures.

In contrast, for large pulses,  $U_m(t)$  reaches significantly higher values and then suddenly drops rapidly. Still bigger pulses led to even more rapid membrane discharge. These very different outcomes can be quantitatively described by a transient aqueous pore model [46,47].

### 9.3 Cell Membrane Electromechanical Phenomena

A cell membrane is much more complex than an artificial planar BLM. But even if the detailed biochemical composition is neglected, there are still important physical differences. These include the membrane's non-planar shape, the closed membrane topology, the non-zero trans-membrane voltage due to pump activity, and the presence of membrane proteins, and membrane cytoskeleton interactions [52]. Although real cells range from spherical to elongated cylinders to irregular three dimensional shapes, a local membrane area can often be regarded as a small subsystem with a planar geometry. Consider the most commonly treated cell shape (spherical) for the small electric field case. As is well known, for small fields a spherical cell develops a change in transmembrane voltage,  $\Delta U_m$ , which varies in position over the membrane and also with time:

$$\Delta U_m = \frac{1.5E_e r_{\text{cell}}}{1 + r_{\text{cell}} G_m (\rho_{e, \text{int}} + 0.5\rho_{e, \text{ext}})} [1 - \exp(-t/\tau_{m, \text{cell}})] \cos \theta, \quad (9.3)$$

where  $r_{\text{cell}}$  is the spherical cell radius,  $\rho_{e, \text{int}}$  and  $\rho_{e, \text{ext}}$  are the resistivities of the intra- and extracellular electrolytes, respectively,  $G_m$  is the (constant; no electroporation) cell membrane conductance,  $\theta$  is the angle between the applied electric field,  $\vec{E}_{\text{app}} = \vec{E}_0 e^{j\omega t}$ , and the site on the cell membrane at which  $U$  is measured. In this classic case the response field,  $\vec{E}_{\text{res}}$ , differs significantly from  $\vec{E}_{\text{app}}$ , in that  $\vec{E}_{\text{res}}$  goes around the cell. In contrast  $\vec{E}_{\text{app}}$  is uniform, as it is the field that would exist if the cell were removed and only aqueous electrolyte was present. Finally, the time constant associated with charging the fixed cell membrane is

$$\tau_{m, \text{cell}} = \frac{r_{\text{cell}} G_m (\rho_{e, \text{int}} + 0.5\rho_{e, \text{ext}})}{1 + r_{\text{cell}} G_m (\rho_{e, \text{int}} + 0.5\rho_{e, \text{ext}})} \quad (9.4)$$

Typical mammalian cells with  $r_{\text{cell}} \approx 10 \mu\text{m}$  have  $\tau_m \approx 1 \mu\text{s}$ . For  $t \gg \tau_m$  and  $G_m$  essentially zero, Equation 9.3 reduces to the widely used simple form

$$\Delta U_m = 1.5E_e r_{\text{cell}} \cos \theta \quad (9.5)$$

However, Equation 9.3 through Equation 9.5 are not valid for electroporation, because these equations do not hold if the membrane conductivity varies with position. Specifically, with electroporation,  $G_m$  varies dramatically with time at the sites of large  $U_m$ , but hardly at all at other sites. This has been convincingly established experimentally by submicrosecond fluorescence measurements with membrane dyes that respond to the transmembrane voltage [48–50]. Moreover, for suspended cells with significantly different extra- and intracellular medium conductivity, membrane deformation is expected [53–55], and has been observed experimentally [56]. With this in mind theoretical models that use Equation 9.3 and Equation 9.4 under conditions with significant electroporation should be viewed with suspicion.

Two irreversible cell membrane electroporation processes have been identified at the cellular level. First, mechanically bounded small regions of the cell membrane, e.g., regions

bounded by cytoskeletal elements and attached to the cytoskeleton by membrane proteins [52], may behave like a small planar membrane and may therefore exhibit prompt rupture even though the plasma membrane typically has a low surface tension [57,58] compared to a artificial planar BLM [9,59]. Surface tension tends to expand a pore once it has formed, so rupture is much less likely in cell plasma membranes unless the cell is osmotically swollen and thus has an elevated tension. In this case the behavior of a BLM is relevant. A topologically closed membrane, e.g., a vesicle, is not expected to rupture globally. Unlike a BLM there is no boundary at which membrane phospholipids can accumulate by expansion of one or a few large pores. The cell membrane surface tension,  $\Gamma_{\text{cell}}$ , is also important, and is expected to be small for some cell or vesicle membranes. But for some vesicles and intracellular organelles  $\Gamma$  is large [60].

In the case of BLM the tension is due to the meniscus at the aperture and is a constant during pore expansion. For cells and vesicles, however, the membrane tension is associated with osmotic pressure difference,  $\Delta\pi$ , through Laplace's law. Following electroporation,  $\Delta\pi$  (if it exists) goes to zero, and the tension,  $\Gamma$ , also tends to zero. In this case pore evolution is more complicated and involves the laws of colloid osmotic lysis. In the second case, reversible electroporation may be accompanied by significant molecular transport between the extra- and intracellular volumes, with the resulting chemical imbalance leading to cell stress and eventual lysis. This is an example of the importance of considering biochemical change due to field exposures (see *BBA*, [Chapter 5](#)).

Minimum size pores are believed to be small, of order  $r_{p,\text{min}} \approx 0.8 \text{ nm}$  [61–63] or in erythrocytes about a factor of 2 smaller [36]. However, significantly larger (several nanometer radii) pores are believed to evolve for longer, e.g., 100  $\mu\text{s}$  electroporating pulses that are used to deliver  $\sim 1\text{kDa}$  drug molecules such as bleomycin into cells. In this case cell membranes discharge during reversible electrical breakdown (REB) through the pores, and the cellular or vesicular membrane returns to its initial state, except for consequences of net ionic or molecular transport through the transient aqueous pore population. The inability to promptly rupture via critical pores to directly lyse a cell is expected to apply to unswollen cells.

Cell membranes are believed to exhibit REB, i.e., a tremendous increase in electrical conduction which can be inferred from transmembrane voltage measurements [48–50], and which is believed due to ionic conduction through transient aqueous pores. Electric field pulses that cause  $U_m$  to rapidly rise into the range 0.5 to 1.5 V cause onset of REB with essentially no dependence on membrane composition. For this reason, the onset of electroporation involving REB is believed to be universal, and it has been observed for a wide variety of cell types. The behavior of the transmembrane voltage,  $U_m(t)$ , during membrane charging and the subsequent appearance and evolution of a pore population, is intimately connected with the number and size of pores. The success of a transient aqueous pore model in providing a quantitative description of  $U_m(t)$  under these conditions gives confidence that electroporation is a valid concept. Significantly, a large increase in molecular transport across cell membranes is generally observed for essentially the same pulse conditions (cell exposures) that cause REB.

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## 9.4 Nonpore Theories of Electromechanical Phenomena

One early approach to explaining membrane destabilization for an elevated  $U_m$  involved “punch through” [64] while another was based on electromechanical collapse due to compression of the membrane [65]. Electrocompression models have several important



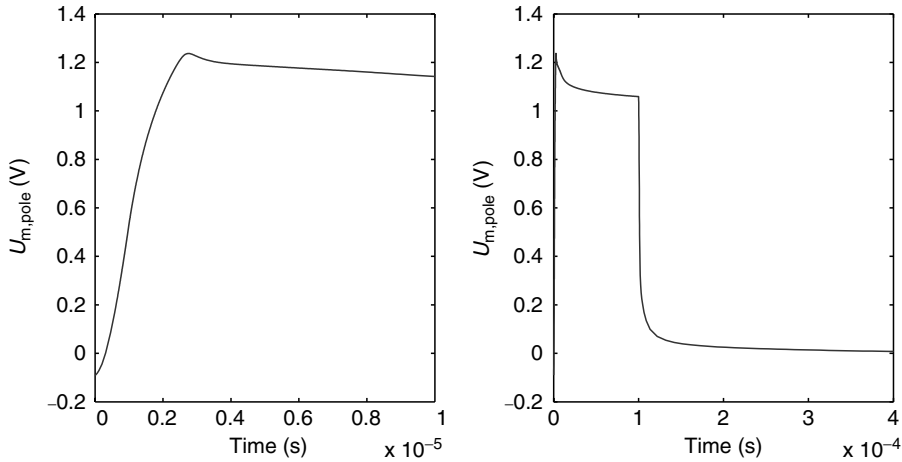
attributes. First, it is deterministic, predicting a critical transmembrane voltage,  $U_{m,c}$ , above which rupture occurs. However, for realistic values of membrane compressibility for solvent-free membranes this model predicts  $U_{m,c} \approx 5 \text{ V}$ , which is about an order of magnitude too large. Further, the absence of a marked change in membrane capacitance,  $C_m$ , before rupture argues strongly against large-scale electrocompression, as an increase in  $C_m$  is an inevitable consequence of a decrease in membrane thickness,  $d_m$ . Moreover, the observed stochastic nature of rupture is in direct conflict with the concept of a deterministic critical voltage. Neither the stochastic nature nor the strong lifetime dependence on  $U_m$  is expected for an electro-mechanical rupture mechanism. Finally, the fate of the membrane in this model depends only on  $U_m$ , but experiments also show a dependence on pulse duration.

Electrohydrodynamic models based on viscoelastic behavior have also been advanced [66], but are intimately related to electrocompression theories. The only difference is that the electrocompression model attributes the increase in system energy to elastic compression energy of the membrane, whereas in the electrohydrodynamic model, it corresponds to the work required to form new membrane surface. In both types of deterministic models the development of instability (“irreversible electric breakdown” or “rupture”) represents a nonlocal process that occurs simultaneously over a large area of the membrane. In contrast, pore models involve highly localized events that involve only a small fraction of the total membrane area for conventional electroporation used for molecular uptake. At another extreme, however, pulses that cause intracellular effects by organelle electroporation are hypothesized to involve supra-electroporation that involves a very large pore density; see Section 9.11).

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## 9.5 Pore Theories of Electromechanical Phenomena

We first provide a qualitative description of how a membrane is believed to respond to an applied pulse. This is illustrated by the example of  $U_m(t)$  given in Figure 9.1. The spontaneous rate of pore formation due to thermal fluctuations is very small. The pore creation rate depends on a Boltzmann factor with an energy contribution that decreases as  $U_m^2$  (Equation 9.6). Even at a resting potential of  $U_{m,\text{rest}} \approx -60 \text{ mV}$  (nerve membrane) to  $-200 \text{ mV}$  (inner mitochondrial membrane) a minimum size pore will only occasionally appear in a membrane [19,63]. Application of an external electric field pulse begins by charging local membrane areas. Initially displacement currents flow as the membrane charges through the extra- and intracellular electrolyte. In the case of a spherical cell membrane the polar regions (Equation 9.3;  $\theta$  small) first reach voltages at which pore creation becomes significant. When  $U_m$  exceeds  $\sim 0.5 \text{ V}$ , pore creation becomes significant. As more pores rapidly appear whereas  $U_m$  rises further, the newly acquired local membrane conductance begins to discharge the membrane through the evolving pore population. When  $U_m$  reaches  $\sim 1 \text{ V}$ , there has been a huge increase in the local pore density and  $U_m$  begins to decrease even though the applied field may itself still be increasing. This is reversible electrical breakdown (REB), the high conductance state associated with significant electropermeabilization of the local membrane area. If the pulse continues after REB, the transmembrane voltage may continue at about the same value (a plateau in  $U_m$ ), or may increase again before reaching a plateau. At the end of the pulse, many local areas in the polar region are so conductive that  $U_m$  quickly drops to  $\sim 0$ . According to what is known, a population of metastable pores remains, with individual pores assumed to vanish stochastically due to local thermal fluctuations.



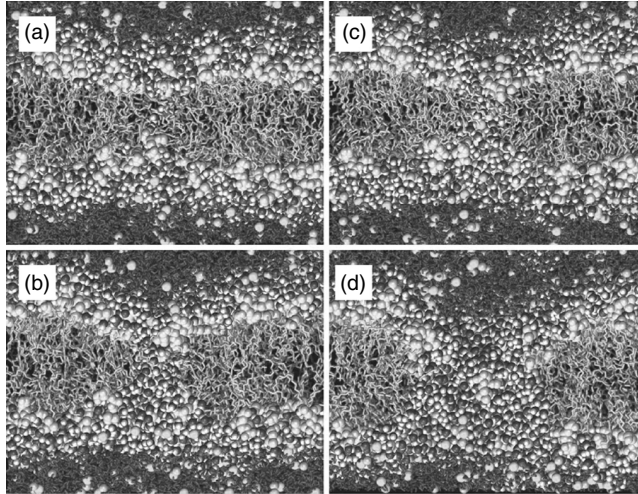
**FIGURE 9.1**

Illustration of the time-dependent transmembrane voltage,  $U_m(t)$ , for electroporation. This example shows the behavior at the pole of a two-dimensional cell model for conditions that cause electroporation and reversible electrical breakdown (REB) at  $U_m \approx 1.2\text{ V}$ . A qualitative description of what is believed to occur is given in the text. This example uses a conventional electroporation electric field pulse of magnitude  $1100\text{ V/cm}$  and a trapezoidal waveform with  $1\text{ }\mu\text{s}$  rise and fall times and a  $98\text{ }\mu\text{s}$  flat peak. Left panel:  $10\text{ }\mu\text{s}$  timescale showing the initial passive charging of the membrane, followed by the spike at which pore conduction onset is so rapid that it arrests the voltage rise and causes a decrease even though the pulse is still on. Right panel:  $400\text{ }\mu\text{s}$  timescale showing REB followed by a transmembrane voltage plateau during the pulse and then a decay when the pulse ends. This model has a distributed resting potential source and membrane resistance that together generate a resting transmembrane voltage of  $U_{m,\text{rest}} = -90\text{ mV}$  [69]. This description uses the asymptotic membrane electroporation model assigned to a circular cell with organelle models (Gowrishankar et al., unpublished results).

Molecular transport is believed to occur during the pulse when the pore population changes rapidly, first expanding in pore size and number, and then shrinking mainly in pore size as  $U_m$  eventually decreases, with pore number decreasing more slowly. Transient aqueous pore models for artificial planar BLMs [19,46,47] and cell membranes are consistent with this view [67–70].

Initial quantitative theories and models used analytical methods to estimate the creation and destruction rates of pores, based on nucleation theory in which pores are regarded as membrane defects [9,19]. Molecular dynamics (MD) simulations were possible only recently, and these support some of the basic transient aqueous pore concepts [71,76]. For example, a toroidal-like geometry is spontaneously achieved (Figure 9.2).

However, computer limitations presently restrict the model volume to be small, imposing a significant limitation on the concentration of soluble ions and molecules that are candidates for transport through a membrane by passing through a transient pore. Entry of water and aqueous electrolytes (large dielectric constant) into a fluid BLM (small dielectric constant) is increasingly favored as the electric field within the lipid region of the bilayer becomes larger. This is the basis of the transient aqueous pore theoretical model, originally introduced in a series of seven back-to-back papers [9–15]. With similar pore creation mechanisms independently proposed several years later [16,17]. Nucleation theory is based on the absolute rate equation and involves an estimate of the free energy change,  $(\Delta W)_p(r_p, U_m)$ , associated with formation of a large dielectric constant aqueous pore within the small dielectric constant lipid portion of the membrane. The initial,



**FIGURE 9.2 (See color insert following page 412)**

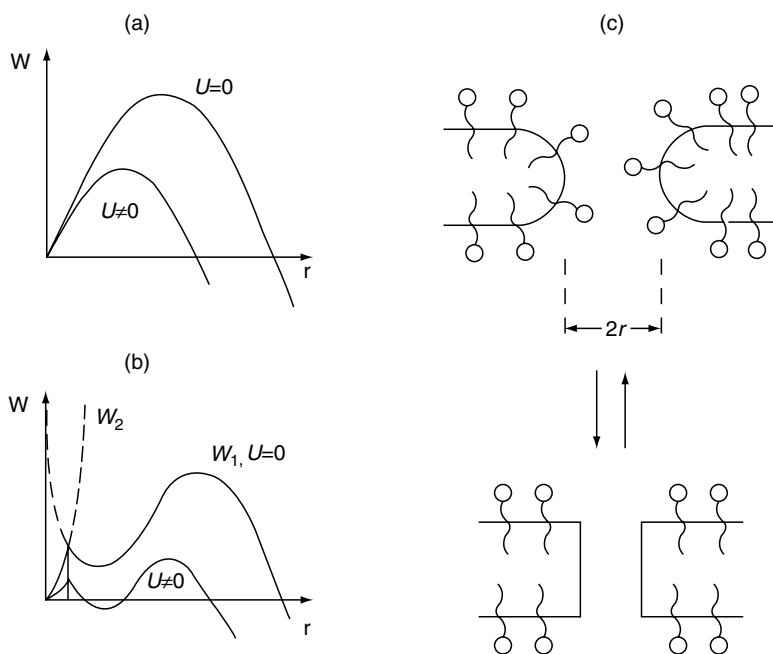
Snapshots of pore creation according to a molecular dynamics simulation. An important, partial test of electroporation was obtained by imposing a constant electric field,  $E = 0.5 \text{ V/nm}$  ( $5 \times 10^6 \text{ V/cm}$ ) throughout a small volume that contains aqueous electrolyte and a small area of phospholipid bilayer membrane (Tieleman, D.P. *BMC Biochem.*, 5, 10, 2004). The phospholipid headgroups are white, the hydrocarbon chains are gray, and water is dark gray. The chloride and sodium ions are somewhat difficult to make out in this gray-scale reproduction, so the reader is referred to the original paper for a color depiction. The snapshots are at times (a) 5.33, (b) 5.45, (c) 5.50, and (d) 5.70 ns from starting the simulation. As described in the original paper (Tieleman, D.P. *BMC Biochem.*, 5, 10, 2004) initially water molecules stochastically enter into the bilayer interior, leading to “formation of single-file like water defects penetrating into the bilayer.” These and other MD results [71–76] show that pores can form on a nanosecond time scale, and are hydrophilic, with phospholipids present on the interior of the fluctuating pore. These hourglass or toroidal-like pores are reminiscent of the simple drawings used to motivate early models of electroporation. Present MD models are “noisy” in the sense that for a typical computational volumes and times only a few transported ions are involved. However this approach has great promise, depending on improved computational power.

simplest form neglected the “spreading resistance” (access resistance) and “Born energy change for ion insertion” (partitioning) which were included in some later versions, and gave (see Figure 9.3)

$$(\Delta W)_p(r_p, U_m) = 2\pi\gamma r_p - \pi\Gamma r_p^2 - 0.5C_{1 \rightarrow w} U_m^2 \pi r_p^2 \quad (9.6)$$

where  $\gamma$  is the line tension associated with a pore edge,  $\Gamma$  is the surface tension of a pore-free planar membrane, and  $C_{1 \rightarrow w} = \epsilon_0[\epsilon - \epsilon_1]/d_m$  is the difference in specific capacitance due to replacement of lipid by water. At zero transmembrane voltage  $(\Delta W)_p(r_p, U_m)$  is a parabola with a maximum of  $\sim 100 kT$ , consistent with infrequent spontaneous BLM rupture.

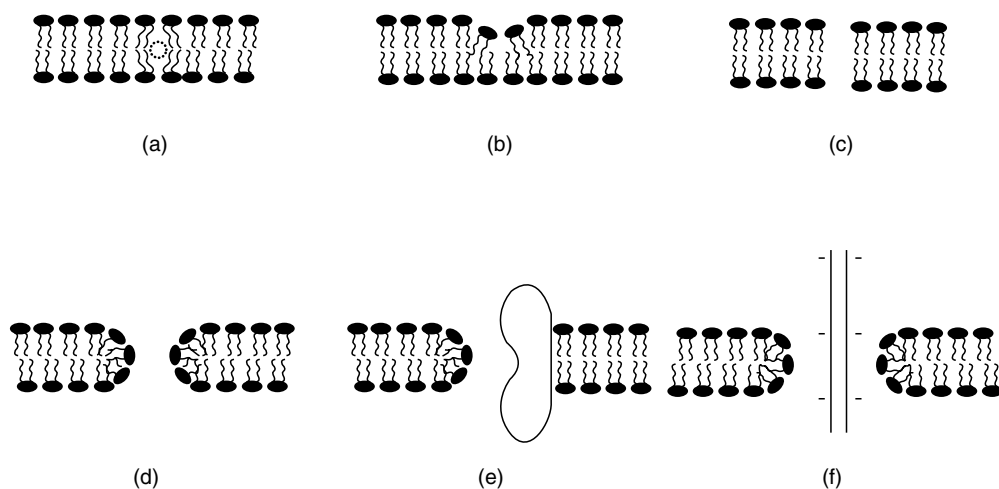
The initial suggestion that pore formation could occur and lead to membrane rupture did not involve electrical behavior. The basic idea was that a “cookie cutter” model for a pore formation energy at zero membrane potential,  $(\Delta W)_p(r_p)$  could be used, based on a gain in “edge energy,”  $2\pi\gamma$ , as a pore is created, and a simultaneous reduction in surface energy,  $\pi r_p^2 \Gamma$ , due to the loss of a circular patch of membrane [77]. The interpretation is simple: a pore-free membrane is envisioned, then a circular region is cut out of the membrane, and the difference in energy between these two states calculated and identified as  $(\Delta W)_p(r_p)$ . The change in pore energy due to an elevated transmembrane voltage is contained in the third term of the right-hand side of Equation 9.6. The membrane is



**FIGURE 9.3**

Drawing of imagined pore structures and associated pore creation energy. Early transient aqueous pore theory was based on two related concepts, hydrophilic pores and hydrophobic pores, with some models considering both and others only hydrophilic pores. (a) Hydrophilic pore creation energy,  $W$ , plotted as a function of pore radius,  $r$ , for zero transmembrane voltage ( $U = 0$ ) at which  $W$  is maximal, and a representative elevated voltages, at which  $W$  is decreased (favoring hydrophilic pore formation). (b) More complicated energy landscape for the hypothesis that small hydrophobic pores (curve  $W_2$  at  $U = 0$ ) form first, followed by thermal activation to hydrophilic pores (curve  $W_1$  at  $U = 0$ ). For elevated  $U$  the overall energy decreases, with two peaks, which favors increased hydrophobic pore formation and also transitions to hydrophilic pores. Dependent on time and  $U$ , hydrophilic pores can surmount the right-most barrier and expand to rupture the membrane (expected for artificial planar bilayer membranes, much less plausible for cell membranes with small surface tension). (c) Envisioned transitions between hydrophobic pores (bottom) and hydrophilic pores (top). In this simple view hydrophobic pores are imagined to be cylindrical, with an interior surface characterized by a hydrocarbon–water interface (large surface energy), and hydrophilic pores are “inverted,” with phospholipid head groups lining the pore interior. Recent molecular dynamics simulations show that hydrophilic pores are expected, and the precursor membrane conformations involve water entry, achieving a water chain (analogous to a hydrophobic pore) that penetrates the membrane before a phospholipid-lined pore forms.

regarded as a capacitor, with the  $U_m$ -dependent term representing the change in electrical energy resulting from replacing membrane material with dielectric constant  $\epsilon_1$  by water with dielectric constant  $\epsilon_w$  in a cylindrical pore of radius  $r_p$ . In other words, creation of a hydrophilic pore (hereafter simply “pore”) is described as a change in specific capacitance,  $C_1 \rightarrow_w$  in the region occupied by the pore. However, for small hydrophilic pores, even if bulk electrolyte exists within the pores, the permittivity would be  $\epsilon \approx 70\epsilon_0$ , only about ten percent smaller than for pure water. Moreover, the pore interior resistance would still be large,  $R_{p,int} \approx \rho_e d_m / \pi r_p^2$  compared to the spreading resistance (see below), so the voltage across the pore would be very close to  $U_m$ . Although each of the “ingredients” of the pore model is plausible, if they are combined to represent a complete experimental situation, the overall model is too complex to solve in closed form. Other membrane deformations, conformational changes and hypothesized pore structures are shown in [Figure 9.4](#) [78].



**FIGURE 9.4**

Hypothesized pore structures. Illustrations of hypothetical structures of both transient and metastable membrane conformations that may be involved in electroporation. Hypothetical bilayer membrane structures related to electroporation: (a) membrane free volume fluctuation; (b) aqueous protrusion into the membrane ("dimple"); (c) hydrophobic pore proposed by Chizmadzhev and coworkers [9]; (d) hydrophilic pore proposed by Litster and by Taupin and coworkers, usually regarded as the "primary pores" through which ion and molecules pass during electroporation; (e) composite pore with one or more proteins at the pore's inner edge; (f) composite pore with "foot-in-the-door" charged macromolecule inserted into a hydrophilic pore. Although the actual transitions are not known, the transient aqueous pore model assumes that transitions from  $A \rightarrow B \rightarrow C$  or  $D$  occur with increasing frequency as  $U_m$  increases. Type E may form by entry of a tethered macromolecule when  $U_m$  is large, and then persist after  $U_m$  has decayed by pore conduction. These hypothetical structures have not been directly observed, but are consistent with a variety of experimental observations and with theoretical models. (Reproduced from Weaver, *J. Cell. Biochem.* 51, 426–435, 1993. With permission.)

Such models are, however, now routinely solved numerically on computers to generate qualitative and quantitative predictions, and these results can be compared with experimental observations. Solutions for artificial planar BLMs have been developed, and these produce reasonable, but not completely correct, quantitative descriptions of measurable quantities such as the transmembrane voltage,  $U_m(t)$  the pore size distribution, and also the membrane conductance,  $G_m$ , which increases tremendously during REB. The transport of small ions and small molecules can also be predicted approximately (see Section 9.6). In general agreement with experimental observation [31], a model solved for different pulse magnitudes also predicts the four distinguishable outcomes that are found experimentally for an oxidized cholesterol planar membrane subjected to a 400 ns second square charging pulse: (i) simple, passive charging of the membrane capacitance for small pulses; (ii) charging to about 0.5 V, followed by a sigmoidal decay of  $U_m$  as the membrane ruptures for a moderate pulse; (3) incomplete reversible electrical breakdown in which the membrane achieves a high conductance state, but not enough to discharge the membrane fully to zero, for a larger pulse; and (4) reversible electrical breakdown due to a still larger but transient high conductance that discharges the membrane completely [46]. This behavior reflects the interplay between the rapidly changing electrical conductance and the mechanical expansion and contraction of the individual pores in a heterogeneous, dynamic pore population.

An extension of the single artificial planar BLM to two identical membranes in series can be used as a very simple model of a "cubic cell" [79]. By using a very low value of  $\Gamma$  the inability of a spherical membrane to rupture can be approximated, and this allows the

behavior of cell membranes to longer pulses to be explored. Such an approach is ongoing and generates predictions of  $U_m(t)$  and the transport of small charged molecules of type “s”,  $\bar{n}_s$ , that are reasonable. Investigation of a planar membrane has also generated a surprise, the prediction of an approximate intrapulse plateau in  $U_m$  as a function of exponential pulse amplitude [47]. Whereas too simple to fully represent cellular electroporation, this prediction may be related to experimentally observed plateaus in charged molecular transport (see Section 9.6).

The various theoretical models support the idea that the reversibility of electroporation in planar membranes is due to (i) the chemical composition of the membrane, (ii) the pulse protocol, and (iii) feedback at the pore level that is associated with the spreading resistance, such that as a pore expands, the local transmembrane voltage across the pore decreases. Overall, considerable progress has been made in devising theoretical models that can quantitatively describe some aspects of electroporation, whereas others are still poorly understood. Mechanical and electrical aspects of electroporation of a relatively simple artificial planar BLM do account for (i) the approximate magnitude of  $U$  at which membrane rupture (irreversible breakdown) occurs, (ii) the strong dependence of membrane lifetime on  $U_m$ , and (iii) the stochastic nature of rupture. Moreover, a reasonable but not exact quantitative description of  $U_m$  is given by a transient aqueous pore theory, and the maximum fractional aqueous area of the membrane during reversible electroporation [47] agrees with limited experimental observations [48,49]. There has been some progress in predicting molecular transport, but much remains to be done, and the mechanisms of membrane recovery, cellular stress, and the determinants of cell survival or death are essentially not understood. Moreover, single pulse electroporation has been emphasized by theories and models to date, and relatively little is known about the importance of pulse shape, repetition and spacing of multiple pulses. The challenge is to understand what changes persist and therefore represent the state of the membrane when second and then additional pulses are applied.

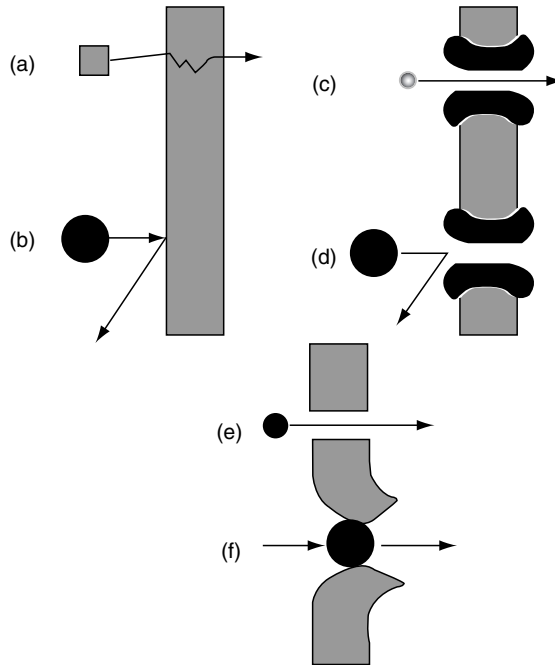
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## 9.6 Molecular and Ionic Transport

Greatly enhanced molecular and ionic transport is the basis of most all applications of electroporation. DNA introduction into cells for transfection was first reported about 25 years ago [41] and “transfection” has almost become synonymous with “electroporation” in some areas of biological research [80–83]. Delivery of drugs, proteins, and fluorescent indicators into cells and tissues is a basic manipulation that is also widely used in research, and is being adopted for clinical treatment of local cancer tumors [84–86]. Accordingly, by now numerous potential applications in biology, biotechnology, and medicine have been suggested, and many other applications are explored. The basic idea is qualitatively simple: application of one or more electrical pulses leads to elevated  $U_m$ , which if sufficiently high and long leads to membrane pore formation and subsequent expansion to transport water-soluble molecules under the influence of local driving forces (Figure 9.5). Quantitatively, however, surprisingly little is established theoretically using cell and tissue models. In the case of potential biomedical applications involving *in vivo* electroporation, more research will be needed to understand both efficacy and side effects.

Early studies of transport reported molecular release from biological vesicles due to a field-induced permeability increase [25]. Red blood cells were subsequently used to demonstrate DNA delivery into a cell that was associated with dielectric breakdown of





**FIGURE 9.5**

Schematicized drawing of molecular transport pathways. These pathways are envisioned to exist in the intact or permeabilized lipid bilayer portion of a cell membrane or of an artificial bilayer membrane. The membrane may contain preexisting channels or induced pores. This cartoon can, with some restriction, be considered valid for multilayer structures, e.g., in the skin's stratum corneum. (a) Hydrophobic molecule, which is shown as a gray square can cross the lipid bilayer via partitioning and diffusion. (b) Hydrophilic molecule (large black dot), which is repelled from the lipid phase, can not overcome a hydrophobic barrier. (c) Small ions (gray dot) can cross a membrane through narrow preexisting defects or proteinous channels. (d) Large polar molecules (large black dot) cannot pass through ion channels evolved to regulate small ion transport. (e) Electropores are large enough to provide effective transport of ions and polar particles of moderate size, with the size dependent on evolution of pores during a particular electrical pulse. (f) Bigger charged molecule (large black dot), e.g., plasmid DNA, can be forced by the local electric field to enlarge an electropore and then move across the membrane.

the cell membrane [40]. This experiment did not, however, involve the critical demonstration of cell transfection. This achievement was reported several years later [41,87]. Molecular uptake into cells has now been widely reported, with experiments typically using a large number of cells. These measurements determine the average molecular transport per cell by measuring a total cell population effect. Examples of delivered molecules in such experiments include antibodies [88–90], enzymes [35], and small molecules [91–95]. A few experiments obtained calibrated measurements that yielded that approximate number of molecules delivered into individual cells [91–95], with quantitative results needed for the development of theories and models for transport. Some of these experiments show that there is significant cell–cell variation in molecular transport.

Taken together these studies show that significant molecular transport occurs under approximately the same conditions that cause REB. This is an important finding, but does not address the question of how much molecular transport occurred for various pulsing conditions, nor was the question of cell–cell variation considered. One particularly interesting study showed that uptake of a first fluorescence-labeled molecule (fluorescence-labeled

dextran; 19 kDa) was a reliable indicator for the uptake of a second, larger fluorescent molecule (phycoerythrin, 240 kDa) [96]. This report is consistent with the idea that electroporative molecular transport is a physical process (movement through aqueous pathways), which is relatively nonspecific if the pathway is not too close to the molecule's size. As mentioned earlier, studies with artificial planar BLMs show that electroporation depends on the history of the transmembrane voltage,  $U_m(t)$ . This implies that physical factors which govern field-induced change in  $U_m$  over a cell membrane should be important, i.e., the shape, size and orientation of cells with respect to the cells with respect to the local electric field. In addition to isolated cell models, a didactic multicellular model of a hypothetical local tissue region shows that individual cells can experience significantly different changes in  $U_m$  over each cell membrane [7].

Biological systems are also well known to be variable, e.g., displaying cell-to-cell variation in many properties ("biological variability"). At a nanometer scale electroporation is believed to arise from fluctuations of water molecules and phospholipid molecules and also nearby ions or molecules that are transported through the membrane in the presence of an average elevated  $U_m$ . This view intrinsically arises in molecular dynamics (MD) simulations of artificial membrane electroporation [71–76] and is consistent with stochastic models based on analytic estimates [9,16–19,97]. For both analytic and MD analysis the overall process of forming pores is fundamentally stochastic. Existing continuum models that exhibit REB have significant internal feedback between pore population ionic conduction and  $U_m(t)$ . This intrinsic regulation of the local transmembrane voltage is missing from the present MD analysis of electroporation.

Pore formation is expected to become more deterministic for pulses significantly shorter than 1 ns or for pores created in spatially restricted regions of a cell membrane, e.g., small phospholipid regions within the mitochondrial inner membrane [98]. However, conditions favoring deterministic pore creation have been uncommon to date. With this in mind, electroporation-related phenomena are expected to exhibit significant cell-to-cell variability. This in turn supports the idea that quantitative molecular transport determinations at both the population level and the individual cell level are important to understanding the magnitude and mechanisms of molecular transport.

Of the many possible molecular transport measurement methods, optical methods have the best combination of sensitivity, specificity, and speed. Both image analysis (quantitative microscopy) and flow cytometry make quantitative measurements at the individual cell level. This allows measurement of the number of molecules present by light absorbance (least sensitive) and by fluorescence or luminescence (most sensitive). With corrections for background and non-specific binding to external cell surfaces, measurements can determine the number of molecules of type "s" taken up (or released),  $n_s$ , from individual cells. Image analysis and flow cytometry are generally complimentary. Flow cytometry makes spatially-unresolved individual cell measurements at rates of  $10^2$  to  $10^4$  cell/s, and therefore routinely analyzes large cell populations. Image analysis excels at measurements with spatial resolution (of order 1  $\mu\text{m}$ ) but with increasingly improved temporal resolution that is best (fastest) for gated (strobed) image acquisition and generally less (slower) for rastered image acquisition. Both flow cytometric and image-based molecular uptake measurements are at their best when using molecular probes such as propidium iodide (PI) that exhibits major changes in fluorescence upon binding. Such test molecules are attractive because wash steps are avoided and measurement speed is governed by molecular (ionic) transport and binding kinetics combined with optical signal-to-noise ratio considerations. If confocal or two-photon microscopy were used with sufficiently large cells that a portion of the intracellular region can be volumetrically resolved, then molecular transport into an intracellular volume could be quantitatively monitored during the uptake process.



Image analysis also provides information about localization of molecular transport [99–102]. This has allowed observation of the degree of cellular uptake asymmetry. In the case of suspended spherical cells the instantaneous transmembrane voltage,  $U_m(t)$ , at the two poles (consider  $\theta = 0, \pi$  in Equation 9.3 and Equation 9.5 for pre-electroporated cells). These equations suggest that  $U_m$  will be increased at one pole and diminished at the other. A mammalian cell's resting potential (transmembrane voltage) is  $U_{m,\text{rest}} \approx 60$  to 90 mV (cell interior negative with respect to exterior). Qualitatively, it could be expected that  $U_{m,\text{rest}}$  adds at one pole and subtracts at the other. Quantitatively, this has been addressed by describing  $U_m$  during electroporation and finding that the fractional change is small except within the equatorial region [67]. Electroporation asymmetry can also be hypothesized as arising from a metabolically driven ionic current that passes through membrane leaks (the passive cell membrane resistance) to establish  $U_{m,\text{rest}}$  and to also estimate changes due to electroporation shunting current through electropores [69]. Consideration of  $U_m$  alone is not expected to provide a full explanation. Whereas some experiments measure  $U_m$  [49,50], other experiments reporting asymmetry involve molecular transport observations [99–102] and in these cases net transport should be understood.

An example of quantitative, individual cell level molecular transport determinations involved yeast cells in a medium containing 80  $\mu\text{M}$  PI (668 Da; charge of  $z = +2$ ). Without electroporation there is negligible uptake. This is expected because of the large Born energy barrier (Equation 9.1) for small charged molecules, and doubly charged PI is a well established “membrane exclusion dye” that is routinely used to test for cell membrane integrity [103]. However a single square electric field pulse within the electrolyte ( $E_e = 5 \times 10^3 \text{ V/cm}$  for a pulse with duration  $t_{\text{pulse}} = 50 \mu\text{s}$ ) results in a significant average uptake, viz.  $\bar{n}_{\text{PI}} \approx 10^8$  molecules per cell. Flow cytometric fluorescence measurement is aided by PI binding to doubly stranded nucleic acids within the cell, which significantly increases PI fluorescence [92]. But for typical experimental conditions PI fluorescence is weaker than calcein. Accordingly, in a subsequent example, red blood cell ghosts were suspended in an aqueous electrolyte with the green fluorescent polar tracer molecule, calcein (623 Da; net charge of  $z = -4$ ) at a concentration of 1 mM. Calcein-exposed cells emit only very slight green fluorescence if unpulsed. This control case fluorescence is attributed to weak surface binding and autofluorescence. However, if a single “exponential pulse”  $E_e(t) = E_{e,0} \exp -t/\tau_{\text{pulse}}$  with  $\tau_{\text{pulse}} \approx 3 \text{ ms}$  and  $E_{e,0} \geq 3 \times 10^3 \text{ V/cm}^{-1}$  then a plateau in molecular uptake  $\bar{n}_{\text{Cal}} \approx 7 \times 10^4$  molecules per ghost is observed. For the same conditions, but with fluorescence-labeled macromolecules 10  $\mu\text{M}$  lactalbumin (14.5 kDa;  $z \approx -15$ ) and 10  $\mu\text{M}$  bovine serum albumin (BSA; 68 kDa;  $z \approx -25$ ) an uptake plateau is also found,  $\approx 7 \times 10^4$  molecules per ghost [104]. Surprisingly, for these single exponential pulse experiments, the plateau uptake per ghost is well below the estimated equilibrium value obtained by multiplying the supplied extracellular concentration and the ghost volume,  $\bar{n}_{s,\text{equi}} = c_{\text{ext}} V_{\text{ghost}}$ . Extension of these experiments to multiple pulses for BSA uptake shows that the plateau value for  $\bar{n}_{\text{BSA}}$  increases with pulse number, but remains well below  $\bar{n}_{\text{BSA},\text{equi}}$ . Finally, observation of an uptake plateau well below equilibrium holds for yeast experiments in which the effective partition coefficient between the extracellular medium and cell interior for calcein was estimated experimentally [105] and for other experiments in which calcein uptake and clonal growth were both measured at the individual cell level [95].

The variation in individual cell uptake can also be determined and is significant. Yeast uptake of PI by a 50  $\mu\text{s}$  square pulse and erythrocyte ghost uptake of calcein, fluorescence-labeled lactalbumin, and fluorescence-labeled BSA due to single  $\sim 1 \text{ ms}$  exponential pulses show broad population distributions in the amount of uptake per cell without distinct subpopulations. In contrast, molecular uptake of calcein by a suspension of yeast

(*Saccharomyces cerevisiae*) subjected to a single  $\sim 1$  ms exponential pulse exhibits several subpopulations that change markedly as the pulse amplitude is increased from an approximate threshold  $E_{e,0} \approx 1 \times 10^3$  V/cm and above threshold to  $\sim 7 \times 10^3$  V/cm [105]. In spite of this significant heterogeneity the average uptake per cell,  $\bar{n}_{\text{Cal}}$  exhibits plateaus, with separate plateaus found for both a “major uptake subpopulation” and a “minor uptake subpopulation.” Surprisingly, for these single exponential pulse experiments with yeast (elongated, budding), the plateau uptake per cell is well below (more than the order of magnitude smaller) than  $\bar{n}_{\text{equi}}$ , even after the intra- or extracellular partition coefficient is included. In summary, several single pulse experiments have found uptake plateaus corresponding to nonequilibrium amounts. These results suggest that the plateau may be a consequence of molecular transport driven by local electric fields, e.g., electrophoresis. An approximate plateau in  $U_m$  is predicted by a transient aqueous pore model for an exponential pulse that causes electroporation [47]. If molecular transport is dominated by electrophoresis (or electro-osmosis) through rapidly changing pores during a pulse, then nonequilibrium net transport may therefore result. If instead molecular transport is dominated by diffusion during and after a pulse [106], then net transport can approach equilibrium values or can be less, depending on the number of pores and their lifetime. The total intra- and extracellular volumes of the system, which is usually quite different for *in vitro* and *in vivo* conditions, (Equation 9.7) is also important [107].

Presently quantitative molecular transport measurements at the individual cell level have been made on (i) red blood cell ghosts, for which the uptake per ghost was determined for calcein, lactalbumin, and BSA due to a single exponential pulse with a  $\sim 1$  ms time constant and (ii) intact yeast (*S. cerevisiae*) cells, for which the uptake per cell was determined for PI because of a single 50  $\mu\text{s}$  square pulse, and calcein, lactalbumin, and BSA because of an exponential pulse with a 1 ms time constant. It is therefore not yet established whether nonequilibrium uptake is a general feature, particularly for small charged molecules. Similarly, it is not yet known whether multiple pulses, pulses of other shapes, or certain features of the transported molecules are particularly important. In short, the mechanism of molecular transport is not yet understood in detail. For small molecules, the creation of a pore population probably occurs independently of molecular transport, but for larger molecules, particularly highly charged macromolecules such as DNA, electrically created pores may be significantly enlarged by interaction with approaching molecules driven by the local field (Figure 9.4).

In addition to electrophoresis through pores involving local, inhomogeneous electric fields, and time-dependent diffusion through pores, pulsed cells may be stimulated by presently unknown mechanisms to take up molecules by endocytosis [108]. External additional driving forces such as a pressure difference due to simultaneous or subsequent centrifugation may act to both enlarge primary (electric field-created) pores and to drive flow through the enlarged pores. Quantitative uptake measurements were also reported for mammalian cell experiments with submicrosecond (10 to 100 ns) megavolt-per meter scale (150 kV/cm) pulses in which extracellular media with smaller conductivity were used [56]. For these conditions with low extracellular conductivity media significant cell deformation occurs. Cell deformation increases the challenge of understanding molecular transport through pores. Overall, the strongest evidence to date supports involvement of electrical drift (electrophoresis) for moving small charged molecules through pores, with diffusion contributing to both neutral and charged molecules. The interpretation of experiments that introduce highly charged DNA is consistent with local electrophoresis.

Moreover, a transient aqueous pore theoretical model involving only electrophoretic transport through a dynamic pore population created by a single exponential pulse, predicts some of the plateau behavior found for red blood cell ghosts and yeast cells.

A tentative interpretation is that electroporation involves a rapid interaction between the membrane conductance due to pores, and the transmembrane voltage,  $U_m(t)$ . A slight increase in  $U_m$  results in rapid creation of many pores, which by a voltage divider effect tends to reduce  $U_m$ . This tendency to a nearly constant  $U_m$  seems to prevail during much, but not all, of the pulse duration, and this is qualitatively consistent with a local transmembrane electrophoretic transport that is independent of the pulse magnitude once a significant number of pores exist. Given the central importance of molecular transport to applications, considerably more development of basic insight into molecular transport mechanism is needed. Both the average and the variation in molecular uptake or release per cell is of basic importance to cellular electroporation, yet has even now received relatively little attention.

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## 9.7 Electrotransfection

The delivery of DNA into cells *in vitro* is the predominant application of electroporation [80–83,109,110] and is extremely important. Molecular transport of DNA into cells by electric field pulsing was first reported in 1976 [40], but only as a demonstration of principle, since it did not alter cell function. Indeed, the first electrically induced gene transfer *in vitro* with subsequent expression was demonstrated in 1982 [41], and this stimulated a large number of papers demonstrating the applicability of electrotransfection to mammalian, plant, bacterial, and yeast protoplasts and cells. Electrotransfection has significant advantages: it is physical and therefore universal, effective, and often is the only method that gives positive results. For this reason, electrotransfection is actively pursued for human gene therapy based on two general approaches: (i) cells treated *in vitro* followed by reimplantation into the body [83,111–113] and (ii) cells transfected either in isolated tissues (e.g., plant tissue) or mammalian tissue *in vivo* [83,110,114–117]. For maximum utility, the efficiency of introduction of active DNA and cell survival must be simultaneously optimized, but understanding mechanisms involved in electroporation and downstream events remains inadequate.

Many investigators have reported a correlation between electroporation and electrotransfection. This could be interpreted as support for the role of electropores as the actual pathways for DNA transport. However, primary pores caused solely by an increased  $U_m$  by a short pulse are too small ( $r_{p,\min} \approx 10$  nm [62]) to accommodate DNA. Passive entry and diffusion through a moderately large pore ( $r_p \approx 10$  nm) may allow DNA entry [118]. The frequency-of-occurrence of large pores ( $r_p > 10$  nm) [47,119,120] and the possibility that entering DNA drives enlargement of a pore should also be considered. In any case, DNA uptake is usually not due to passive diffusion. Instead, the following driving forces were considered: (i) flow of water occurring due to the colloid–osmotic cell swelling following electroporation [121], (ii) electro-osmotic flow through a pore with a charged interior surface [122], (iii) electrophoretic transfer of DNA through pores [123,124], and (iv) electrophoretically driven adsorption of DNA onto the membrane, followed by endocytosis which engulfs the DNA [108,125]. Other, relatively recent experiments support the mechanism of electrophoresis by local electric fields focused (by the spreading resistance) through pores, such that DNA is carried into and through a pore that is actively enlarged by the electrically driven DNA [123,124].

The introduction of DNA by electroporation also increases the uptake of dextrans  $> 20$  kDa supplied after pulsing. This occurs even if the dextran is added a few minutes after

pulsing, implying a pore lifetime of 10 to 100 s. Moreover, the larger the DNA fragment, the greater the increase in permeability to the dextrans. The interpretation is that the use of a two-pulse technique (a large first pulse to create pores, a second long but much smaller to cause electrophoresis) provides separation of the primary electroporation process and subsequent field-driven transport through long lifetime pores. A DNA molecule may also prevent a DNA-occupied pore from closing, a foot-in-the-door mechanism that may lead to prolonged pore lifetimes. A fluctuating pore temporarily occupied by a macromolecule can allow small molecules to continue to be transported [78]. The introduction of highly charged heparin into the skin's stratum corneum by pulses believed to cause electroporation results in co-transport of small molecules that are consistent with this possibility [126].

An associated theory predicts that DNA in the electroporating electric field exerts high pressure on the membrane, which can then induce structural rearrangements of the membrane [127]. In this view, electrophoresis is the transport mechanism for DNA uptake, as it brings DNA to the cell surface and then drags DNA through the membrane, initially entering preexisting pores, which are then enlarged and deformed in course of translocation. This is an example of a process that leads to secondary pores, which are significantly different than the primary pores that arise solely by the interaction of the electric field with the membrane.

Molecular electroinsertion is distinct from transport of ions or molecules through a membrane. Experiments have shown that certain macromolecules can be inserted stably into cell membranes by pulses associated with electroporation [128–132]. A qualitative picture is that transient openings in the cell membrane provide an opportunity for such macromolecules to partially enter a pore and then become trapped as the pore shrinks. Perhaps one end of a membrane-spanning molecule is loosely bound to part of the membrane, such that the molecule can rotate into a pore, only to become trapped as the pore shrinks and eventually vanishes. Electroinsertion is such a fascinating event that it deserves conceptual emphasis, even though relatively few studies have been made since its discovery.

Electrofusion provides somatic hybridization of cells [111,112,133–139]. This has led to demonstrations of cell-tissue fusion [140,141], hybridomas for antibody production [81,142], individual cell and vesicle manipulation [143,144], tumor vaccine production [145,146], and other applications [80,82,147]. But the mechanism of cell electrofusion is still not fully understood. The understanding we do have comes from different types of studies. Experiments using model systems consisting of two planar lipid bilayers have provided significant insight [59]. After pressure application, the bilayers reach a state of close contact. Close proximity is usually necessary for cell electrofusion. The distance between bilayers is determined by the equilibrium of all acting forces: molecular, electrostatic, hydrostatic pressure, and hydrational. After a lag time, one or more bridges (stalks) form spontaneously between two adjacent monolayers. These bridges then expand, leading to a so-called trilaminar structure, which contains a single bilayer in contact with two bilayers along a perimeter. A trilaminar structure is rather stable, but after a voltage pulse is applied, electroporation occurs, followed by an irreversible rupture of the contact bilayer with its transition to so-called membrane tube. This stage of the process results in fusion because now there are joined lipid phase and water compartments. It is important to note that two different structural rearrangements take place: (i) stalk (or bridge) formation between adjacent monolayers and (ii) pore formation in contact bilayer with measurable time lag. Note that the first stage is essentially spontaneous, and that the electric field is involved only during the second stage of the fusion process.

Additional insight follows from noting that the probability of stalk formation depends on its energy, which in turn is determined by the favorable molecular geometry of

phospholipid molecules. From the viewpoint of continuous media mechanics, this implies a dependence on the spontaneous curvature of monolayers. This theory was compared with the results of experiments in which lag times were measured for membranes with different spontaneous curvatures. Good agreement was obtained, supporting the view that the stalk mechanism is involved in lipid bilayers fusion.

Cell fusion is much more complicated. Attempts to find evidence for trilaminar structures in electrically pulsed cell suspensions have not been successful. For this reason, another fusion mechanism and the interaction of coaxial electropores at adjacent membranes have been considered [148]. In this case it was shown that attractive forces due to the Maxwell tension between two parallel bilayers with coaxial pores are large enough to almost bend the membranes and to bring the pores together until a fusion of pore edges is achieved. These two mechanisms differ in their sequence of events: (i) monolayer fusion and followed by electroporation (the first case; see above) and (ii) electroporation followed by fusion of pore edges (the second case). Significantly, it has been experimentally demonstrated [149,150] that fusion can also proceed by using a protocol in which pulsing precedes cell contact. This supports the mechanism of fusion through electropores. Additional understanding of electrofusion should therefore depend on further developments in the understanding of electroporation.

Extension of cell–cell electrofusion *in vitro* to cell–tissue *in vivo* has been briefly described [140,141] and offers the prospect of many applications in research and medicine. In this case critical issues involve making contact between the introduced cells or vesicles to the target tissue and then causing a high probability of fusion while retaining the viability of both the introduced cells and the target tissue.

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## 9.8 Membrane Recovery

Membrane recovery after pulsing is fundamentally important to understanding whether an electroporation protocol is reversible at the membrane level and at the cellular level. Terminology includes “resealing” and “pore lifetime.” According to the transient aqueous pore hypothesis, recovery is expected to involve shrinkage and disappearance of pores, with some models further assuming that pores shrink to a minimum size before stochastically vanishing. However, quantitative data that constrain membrane recovery mechanisms remain relatively scarce and are often indirect. For example, molecular uptake observations are often used, which depend on more than pore shrinkage and destruction.

The scientific literature contains reports with widely varied membrane recovery times, from nanoseconds to minutes at room temperature to 37°C and even longer at low temperatures. Here we cite several examples, beginning with several papers with data relevant to membrane barrier recovery. These include molecular and ionic uptake on human erythrocytes ( $\tau_p \approx 1$  to 10 h and strongly dependent on temperature, with much longer resealing at 3°C than at 37°C) [36], on isolated rat skeletal muscle cells ( $\tau_p \approx 500$  s) [151], PI uptake by electropermeabilized myeloma cells ( $\tau_p \approx 100$  s) [152], dye uptake ( $\tau_p \approx 120$  s) [153],  $\text{Ca}^{2+}$  influx into vesicles ( $\tau_p \approx 165$  ms) [101], and into adherent cultured cells ( $\tau_p$  timescale of milliseconds to seconds) [154]. As these examples illustrate, there is a wide range of membrane recovery timescales for molecular uptake, but with artificial membranes often faster than cellular membranes. Electrical measurements can also be used and have the advantage that the transported species (small ions at a high

concentration:  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) are preexisting throughout cells, so they do not require delivery by mixing and diffusion to reach cell membranes. Examples are electrical measurements on isolated frog muscle cell membranes [155] ( $\tau_p \approx 500$  s), on cell suspensions and cell pellets, and on artificial planar BLM. Molecular dynamics simulations of electroporation are relatively recent and suggest pore lifetimes of a only few nanoseconds [75], which is difficult to reconcile with experimental values based on ionic and molecular transport.

Both reversible and irreversible electroporation lead rapidly (typically 1 to 100  $\mu\text{s}$ ) to a high conductance state, which discharges the membrane after pulsing or provides a small membrane resistance that participates in a voltage divider effect to reduce the transmembrane voltage, even during the pulse [47]. By definition irreversible behavior has no physical membrane recovery, and recovery by biological repair of significant membrane openings due to large magnitude or longlasting pulses has not yet been investigated. In considering what is known about membrane recovery, it was noted above that both electrical and molecular assessments of membrane barrier function can be made.

Early experiments used electrical measurements and planar membranes to show that after a decrease in the voltage, there is a very rapid ( $< 5$   $\mu\text{s}$ ) decrease in the membrane conductance [51,156]. Initially this result was interpreted as reflecting rapid resealing of pores [156]. It was later shown, however, that a rapid decrease in conductance is associated with the nonlinear behavior of a pore's current-voltage curve [45,157,158]. The number and the mean radius of the pores were unchanged on a timescale of microseconds, but in the timescale of 1 to 10 ms the decrease in conductance is governed by a change in the size of the pores. For artificial planar BLMs pores disappear from the membrane on a timescale of milliseconds. For cell membranes the timescale for the disappearance of pores is much longer, ranging from seconds to hours [45].

Electrical assessment of recovery has the advantage of speed and convenience, but testing for restoration of membrane barrier function by probing with molecules of different sizes and charges may fundamentally be more revealing as to the extent and kinetics of recovery. Indeed, several studies have reported the results of delayed addition experiments, with probe molecules supplied to pulsed cells at different times after pulsing. As expected, the qualitative observation from experiments using total population measurements is that significantly less uptake occurs if molecules are added to the extracellular medium after the pulse. Typically the added molecules are small [36,159], but sometimes macromolecules, including DNA, were used [124]. An important finding is that if analytical techniques with individual cell level capability (microscopy, flow cytometry, image analysis) are used, then subpopulations with uptake of delayed addition molecules are observed [105,142,160,161]. Factors affecting membrane recovery kinetics have not yet been thoroughly investigated. However, some experiments show that reduced temperatures lead to much longer membrane recovery times [36,162]. This is not surprising, as the diffusive pore shrinking that is expected as the membrane discharges are expected to have this qualitative temperature dependence. Moreover, most physical and chemical processes of multi-molecular systems are characterized by one or more rates that have Boltzmann factors, with their strong, nonlinear reduction in rate at lower temperature.

Pulses that lead to pore formation and evolution are expected to involve a dynamic pore population with time-dependent pore size distributions [46,47]. For this reason the change with time of the membrane's ability to exclude molecules of different sizes and charge should provide additional insight into the membrane recovery process. Indeed, experiments have shown that if molecules are first presented to cells at increasing time delays after pulsing, then a decreasing fraction of cells have a persistent ability to take up molecules [160]. This suggests that a subpopulation of cells has metastable pores with a distribution of pore lifetimes. Enhanced recovery by adding certain surfactants

has been demonstrated [163,164]. The discovery of enhancement both challenges our understanding of what membrane recovery is and offers promise as a therapy for electrical injury that involves nonthermal membrane damage by otherwise irreversible electroporation.

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## 9.9 Cell Stress and Survival

Most *in vitro* applications of electroporation benefit from some understanding of cell survival, but for *in vivo* applications it is becoming essential. *In vitro* electroporation often focuses on delivery of molecules into the cytosol, but for some research studies [165–167] related to nonthermal electrical injury [164,168,169] and harvesting of cellular products [170] release of molecules is sought.

A general observation over many experiments is that as the field magnitude is increased for a given pulse shape, phenomena such as molecular uptake become significant at a “fuzzy threshold” (dependent on pulse shape, including pulse duration), and then increase. However, at about the same “fuzzy threshold,” cell viability begins to decrease, with almost all cells getting killed as the electric field is increased. Although the mechanism of cell killing by electroporation is not fully established, a plausible hypothesis is that cell survival or death is mainly due to the degree of molecular exchange between cells and their environment and the resulting cell stress due to chemical imbalances [160,171,172]. This is consistent with the observation that cell killing can occur without significant heating [22–24,164,173] and that a tremendous increase in membrane permeability and associated molecular transport occurs.

A transient pore population and also possibly a small number of metastable pores are expected, and all may contribute to stress-inducing transport. Thus, reversible membrane electroporation (complete resealing or pore disappearance) may still lead to net transport (uptake or release). This means that relatively nonspecific molecular exchange may occur between the intra- and extracellular volumes and can lead to stress-inducing chemical imbalances. Depending on the ratio of intra- and extracellular volumes, the composition of the extracellular medium and the cell type, a cell may not recover from the associated biochemical imbalance (stress) and will therefore die. Both reversible and irreversible electroporations result in transient openings (perforations) of the membrane, which are often large enough that the pores are not selective. In this case, molecular transport is expected to be nonspecific. It is plausible that a portion of the cell membrane behaves much like a small planar membrane and undergoes rupture (irreversible electroporation). Just as in the case of reversible electroporation, significant molecular transport between the intra- and extracellular volumes may lead to a significant chemical imbalance. If this imbalance is too large, recovery may not occur, resulting in cell death. It has been hypothesized [172] that the volumetric ratio

$$F_{\text{vol}} = \frac{V_{\text{extracellular}}}{V_{\text{intracellular}}} \quad (9.7)$$

should correlate with cell survival or death. In this equation  $V_{\text{extracellular}}$  is the volume of liquid medium outside the cell and  $V_{\text{intracellular}}$  is the volume of liquid in the cell interior. The idea is that, for a given cell type and extracellular medium composition,  $F_{\text{vol}} \gg 1$  (typical of *in vitro* conditions such as cell suspensions and anchorage-dependent cell culture) should tend to favor cell death. The other extreme is typical of *in vivo* tissue conditions ( $F_{\text{vol}} \approx 0.15$ , a higher interstitial fluid fraction) and this should favor cell

survival. The dilution of released molecules is governed by  $F_{\text{vol}}$  and partitioning between the extra- and intracellular environments [105]. For example, if the partition coefficient is one, the maximum *in vivo* initial dilution factor in solid tissue should be  $1/0.15 \approx 7$ , whereas the expected dilution is several orders of magnitude larger for typical *in vitro* conditions. This suggests that for the same degree of electroporation, significantly less damage may occur in tissue than in body fluids or under most *in vitro* conditions. With time, however, released molecules can diffuse within the interstitial space, be cleared by blood perfusion, and then distributed by the systemic circulation. The ultimate fate of released molecules is governed by excretion, metabolism, and storage, which is traditionally described by pharmacokinetics.

Cell viability of culturable cells can be stringently tested by clonal growth assays, whereas differentiated cells are often assessed by assaying particular functions, such as membrane exclusion dyes. However, caution should be exercised in assessing cell viability after electroporation protocols. After all, there is little *a priori* justification for using membrane-exclusion dyes (e.g., trypan blue [960 D,  $z = -4$ ], PI [668 D,  $z = +2$ ]), because electroporation causes the membrane permeability to significantly increase by unknown amounts for unknown times. Membrane-exclusion dyes are generally larger than some small ions and molecules (e.g.,  $\text{Ca}^{2+}$ ). Uptake or release of such ions and molecules may occur even if membrane recovery has progressed to the point that membrane-exclusion dyes are excluded by the membrane. Without validation it cannot be assured that the cell membrane has recovered sufficiently that fatal chemical imbalances are avoided. Instead, relevant functional tests should be considered. If electroporation is to be optimally used, a significantly better understanding of the mechanism of cell death will be needed.

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## 9.10 Tissue Electroporation and *In Vivo* Delivery

A purposeful electroporation of tissue *in vivo* and *in vitro* has been motivated by therapeutic interventions such as tumor treatment by delivery of anticancer drugs [86,174], gene therapy by delivery of DNA, and other genetic material [83,116,117,175], and delivery of various sized molecules into and across the skin [174,176–178]. Undesirable consequences of tissue electroporation have also been identified. A major insight is that tissue electroporation is the source of a nonthermal contribution to electrical injury [164,168,169,173]. Tissue electroporation has also been identified as a side effect to defibrillation interventions [61,179–181]. Finally, tissue electroporation may be relevant to neuromuscular incapacitation (stunning) pulses [182].

Describing the response of many closely spaced cells to an applied pulse is central to understanding electroporation of solid tissue. A fundamental attribute of cells is that applied electric fields result in response fields that are largest in lipid-based membranes, which have an effective high resistivity even in the presence of many ion channels. This is the basis of the amplification associated with Equation 9.3. Although well established at the cellular level [5,6,43], the corresponding concentration of electric fields in tissues has received less attention [7]. Here we emphasize that this voltage-concentrating effect means that electroporation of other lipid-based barriers should occur preferentially. For preferential electroporation, two features should be sought: (i) tissue barriers comprised mainly of lipids and (ii) mechanical deformability (compliance) of membranes comprise of the particular lipids so that the electrostatically favored entry of water into a deformable phospholipid-based membrane results in the creation of aqueous pathways.



In many solid tissues there are significant spaces between most cells, such that for small electric fields the electrical currents at low frequencies flow mainly between the cells. In this case, tissue electroporation consists of electroporation of individual cells whose behavior is expected in many respects to be similar to the electroporation of isolated cells, but with two major differences: (i) the local extracellular electric field depends in a complicated way on many other neighboring cells [7,183] and (ii) the ratio of the extra- to intracellular volumes (Equation 9.7) is usually small, just the opposite of most *in vitro* electroporation conditions. This means that if chemical exchange between the intra- and extracellular volumes is the main cause of cell stress and therefore cell death, tissue electroporation may be intrinsically less damaging than most *in vitro* electroporation conditions.

Tumor tissue is an important example of tissue for which many cells have intercellular aqueous pathways. Even without electroporation there is a significant physiological resistance to entry of anticancer drugs because of limited blood perfusion, elevated interstitial pressure, and relatively large distances to blood vessels [184]. Not only does electroporation greatly reduce the main barrier (that of a cell membrane itself), tissue electroporation should also reduce these physiological resistances. For typical cancer chemotherapy conditions blood perfusion delivers the anticancer drug to sites within a tumor and the drug then diffuses across the avascular regions. Local tissue electroporation should create aqueous pathways that assist drug movement and that may also relieve pressure, but the fourth power dependence of volumetric flow on pathway size implies that significant water flow may be more difficult than diffusion and drift of small drugs. But if the primary pores created electrically subsequently expand, then flow may be successful in reducing the pressure, and perfusion should increase. Diffusional barriers should also be reduced because of the new aqueous pathways. Striking results have, in fact, been reported in experimental tumor systems, mainly using the drug bleomycin, which is a potent drug that ordinarily does not cross the membrane readily [109,185–187]. Importantly, clinical use has been vigorously pursued [84,86,188–190]. Initial results were often obtained by treating surface tumors (nodules) [84]. More generalized use requires electrode access to provide localized electric field pulses to the targeted tumors [191–193]. As a partial guide, the use of electrical impedance measurements or even electrical impedance imaging can be considered [194,195].

Interestingly, there is a significant economic barrier to a general approach to local tumor treatment and ECT-specialized drugs (e.g., molecules with significant charge) that do not passively cross the cell membrane. Almost all approved anticancer drugs (the striking exception is bleomycin) readily cross the cell membrane, actively or passively. Accordingly, such molecules do not enjoy a tremendous increase in delivery by electroporation. Thus, even though it should be fundamentally attractive to use tissue electroporation with cell membrane-impermeant drugs, e.g., an established drug with several charge groups added that are quickly cleaved by intracellular enzymes to greatly reduce side effects, the modified drug will be regarded as new; and the expensive and lengthy approval process for new drugs is a significant disincentive.

Tissues that are comprised of one or more monolayers of cells in which the cells are connected by tight junctions are somewhat favored to experience voltage concentration across their membranes. Such tissues form the linings of organs and other specialized structures, such as the lining of sweat gland ducts [196]. An example of such behavior is found in a didactic, multicellular model [7,183]. Qualitatively, the main barrier of each cell monolayer consists of two cell membranes in series and can therefore be regarded approximately as two planar BLMs in series. In fact, some tissues such as frog skin and toad bladder are plausible approximations to an ideal single cell monolayer tissue, in which a single cell monolayer is the main barrier. For this reason, such tissues provide a

convenient experimental system that allows a convenient investigation of electrical behavior of tissue electroporation [197]. In contrast, recent experiments have focused on molecular transport into or through epithelia [198–201]. More complicated and realistic models of regions of a tissue with a layer of cells connected by tight junctions are now becoming available [7,183].

Transdermal drug delivery has long been of interest, with both chemical and physical “enhancers” being pursued [178]. Skin electroporation is one of the approaches based on physical intervention [174,176,177]. Mammalian skin is a complicated tissue. It has both the specialized barrier structure, the stratum corneum (SC), and appendages, sweat ducts and hair follicles, which perforate the SC [202–204]. The SC is the skin’s main barrier. It is a dead tissue whose barrier properties are often described by using a “brick wall model” [205]. The “bricks” are corneocytes, which contain cross-linked keratin and increase in volume by up to a factor of 5 if the SC is hydrated, the usual condition for transdermal drug delivery. The “mortar” is the intervening lipid, which surrounds the corneocytes, and exists as mostly parallel bilayers made of special (nonphospholipid) lipids, with about five to six bilayers between each of the approximately 20 rows of corneocytes. The SC is mechanically flexible but tough, essentially impenetrable to most infectious microorganisms and very impermeable to water-soluble molecules, particularly charged molecules, as the lipids are present as about 100 bilayers in series. The corneocytes are residual entities, left over from cells that migrated outwards to form the SC, with the multilamellar lipid bilayer providing most of the barrier function of the skin. Clearly this very effective barrier has evolved to provide protection, but it also presents a major barrier to potential medical interventions, viz. transdermal drug delivery [178] and extraction of chemical analytes, e.g., glucose within the subcutaneous interstitial fluid, for minimally invasive sensing [206–208].

The skin’s appendages (sweat gland ducts and hair follicles) are usually lined with an extension of the SC, or, deeper within the skin, by a double layer of living cells connected by tight junctions [196]. Both the appendageal barriers and the SC barrier are candidates for electroporation, but at very different transbarrier voltages,  $U_{\text{barrier}}$ . The double cell lining of sweat ducts should experience electroporation at about  $U_{\text{barrier}} \approx 2$  to 4 V, but the approximately 100 multilamellar bilayers of the SC need  $U_{\text{barrier}} \approx 50$  to 100 V for pulses with duration of 100  $\mu\text{s}$  to 1 ms, i.e., about 0.5 to 1 V per lipid bilayer [209–212].

Electroporation of skin and other tissues can be studied *in vitro* using a well-established apparatus, a permeation chamber [213], which is a version of the Ussing chamber originally used for molecular flux and electrical studies of epithelial tissues [214]. Two- and four-electrode systems can be used for electrical stimulation and measurement, and in addition, molecular transport measurements can be made by supplying molecules in the donor chamber and removing aliquots at intervals from the acceptor chamber for chemical assay. Experiments of this type with human skin show that if exponential pulses with  $U_{\text{SC},0} \approx 50$  to 300 V and time constant  $\tau_{\text{pulse}} \approx 1$  ms are applied every 5 s for 1 h, then there is an enhancement by up to a factor of  $10^4$  in the flux of charged molecules of up to about 1 kDa [176,215]. Companion electrical impedance measurements show a rapid ( $\leq 25$  ms) decrease in skin resistance [216], and both molecular flux and electrical measurements show that either reversible or irreversible behavior occurs, depending on the transdermal pulse amplitude,  $U_{\text{SC},0}$ . Several *in vivo* experiments show that transdermal delivery can be achieved with minimal damage [176,217–219]. This is consistent with the theoretical estimates of associated heating within the nearby viable epidermis [220].

Extension of this type of *in vitro* method to almost continuous measurement of transdermal molecular flux uses a sampling stream and quantitative, computer-controlled spectrofluorimetry to measure two fluorescent molecule concentrations at alternating intervals, so that with deconvolution, two molecular fluxes and passive electrical

properties can be determined for each skin preparation [221]. A faster responding, single flux version of the same method allows the demonstration of both the large magnitude and the rapid onset and cessation of the flux of calcein (623 D,  $z = -4$ ) and supports the view that skin electroporation can deliver small pharmaceuticals with rapid delivery onset [222]. Such methods provide a flexible approach to characterizing new aqueous pathways created by skin electroporation.

Gene therapy based on physical introduction of genetic material into cells *in vivo* is of great and growing interest and includes tissue electroporation [83,116,117,175,223]. Electroporation-based gene therapy is related to electrochemotherapy of tumors by introduction of bleomycin or other anticancer drugs, but with the significant difference that gene therapy requires delivery of large molecules (e.g., DNA) whereas traditional anticancer drugs are small. This is consistent with the use of smaller, longer electroporating pulses in gene therapy [119,120,224]. If the desired therapy does not require that all cells in a tissue be modified, local tissue electroporation can be used to treat some cells. The modified cells can, for example, secrete a therapeutic molecule. An early, partial demonstration was based on subcutaneous injection of DNA followed by surface electrodes for *in vivo* electroporation, leading to transformation of some cells [114]. Major advances have since occurred [83,116,117,175]. As with all new technologies, undesired side effects must be considered and understood along with possible uses.

Thus, the possibility of tissue damage must also be considered. Fortunately, major advances in understanding the role of electroporation in tissue damage has been achieved, through a series of studies motivated by the hypothesis that electroporation can account for a nonthermal permeabilization of cell membranes [164,168,169]. Although electrical injury to tissue has in the past been interpreted as a thermal denaturation phenomenon, more recent studies make a convincing case that electroporation can be a significant factor, particularly for the larger cells such as skeletal muscle cells and nerve cells [173]. Moreover, the demonstration that certain surfactants can significantly accelerate membrane barrier function recovery has offers a basis for therapy after electrical shock injury [163,164,225]. Not only have surfactants such as Polozamer-188 been pursued as a damage-minimizing therapeutic for electrical injury, this polymer is active against damage due to ionizing radiation that also causes cell membrane permeabilization [226] (see chapter 10 in *BMA*). Damaging consequences of tissue electroporation are also relevant to the strong electrical pulses which are widely used to stimulate fibrillating hearts [61,179–181], and this is an active research area. In this case, alteration of electrical behavior has often been emphasized.

Tissue electroporation thus has compelling potential advantages for medical interventions. A general attribute is that it achieves chemical results, transport of molecules into desired sites, without leaving another chemical residue such as the transdermal drug delivery enhancer dimethyl sulfoxide. Perhaps even more important, however, is the fact that electroporation is caused electrically, and the tissue experiencing electroporation can be rapidly assessed electrically, e.g., by impedance measurements. This means that much better control of molecular transport for drug delivery and analyte extraction may be possible. In spite of tremendous progress it is nevertheless true that a better fundamental understanding of electroporation mechanism is needed. The characteristics of such pathways need to be better established quantitatively, in terms of both molecular transport and electrical properties. Also, the potential side effects of possible tissue damage must be confronted, and the trade-off between desired and undesired consequences understood. This is a classic challenge.

Several potential applications of tissue electroporation have now been identified and are vigorously pursued [83,86,116,117,174]. Understanding tissue electroporation thus has several motivations. Unintended exposure to electric fields such as those due to

lightning and contact or close approach to electrical power sources or distribution wiring can cause nonthermal tissue damage [164,168,169]. Stunning (neuromuscular incapacitation) devices may also cause tissue electroporation or membrane channel denaturation. Although some acute side effect possibilities have been considered [182,227–230], the spatially distributed fields within the body should be investigated to obtain a thorough understanding of both efficacy and potential side effects.

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## 9.11 Electroporation of Organelles

Most models of cells focus on the outer, plasma membrane. At low frequencies and small applied field amplitudes, the response field is excluded from the cell interior, the site of organelles with their own single or double membranes. However, very-short field pulses have high-frequency content and can lead to displacement currents and associated transient intracellular electric fields [69,70,231]. A recent analysis shows that absolute rate theory can plausibly account for pore creation on a nanosecond timescale at a transmembrane voltage of  $U_m \approx 1.2$  V [19]. This supports the use of spatially distributed cell models that are based on the asymptotic model of membrane electroporation [69,70]. Such models show that extensive poration occurs for submicrosecond, megavolt-per-meter pulses. Further, the area pore density is exceptionally large. In the plasma membrane this leads to intracellular ionic conduction currents and associated large fields within the cell [50,69,70]. According to initial cell models involving the supraelectroporation hypothesis [69,70] an extremely large pore density (pores per area) is predicted for the cell's outer plasma membrane and also internal organelle membranes. This means that pores are extremely close together, and this in turn suggests that deterministic membrane deformation should be expected [98] in order to create a large membrane conductance that limits the magnitude of  $U_m$  to  $\sim 1$  V [50].

The supraelectroporation hypothesis predicts that for very large intracellular fields the membranes of organelles should also electroporate [70]. Indeed, a striking experimental observation shows that intracellular granules take up calcein from the cytosol [232], with organelle membrane electroporation as the likely cause. Following this report of *in situ* organelle electroporation, there have been a growing number of studies supporting the view that extremely large field pulses cause intracellular effects.

Many of these investigations find evidence for apoptosis, not the prompt necrosis that is consistent with a large increase in plasma membrane permeability by conventional electroporation [233–243]. These experiments involve electric field pulses of durations between 7 and 300 ns and with intensities from 360 to 10 kV/cm. Such pulses cause intracellular effects such as cytochrome *c* release, caspase activation, phosphatidylserine (PS) translocation, disruption of nuclear DNA, vesicle membrane electroporation, and molecular uptake into subcellular granules [232,234–242,244]. It is important to note that apoptosis and necrosis are distinguished as the two major types of cell death [245–249]. Prompt necrosis is known to be an outcome of excessive conventional electroporation when the pulses are sufficiently long, intense, or repeated enough times [98,164,169, 250–252]. Apoptosis (programmed cell death) is usually stimulated by biochemical perturbation of molecular signaling pathways [248,253–257] and not by the application of electrical fields. Indeed, apoptosis has only occasionally been reported to follow conventional electroporation [258,259]. Although significant phosphatidylserine (PS) translocation occurs in the plasma membrane (PM) of red blood cells for conventional electroporation pulses ( $\text{kV/cm}^{-1}$ ) [260–262], some of these recent studies with extremely

large ( $\sim 100 \text{ kV/cm}^{-1}$ ) field pulses show extensive PS translocation [242,263]. Nevertheless some experiments report insignificant uptake of membrane integrity dyes such as PI, and report that plasma membrane electroporation is minimal or has not occurred [233,235,236,238,240–242,263,264]. This suggests that the PM permeabilization for at least some molecules is only minimally increased by these pulses. Even though the applied fields are extremely strong, their effects are nonthermal due to the limited pulse duration (from 7 to 300 ns). Often, the pulse intensity is adjusted to limit the Joule heating per pulse to  $\sigma_e E_{\text{app}}^2 \Delta t \sim 1.7 \text{ J/ml}$ , where  $\sigma_e$  is the electrolyte conductivity,  $E_{\text{app}}$  is the applied field intensity, and  $\Delta t$  is the pulse duration. In this case the corresponding adiabatic (assumption of zero heat conduction; a worst case) temperature rise is only  $\sim 0.4^\circ\text{C}$  per pulse.

The use of these ultrashort (submicrosecond), extreme (10 to 300 k V/cm) pulses is relatively new, but has generated intense interest. The mechanism underlying the observed intracellular effects has not yet been established. One hypothesis is that ultrashort, extreme pulses cause irreversible effects at the membrane level, either membrane rupture or electrically based membrane protein denaturation [74,265–269]. Supraelectroporation is another hypothesis. It predicts that there is a massive creation of transient pores, which expand negligibly because of the short pulse duration, and this leads to full reversibility at the membrane level [69,70]. This is qualitatively consistent with avoiding prompt necrosis, because most ions and molecules should be retained by membranes with minimum size ( $\sim 0.8 \text{ nm}$ ) pores. The observed irreversible effects at the cellular level then arise from ionic and molecular transport through pores, which includes phospholipid translocation and other downstream events. As noted above, a contribution to irreversibility may also arise from electro-denaturation (long-lived membrane channel conformational changes induced by the supra-physiologic transmembrane voltage;  $\sim 0.3 \text{ V}$ ) [270–272], a magnitude that is achieved by both conventional and supraelectroporation. However, the duration of  $U_m \approx 1 \text{ V}$  decreases by several orders of magnitude in going from conventional to supraelectroporation, and the kinetics of the membrane protein conformational changes are not yet known. In any case, the basic idea is that for the largest of these fields, a very large number of pores are created in all of a cell's membranes, such that the field penetrates into the cell interior and also the organelle membranes, with the intracellular electric field nearly equal to the applied electric field, even after displacement currents have decayed [69]. That is, poration is so extensive that ionic current flows through the cell's membrane, in marked contrast to low-field responses in which current flows predominantly around the cell [69,70]. This opens the conceptual possibility of distinct effects due to electroporation of various types of organelles. For example, if pore lifetimes are long (as often inferred from conventional electroporation experiments), then it may be possible to gate open the mitochondrial permeability transition pore (MPTP) [98], which appears to require depolarizing the inner mitochondrial membrane (IMM) for many seconds [273]. However, other mechanism hypotheses are proposed. At the time of this writing, the cause of intracellular effects by extreme field pulses has not yet been established, but is likely to involve electroporation.

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## *Electrical Shock Trauma*

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### 10.1 Introduction

No engineering achievement has had a greater impact on human culture than electrical power. As electric power gains its significance and vital importance in today’s modern society, it poses equal threat to human society in terms of safety. Most citizens have experienced an electric shock at least once in their lifetime. The fear reflex generated by the bad experience of pain usually prevents us from further tampering with electricity. However, no matter how careful we are, accidents do and will occur, especially among electrical workers who have to handle commercial electrical power lines everyday. Recently, use of electrical power has made it into the mainstream of law enforcement. Nonlethal electrical weapons now provide a new intermediate force option for the police. The purpose of this chapter is to provide a basic overview of harmful effects of electrical force from both the engineering and the medical perspectives.

Basically, the range of clinical manifestations of electrical shock is not well documented. Many survivors of accidental electrical shock never seek medical attention. The data that exists is from those who do seek attention or from few case studies of electrical workers.

Furthermore, there is considerable variation in how electrical injury and safety is managed across various countries and cultures. In industrializing countries safety practices are often not the top priority, resulting in high rates of injury. A study of burn injuries by Nursal et al. [1] during a 1 y (2000–2001) period indicated that 21% of burn subjects were victims of electrical injury. In highly industrialized nations, electrical shock rates are on the decline. In the United States, workplace electrocution remains the fifth leading cause of fatal occupational injury with an estimated economic impact of more than \$1 billion annually [2]. The rates of injury may be the highest among electrical workers, mostly caused by working on “live” electrical equipment, wiring, light fixtures, and overhead power lines [3]. A study in Virginia suggested that public utilities have the highest rate of fatal electrical injuries among all industrial sectors. More than 90% of these injuries occur in men, mostly between the ages of 20 and 34, with 4 to 8 y of experience on the job [4]. Another source [5] suggests that the average age of victims was 37.5 y and the average years of experience amounted to 11.3 y. In one of the author’s own (German) study, the incidence in a 9 y period was 8.3%, 96% of victims were male, and the mean age was 32.7 y [6]. For survivors, the injury pattern is very complex, with a high disability rate due to accompanying neurologic damages and loss of limbs.

Away from the workplace, most electrical injuries are due to either indoor household low-voltage (<1000 V) electrical contact or outdoor lightning strikes [7]. Domestic household 60-Hz electrical shocks are common and usually result in minor peripheral neurological symptoms or occasionally in skin surface burns. However, more complex injuries may result depending on the current path, particularly following oral contact with household appliance cord disclosures or outlets in small children [8]. Compared to a high-voltage shock that usually is mediated by an arc, low-voltage shocks are more likely to produce a prolonged, “no-let-go” contact with the power source. This “no-let-go” phenomenon is caused by an involuntary, current-induced, muscle spasm [9]. For 60 Hz electrical current the “no-let-go” threshold for axial current passage through the forearm is 16 mA for males and 11 mA for females [10,11].

There are roughly 200 human deaths annually in the United States due to lightning strikes and there are three times those many who survive. The range of lightning injury extent is quite broad, depending upon the magnitude of exposure and the condition of the victim. Usually lightning hits result in surface burns, complex neurological damage similar to blunt head trauma, peripheral neurologic injury, and cardiac damage [12]. Radio frequency (RF) and microwave injuries are less common. Nonetheless, they represent an important medical problem to understand. In short, electrical trauma may produce a very complex pattern of injury because of the multiple modes of frequency-dependent tissue–current interactions, the variation in current density along its pathway through the body, as well as variations in body size, body position, and use of protective gear. No two cases are the same.

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## 10.2 Electrical Transport within Tissues

The fundamental bioengineering perspective is that the human body is considered to be a compartmentalized (or lumped element) conducting dielectric. It consists of about 60% of water by weight, in which 33% is intracellular and 27% is extracellular [13]. Body fluid in both the intracellular and the extracellular compartments is highly electrolytic, and these two compartments are separated by a relatively impermeable, highly resistive plasma membrane. Current within the body is carried by mobile ions in the body fluid.

The concentration of mobile ions results in a conductivity of approximately 1.4 S/m in physiological saline. Because electrons are the charge carriers in metallic conductors or electrical arcs, when in contact with the human body the current carrier changes from electrons to ions. This conversion occurs at the skin surface through electrochemical reactions [14].

At low frequencies (i.e., below radio frequencies), the electrical current passing across the body distributes such that the electric field strength is nearly uniform throughout any plane perpendicular to the current path [15–17]. As a consequence, current density distribution depends on the relative electrical conductivity of various tissues and the frequency of the current. Experimental data support this basic concept. Sances et al. [17] measured the current distribution in the hind limb of anesthetized hogs. They found that major arteries and nerves experienced the largest current density because of their higher conductivity. It was also observed that skeletal muscles carried the majority of the current due to their predominant volumetric proportions.

At a more microscopic scale, low-frequency current distribution within tissue is determined by the density, shape, and size of cells. The cell membrane is an insulating ion transport barrier that mostly shields the cytoplasmic fluid from low-frequency electrical current. In addition, the presence of cells diminishes the area available for ionic current and, in effect, makes tissues less conductive. As cell size increases, the membrane has less impact on a cell's electrical properties, because the volume fraction of the cell occupied by the membrane is proportionately decreased [18]. Similarly, the resistivity of skeletal muscle that is measured parallel to the long axis of the muscle cells is less than what is measured perpendicular to the axis. Solid volume fraction of the extracellular matrix can also be important in certain tissues and anatomic locations. For example, the resistivity of cortical bone and epidermis is higher than other tissues because their free water content is lower. This is also evident in the recent work by Kalkan et al. [19].

At higher frequencies, in the RF and microwave ranges, the current distribution is dependent on different parameters. The cell membrane is no longer an effective barrier to current passage, and capacitive coupling of power across the membrane readily permits current passage into the cytoplasm. Frequency-dependent factors like energy absorption and skin-depth effects govern the field distribution in tissues. At the highest frequency ranges, including light and shorter wavelengths, other effects such as scattering and quantum absorption effects become important in governing field distribution in tissues. Table 10.1 provides a categorization of frequency regimes, with corresponding wavelength spectrum, their common applications and their effects on tissues, as a result of electrical injury. Mechanisms of biological effects are different in each regime. A discussion of injury mechanisms must also be separated according to the frequency regime.

**TABLE 10.1**

Frequency—Wavelength Regimes with General Applications and Harmful Effects

Field Frequency (cycles/sec)	Energy Coupling Mechanism	Tissue Damage
dc to $10^3$	Ionic currents Forces on cell structures	Joule heating Membrane poration
$10^3$ to $10^7$	Ionic currents Field energy absorption by cells	Joule heating Cell spinning
$10^7$ to $10^9$	Field energy absorption by proteins	Macromolecular heating
$10^9$ to $10^{11}$	Field energy absorption by water	Microwave heating of water
$10^{11}$ to $10^{15}$	Field energy absorption by atomic bonds	Photo-optical protein damage

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## 10.3 Physicochemistry of Tissue Injury

### 10.3.1 Low-Frequency Electric Shocks

The biophysical mechanism of injuries caused by contacting electrical power sources remain controversial [9]. It has been shown that the pathophysiology of tissue electrical injury is more complex, involving thermal, electroporation (EP), and electrochemical interactions [19–22], and blunt mechanical trauma secondary to thermoacoustic blast from high-energy arc [23]. The various modes of trauma lead to complex patterns of injury which remain incompletely described until today.

In the most general terms, tissue damage exists when proteins and other biomolecules, cellular organelle membranes or water content is altered. Among all the components of the cells and tissues which can be damaged by the electrical shock, it is the thin cell membrane which has the greatest vulnerability. Thus, the cell membrane appears to be the most important determinate of tissue injury accumulation.

The most important function of the cell membrane is to provide a diffusion barrier against free ion diffusion [24]. Because most metabolic energy of mammalian cells is used in maintaining transmembrane ionic concentration differences [25], the importance of the structural integrity of the lipid bilayer is apparent. The conductance of electroporabilized membranes may increase by several orders of magnitude. ATP production and in turn, ATP-fueled protein ionic pumps, cannot keep pace and lead to metabolic energy exhaustion. If the membrane is not sealed, it results in cell necrosis. Thus, in discussing tissue injury resulting from electrical shock, the principal focus is directed at kinetics of cell membrane injury and the reversibility of that process. A simulation study of the membranes by Tarek [26] explains the EP phenomena in bilayers.

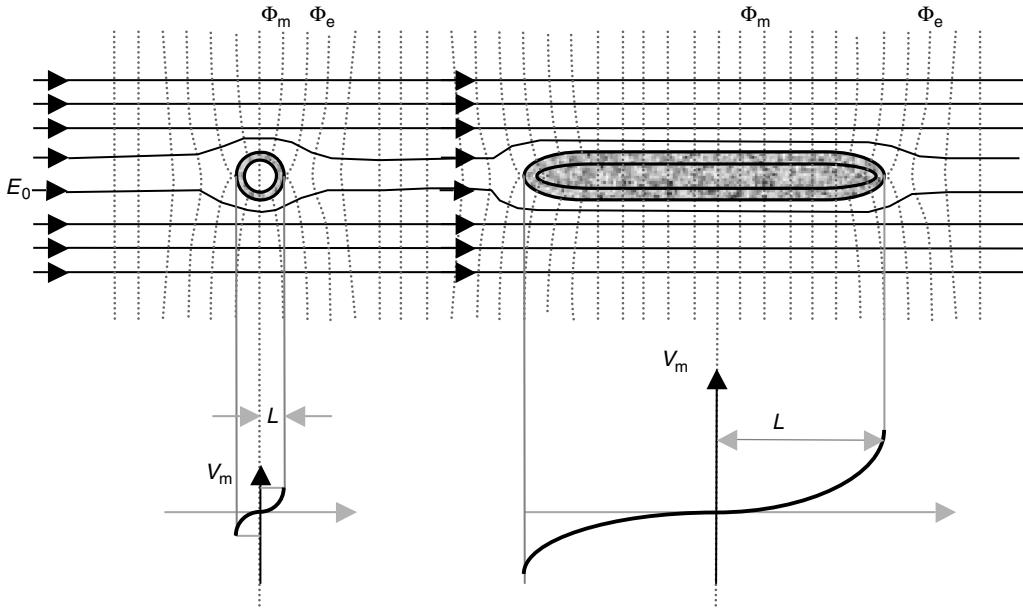
#### 10.3.1.1 Direct Electric Force Damage

A cell within an applied dc or low-frequency electric field will experience electric forces which will act most forcefully across and along the surface of the cell membrane. The forces acting across the membrane can alter membrane protein conformation and disrupt the structural integrity of the lipid bilayer. The magnitude of the forces acting across the membrane is related to the induced transmembrane potential  $V_m$ .  $V_m$  depends on a variety of factors, such as the intra- and extracellular medium conductivity, cell shape and size, the external electric field strength  $E$  as well as how the electric field vector orients with respect to the point of interest on the cell membrane [27–29].

Given that most cells are either spherical or cylindrical in shape, the expressions which describe the relationship between the externally applied electric field and the induced transmembrane potential can be simplified to two simple forms. Considering physiologic conditions, the peak magnitude of induced transmembrane potential  $V_m$  ( $V_m^p$ ) at the electrode-facing poles of spherical cells can be expressed as

$$V_m^p = 1.5R_{\text{cell}} \cos(\phi)(1 + (f/f_s)^2)^{-1/2}E_{\text{peak}}, \quad (10.1)$$

with  $R_{\text{cell}}$  being the radius of the cell,  $E_{\text{peak}}$  is the peak field strength in the tissue surrounding the cell,  $\phi$  is the angle of axis from the field direction,  $f_s$  is the sub- $\beta$ -dispersion frequency limit below which the cell charging time is short compared to rate of field change, and  $f$  is the field frequency. For cylindrical-shaped cells, such as skeletal muscle and nerve cells, which are aligned in the direction of the field (herein assigned the  $z$  coordinate), the induced transmembrane potential takes a different form. Under these



**FIGURE 10.1**

Dependence of cell size on the spatial variation of the induced transmembrane potential difference when cells are exposed to the same electric field. Electrical current lines are the same as electric field lines ( $E_0$ ), and constant voltage lines are dashed.

circumstances, an electrical space constant parameter becomes useful in describing the electrical properties of the cell. The induced transmembrane potential can be expressed as a function of  $z$

$$V_m^P(z) \approx A\lambda_m \sinh(z/\lambda_m)(1 + (f/f_s)^2)^{-1/2}E_{\text{peak}} \quad (10.2)$$

where  $\lambda_m$  is the electrical space constant of the cell,  $A$  is a variable that depends on cell length, the position  $z = 0$  corresponds to the mid-point of the cell [28]. Figure 10.1 illustrates schematically the spatial variation of  $V_m^P(z)$  on the cell size.

Equation 10.1 and Equation 10.2 are valid as long as the electrical properties of the cell membrane remain constant. However, the major theme of this chapter is that the transport properties of the cell membrane are altered by forces that are much greater than the natural physiologic forces. The natural transmembrane potential of mammalian cells has a magnitude of less than 100 mV [30]. When an artificially imposed potential results in a transmembrane potential magnitude of greater than 200 mV, intra-membrane molecular alterations occur which may lead to membrane damage.

The principal mechanisms of damage are EP of the lipid bilayer and electro-conformational denaturation of the membrane proteins. Electro-conformational damage to membrane proteins has been well documented for voltage-gated membrane protein channels. The processes occur quickly, on the order of milliseconds, after strong fields are applied.

EP is the biophysical process of membrane permeabilization owing to electric-field driven reorganization of lipids in the lipid bilayer by suprphysiologic electric fields [27,31,32]. It has been found useful applications at both single and multicellular levels to (1) introduce foreign DNA into cells; (2) introduce enzymes, antibodies, viruses, and other agents or particles for intracellular assays; (3) precipitate cell fusion; (4) insert or embed macromolecules into the cell membrane; (5) sampling of microenvironments

across membranes [33]; (6) gene delivery in human embryonic stem cell [34], gene transfer in whole embryos [35] and gene repair in mammalian cells [36]. Recent reviews and books published have extensively treated this subject [27–29,37–41]. In this chapter, we will briefly examine this phenomenon as it relates to the understanding of electrical injury. (See also Chapter 9 in Vol. 2)

EP can be either transient or stable, depending on the magnitude of the imposed transmembrane potential, duration for which it is imposed, membrane composition, and temperature. The time required for EP ranges from tens of microseconds to milliseconds. The physical state of the lipid bilayer, either liquid crystal or fluidic, is strongly temperature dependent. After application of a brief electroporating field pulse, the transiently electroporated membrane will spontaneously seal. Sealing follows removal of water from the membrane defects. Sealing kinetics are often orders of magnitude slower than the field relaxation because the forces driving the molecular sealing events are not as strong as the electroporating electric field. Sealing of electropores requires reordering of membrane lipids and removal of water molecules from the pore: both time and energy consuming processes [42–44].

The threshold transmembrane potential for induction of membrane EP is remarkably similar across the cell types. The threshold  $V_m$  for EP has been found to be in the range from 300 to 350 mV [42–45]. Several authors have developed models to explain the experimentally observed values of  $V_m$  required for EP and associated transmembrane aqueous dynamics [46,47]. Using empirical data as parameters in an asymptotic approximation [48], the threshold  $V_m$  is predicted to be approximately 250 mV, which is quite consistent with reported experimental data.

Generally, for most media-suspended, isolated cells with a typical diameter of 10–20  $\mu\text{m}$ , the dc field strength threshold for EP is in the range of 1 kV/cm. By comparison, the fields required to alter large cells is much less. Due to their relatively long length, skeletal muscle cells, up to 8 cm long in large animals, and nerve cells, up to 2 m long, have much lower EP thresholds. Therefore, muscle and nerve cell membranes are likely to be damaged with electrical fields as small as 60 V/cm.

The distribution of electropore formation in a cell placed in an applied field was addressed by DeBruin and Krassowska [49,50]. Expanding from previous theoretical models and including the fact that the membrane charging time of about 1  $\mu\text{s}$  is very short compared to a 1 ms field duration, they concluded that suprphysiological  $V_m$  at the pole caps is large enough to create pores, and thereby effectively prevent a further increase in  $V_m$  in these areas.

This confirms early experimental findings which show a saturation of  $V_m$  that is independent of the field strength (for high-voltage shocks, [51–53]). After the effect of ionic concentrations is included in the model, it is even able to confirm asymmetries in  $V_m$  observed in respect to the hyperpolarized (anode-facing) and hypopolarized (cathode-facing) pole of a cell [43,44,54]. Although the pore sealing time (time needed for pores to close) in the range of seconds predicted by the model is in agreement with some published experimental results [55], others have found longer sealing times in the range of several minutes [42,43,56]. This might be explained by the fact that [1] this model is based on pure lipid bilayers instead of cell membranes embedded with proteins, and [2] it only considers primary pores formed by  $V_m$  (pores formed during shock) and not those formed after the external field pulse ends (secondary pores), which provide transport routes for macromolecules.

Investigation of EP of many cells within a tissue had been initially driven by the need for a better understanding of the pathophysiology of electrical injury [57,58]. In the early 1990s, it was studied in connection with cardiac defibrillation shocks [59,60]. More recently, tissue EP has begun to be envisioned as a potential therapeutic tool

in the medical field. It has found use in (1) enhanced cancer tumor chemotherapy (electrochemotherapy [61,62]), (2) localized gene therapy [63,64], (3) transdermal drug delivery and body fluid sampling [65–67]. Computational models of human high-voltage electrical shock suggest that the induced tissue electric field strength in the extremities is high enough to electroporate skeletal muscle and peripheral nerve cell membranes [8,68–70] and to possibly cause electroconformational denaturation of membrane proteins.

Bhatt and coworkers [20] measured EP damage accumulation using isolated, cooled *in vitro* rat *biceps femoris* muscles. After the initial impedance measurement, an electric field pulse was delivered to the muscle using current pulses that setup tissue field pulse amplitudes ranging between 30–120 V/cm, which were thought to be typical forearm field strengths in high-voltage electrical shock. The duration of the dc pulses ranged from 0.5 to 10 ms. These short pulses reduce Joule heating to insignificant levels. Field pulses were separated by 10 s to allow thermal relaxation. The drop in the low-frequency electrical impedance in the muscle tissue following the application of short-duration dc pulses indicated skeletal muscle membrane damage. A decrease in muscle impedance magnitude occurs following dc electric field pulses that exceed 60 V/cm magnitude and 1 ms duration. Thus the field strength, pulse duration, and number of pulses are factors that determine the extent of EP damage.

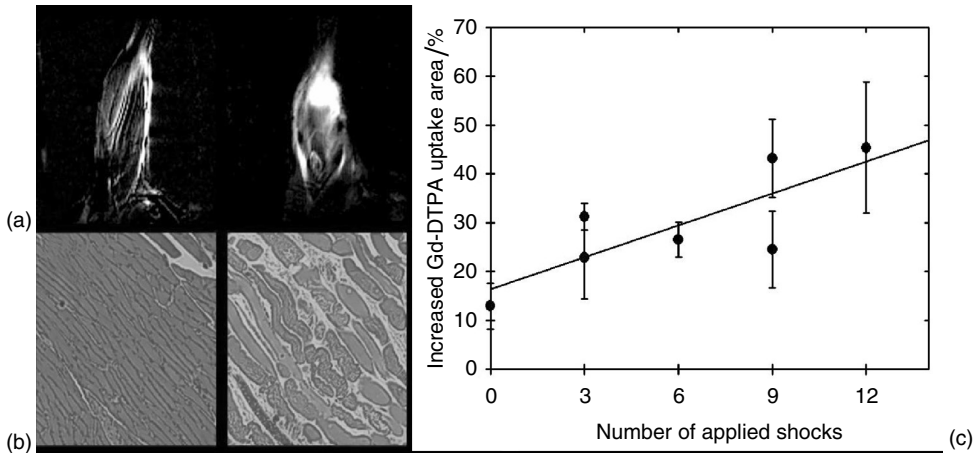
Based on these results, Block et al. [21] electrically shocked fully anesthetized female Sprague–Dawley rats through cuff-type electrodes wrapped around the base of the tail and one ankle, using a current-regulated dc power supply. The objective was to determine whether EP of skeletal muscle tissue *in situ* could lead to substantial necrosis. The study involved histopathological analysis and diagnostic imaging of an anesthetized animal hind limb. A series of 4 ms dc-current pulses, each separated by 10 s to allow complete thermal relaxation back to baseline temperature before the next field pulse, was applied. The electric field strength produced in the thigh muscle was estimated to range from 37 to 150 V/cm, corresponding to applied currents ranging from 0.5 to 2 A. These tissue fields were suggested to be on the same level as that experienced by many victims of high-voltage electrical shock.

Muscle biopsies were obtained from the injured as well as the collateral control legs 6 h post shock and subjected to histopathological analysis. Sections of electrically shocked muscle revealed extensive vacuolization and hypercontraction-induced degeneration band patterns which were not found in unshocked contra-lateral controls (Figure 10.2). The fraction of hypercontracted muscle cells increased with the number of applied pulses. These results are consistent with the investigators hypothesis that nonthermal electrical effects alone can induce cellular necrosis. The pathologic appearance of the shocked muscle was similar to that seen in the disease malignant hyperthermia indicating that EP may lead to  $\text{Ca}^{2+}$  influx into the sarcoplasm. A similar muscle injury pattern has been described in a human electrical injury victim published by DeBono in a clinical case report [71]. These results suggested that direct electrical injury of skeletal muscle *in situ* can lead to the commonly seen pattern of injury in electrical shock victims even in the absence of pathologically significant Joule heating.

### 10.3.1.2 Thermal “Burn” Injury

Passage of electrical current through Ohmic conduction leads to Joule heating, which can lead to severe burn injury in electrical shock victims. Burn injury is used here to specifically refer to tissue injury by damaging suprphysiological temperatures. Burn effects are related to protein lysis of cell membranes and protein denaturation, often followed by recognizable changes in the optical properties of tissue. There are two conceptually





**FIGURE 10.2** (See color insert following page 412)

The effects of multiple applied shocks on muscle tissue. (a) T2-weighted images of control (left) and shocked (right) muscles. (b) Histology sections of control (left) and shocked (right) muscle. (c) Relationship between increased Gd uptake with increasing number of applied shocks. The fraction of hypercontracted muscle cells increased with the number of applied pulses.

different potential outcomes for the denatured protein, which depend on the initial molecular structure and configuration. The first possibility occurs when the native folded conformational state of the protein, held by intra-molecular bonds, is different from the most favored conformation when no intramolecular crosslinks are needed to maintain the native folded state (thermodynamically lowest energy level). When this protein is heated, the intramolecular bonds are broken and it denatures to one of several preferred lower energy states from which it will not spontaneously return to the native conformation. Conceivably, if the primary structure of the protein is undamaged, it may be plausible to reconfigure the protein using similar chaperone-assisted mechanisms which establishes its initial folding after biosynthesis. The second possibility occurs when the native folded state of the protein is the same as the most preferred, energetically the lowest protein conformation in the absence of intramolecular crosslinks.

The speed of the transition from natural to denatured states is governed by the Arrhenius rate equation which states that when the kinetic energy of the molecule exceeds a threshold magnitude  $E_a^i$  (for activation energy), the transition to the  $i$ th state will occur, in this case from natural to denatured state. For a large number of molecules at temperature  $T$  the fraction with a kinetic energy above  $E_a$  is governed by the Maxwell-Boltzmann relation ( $\Gamma$ ) [74],

$$\Gamma^t = \exp(-E_a^i/k_B T) \quad (20.4)$$

where  $k_B$  is Boltzmann's constant. Because the strength of bonds retaining the folding conformation of macromolecules is very dependent on the nature of the chemical bond, the value of  $E_a^i$  is dependent on molecular structure. Despite this complexity the net rate of denaturation of cellular structures containing many different proteins is also often describable in terms of Equation 10.4. For example, the accuracy of this equation in describing thermal damage to cell membranes has been reported [75–77]. Even the thermal injury to intact tissues like human skin is reasonably described by the simple Equation 10.4.

It has been known for more than 50 y that the rate at which damage accumulates in heated skin can be estimated by convolving Equation 10.4 with the temperature history. The resulting expression is called the “heat damage” equation [78],

$$d\Omega/dt = A\Gamma \quad (10.5)$$

where  $\Omega$  is a parameter reflective of the extent of damage, and  $A$  is a frequency factor that describes how often a configuration occurs from which reaction is energetically possible, something which is also very dependent on molecular structure. The shape of the temperature–time curve predicted by Equation 10.5 is indeed the same as the human skin temperature versus time scale burn curve measured by Henriques and Moritz [79]. This temperature–time curve shape has also been obtained for heat damage to isolated cells [77].

Because the lipid bilayer components of the cell membranes are held together only by forces of hydration, the lipid bilayer is the most vulnerable to heat damage [80]. Even at temperatures of only 6°C above normal (i.e., 43°C) the structural integrity of the lipid bilayer is lost [81]. In effect, the warmed lipid bilayer goes into solution, rendering the membrane freely permeable to small ions. At slightly higher temperatures, published reports indicate that the contractile mechanism of muscle cells is destroyed immediately following exposure to 45°C and above [82]. Experiments on fibroblasts demonstrated that heat-induced membrane permeabilization also begins to appear above 45°C [83].

Bischof and coworkers investigated the effect of supraphysiological temperatures on isolated rat muscle cells using a thermally controlled microperfusion stage [84]. Cells were loaded with the membrane permeable fluorescent dye precursor calcein-AM. After entering the cell, the precursor is converted by nonspecific esterases into the membrane impermeable fluorescent Calcein. Using quantitative fluorescent microscopy Bischof et al. measured time-resolved dye leakage from the muscle cells at several supraphysiological temperatures. In addition, using Equation 10.5 and Equation 10.6, the authors determined the activation energy necessary to thermally induce membrane permeabilization in the isolated muscle cells to be 32.9 kcal/mol [84]. Reported activation energy values for thermal damage in other cell types are in the range from 30 to 140 kcal/mol [77].

### 10.3.1.3 *Electro-Conformational Denaturation of Transmembrane Proteins*

Imposed supraphysiologic transmembrane potential differences can produce electroconformational changes of membrane proteins, ion channels, and ion pumps. Approximately 30% of cell membrane consists of proteins, some of them embedded into the bilayer, others spanning across the entire membrane. Many of them carry electric charges from amino acids with acidic or basic side groups that can be acted on directly by an intense  $V_m$  (by, e.g., charge separation or charge induction through dissociation). In addition, each amino acid has an electrical dipole moment of about 3.5 D (1 Debye =  $3.336 \times 10^{-30}$  cm), giving the proteins an overall dipole moment that, in the case of an  $\alpha$ -helical protein structure, can reach 120 D [85]. In a strong external electric field those molecules will orient themselves and thereby change their conformation to increase the effective dipole moment in the direction of that field.

If the field strength becomes sufficiently intense, those field-induced changes can cause irreversible damage to membrane proteins. In particular ion channels and pumps with their selective, voltage-gated charge transport mechanisms (e.g., a  $\text{Ca}^{2+}$ -specific channel) are highly sensitive to differences in  $V_m$ . Chen and coworkers investigated the effects of large magnitude  $V_m$  pulses on voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channel behavior in frog skeletal muscle membrane using a modified double vaseline-gap voltage clamp.

They found in both channel types, but more drastically in  $K^+$  channels, reductions of channel conductance, and ionic selectivity by the imposed  $V_m$  [86]. Chen et al. were able to demonstrate that these changes are not caused by the huge field-induced channel currents (Joule heating damage) but rather by the magnitude and polarity of the imposed  $V_m$  [87]. In the most recent work, Clausen et al. [88] study the effects of shock and EP on the acute loss of force in skeletal muscles and the role of the Na (+) and K (+) pumps in the force recovery after EP. Ionic pumps alone are sufficient to compensate a simple mechanical leakage. They report that EP induces reversible depolarization, partial run-down of Na (+), K (+) gradients, cell membrane leakage, and loss of force. The consequences of this effect may underlie the transient nerve and muscle paralysis in electrical injury victims.

### 10.3.2 Radio Frequency and Microwave Burns

Every year a few cases of RF or microwave field injuries require medical attention in the United States. The victims are usually industrial workers. Above the low-frequency regime ( $>10\text{kHz}$ ), tissue response strongly depends upon the field frequency. In the 10–100 MHz RF range, two types of tissue heating occur, Joule and dielectric heating, with Joule heating outweighing dielectric heating. Small molecules like water, when not bound, are able to follow the field up to the gigahertz range [89]. However, at microwave frequencies (100 MHz–100 GHz), dielectric heating is more significant than Joule heating because both bound and free water are excited by microwaves. Molecular dipoles of macromolecules have lower natural frequencies, so that their most efficient induction frequency is in the radio frequency range.

Exposure to ambient microwave fields is known to cause burn trauma. Microwave burns have different clinical manifestations than low-frequency electrical shocks [90–93]. At low frequency the epidermis is a highly resistive barrier, whereas in the microwave regime, electrical power readily passes the epidermis in the form of “capacitive” coupling with very little energy dissipation. Consequently, the epidermis may not be burned unless it is very moist. The microwave field penetration into tissue has a characteristic depth in the range of 1 cm, resulting in direct heating of subepidermal tissue water. The rate of tissue heating is dependent not only on the amplitude of tissue electric field, but also on the density of dipoles. For example, microwave heating is much slower in fatty tissues [94].

### 10.3.3 Lightning Injury

Lightning arcs result from dielectric breakdown in air caused by build up of free electrical charges on the surface of clouds. The current through an arc can be enormous, but the duration is quite brief (1–10 ms). The primary current is confined to the surface of conducting objects connected by the arc. Peak lightning current ranges between 30,000–50,000 A and is able to generate temperatures near 30,000 K. This abrupt heating generates a high-pressure thermoacoustic blast wave known as thunder.

An individual directly struck by lightning will experience current for a brief period of time. Initially, the surface of the body is charged by the high electric field in the air. This can cause breakdown of the epidermis and several hundred amperes to flow through the body for a 1–10  $\mu\text{s}$  period, which is certainly long enough to induce EP. Following this, a much smaller current persists for several milliseconds, during which time the body is discharging into the ground. The duration of current flow is relatively short, so there is no substantial heating except for a breakdown of the epidermis. However, disruption of cell membrane can wreak havoc on nerve and muscle tissues.

When lightning reaches the ground, it spreads out radially from the contact point. A substantial shock current can be experienced by a person walking nearby, if the feet are widely separated. For example, with an average lightning current of 20,000 A, a step length of 50 cm, and an individual located 10 m away from strike point, the voltage drops between the legs can reach 1500 V. This can induce a 2–3 A current flow through the body between the legs for a 10  $\mu$ s period.

#### 10.3.4 Diagnostic Imaging of Electrical Injury

Because most of the damage caused by electrical shock occurs beneath the skin, it is important to discuss how to achieve tissue injury detection and localization. There are two basic cellular abnormalities in electrical injury, altered protein structure and disrupted cell membranes. In addition, there can be blood coagulation, tissue edema, elevated tissue pressures and other abnormalities that effect molecular transport. From the clinical perspective, it is important to recognize area of damaged cells and interrupted blood flow.

Magnetic resonance imaging (MRI) methods are particularly well suited for detection of changes in protein folding, disruption of cell membranes and tissue edema. In particular, MRI methods in common hospital settings are typically based upon measurement of water proton behavior. As water behavior will change in the presence of denatured proteins and osmotic swelling will follow disruption of cell membranes, the typical MRI equipment in hospitals is well suited for the task. The MRI images in [Figure 10.3](#) demonstrate the tissue changes seen adjacent to tissue-conductor contact points. The surface contact (220 V) was prolonged, resulting in deep burns and possible brain injury. Patient did not manifest significant neurology abnormality despite anatomic evidence of brain surface injury.

Technetium<sup>99m</sup>-PYP (pyrophosphate) is widely used as a radiolabel tracer for various forms of soft tissue injury including electrical trauma; it is believed to follow the calcium movement in cellular function [72]. The increased tracer accumulation in the muscle tissue indicates loss of cell membrane integrity, tissue edema, and predictive of tissue. Using the *in vivo* rat hind limb electrical injury model described by Block, Matthews [73] monitored the uptake of Tc<sup>99m</sup>-PYP in the electrically shocked tissue as a function of the magnitude of the dc current. Either 0.5, 1.0, or 1.85 A of direct current was applied to the rat's hind limb. Intravenous saline infusion was used as sham-treatment. Their results indicated that Tc<sup>99m</sup>-PYP does accumulate in electroporated tissue and the level of the tracer accumulation is positively correlated to the tissue field pulses applied. This indicates that quantitative imaging of Tc<sup>99m</sup>-PYP uptake may be developed further as an indicator of the extent of EP or other membrane injury.

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## 10.4 Summary and Conclusions

Given the importance of electrical power to human culture, the problem of electrical injury is one that will continue to exist for the foreseeable future. Electrical injury has been poorly understood and perhaps less than optimally managed in the past. Better understanding of injury mechanisms, anatomical patterns of injury and therapy are required. A prompt, accurate clinical diagnosis of electrical injury is one of the most difficult tasks



**FIGURE 10.3**

MRI image of electrical contact wound of scalp with extension through skull into brain surface. Dural and brain cortex damage is clear.

in the medical field [8] because it usually calls upon an understanding of the interaction between electric current and human tissue. Specifically speaking, the difficulty involves the following:

1. The exact tissue damage mechanism and damage level depend on a host of parameters: the characteristics of the power source (dc or ac current, voltage, frequency, etc.), path and duration of closed circuit, area and impedance of contact spot, etc. Correspondingly, there is a whole spectrum of damage characteristics depending on the values of these parameters. The physician needs to do 4-dimensional (spatial plus temporal) detective work in order to arrive at a correct diagnosis [95–114].
2. Electrical damage to the tissues is not easily detectable by visual inspection or physical examination. And often times its sequelae will not manifest themselves for a while: electrically injured tissue may initially appear viable, only to become visibly necrotic at a later point (in a number of days) [100,115,116].

The molecular structure of biological systems can be severely altered by the effects of high-energy commercial frequency electrical power. The mechanisms of damage include cell membrane EP, Joule heating, electroconformational changes of proteins (denaturation), and other macromolecules. Rehabilitation and reconstruction needs in the electrically injured are usually not obvious at the initial evaluation. The reintegration of the individual into their preinjury living situation often becomes a real challenge. Aside from physically obvious impairments (loss of limb, etc.) it is not uncommon for an electrician to develop a phobia toward electricity after being injured. The more in-depth understanding of the underlying mechanism of injury will lead to a more specific treatment regime that may prevent some of the late sequelae of electrical injury.

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# 11

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## *Mechanisms and Therapeutic Applications of Time-Varying and Static Magnetic Fields*

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Arthur A. Pilla

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## 11.1 Introduction

It is now commonplace to learn the successful use of weak, nonthermal electromagnetic fields (EMF) in the quest to heal, or relieve the symptoms of a variety of debilitating ailments. This chapter attempts to give the reader an introduction and assessment of EMF modalities that have demonstrated therapeutic benefit for bone and wound repair and chronic and acute pain relief. This chapter will concentrate on the use of exogenous time-varying and static magnetic fields. There is, however, a large body of research, including many clinical studies, describing the successful application of electrical signals via electrodes in electrochemical contact with the skin for pain relief and to enhance wound repair. Consideration of these modalities is beyond the scope of this chapter. The reader is referred to several excellent reviews of such electrical stimulation modalities [1–5]. Electroporation (see Chapter 9 in this volume) [6–8,372], which applies high-amplitude ( $>100$  V/cm), short-duration ( $\leq 1$  ms) voltage pulses with electrodes in contact with the target, allows controlled transient opening of the cell and other membranes, and has shown promise for gene transvection [9] and treatment of certain cancers [10], and is also beyond the scope of this chapter. Finally radio frequency (RF) ( $>100$  MHz) and microwave signals are also beyond the scope of this chapter because these modalities are rarely utilized to enhance bone or wound repair, but rather for tissue heating, thermal ablation, or as surgical tools. Nonthermal bioeffects at these frequencies have been reported, but there are many controversial findings. Excellent reviews are available for the reader interested in detail [11,373].

As of this writing, there are a considerable number of peer-reviewed publications, which show EMF can result in physiologically beneficial *in vivo* and *in vitro* bioeffects. The number of people who have received substantial clinical benefit from exogenous EMF is certainly in the millions worldwide and is increasing rapidly as new clinical indications emerge. The EMF therapies also present as alternatives to many pharmacologic treatments with virtually no toxicity or side effects. The time-varying EMFs consisting of rectangular or arbitrary waveforms, referred to as pulsing electromagnetic fields (PEMFs), the pulse modulated radio frequency waveforms, particularly in the 15–40 MHz range, referred to as pulsed radio frequency fields (PRFs), and the low-frequency sinusoidal waveforms ( $<100$  Hz) have been shown to enhance healing when used as adjunctive therapies for a variety of musculoskeletal injuries. Indeed, peer-reviewed meta-analyses clearly show that both PEMF and PRF modalities, now approved by regulatory bodies worldwide and widely used on patients to enhance bone and wound repair, are clinically effective [12,13]. Although still not completely elucidated, the mechanism of action of EMF signals at the molecular and cellular level is now much better understood and strongly suggests ion or ligand binding in a regulatory cascade could be the signal transduction pathway [14–28]. Furthermore, *a priori* configuration of physiologically effective waveforms via tuning the electrical properties of the exogenous EMF signal to the endogenous electrical properties of ion binding has recently been reported [29,30].

This chapter will provide a brief overview of the basic and clinical evidence that time-varying magnetic fields (EMF) can modulate molecular, cellular, and tissue function in a physiologically significant manner. The fundamental questions relating to the biophysical conditions under which EMF signals could modulate cell and tissue function will be discussed in detail. Particular attention will be paid to the manner by which signal parameters are related to dosimetry. In other words, the properties that render an EMF signal bioeffective. An attempt is made to correlate dosimetry for weak magnetic field with that for electric field effects. The ratio of signal to (endogenous) thermal noise (SNR) in the target is used in an SNR and Dynamical Systems model which has been successful for the *a priori* configuration of physiologically significant waveforms and which the

reader may find useful to decipher the myriad of waveforms that have been utilized. The model may also allow the reader to perform an *a posteriori* analysis of waveforms for dose-related explanations for the presence or absence of a biological effect. Examples of *in vivo* and *in vitro* studies are given, illustrating specific EMF waveforms, including several examples of the use of the model.

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## 11.2 Tissue Repair

### 11.2.1 Orthopedic Applications

Five million bone fractures occur annually in the United States alone. About 5% of these will become delayed or nonunion fractures with associated loss of productivity and independence [31]. Several techniques are available to treat recalcitrant fractures such as internal and external fixation; bone grafts or graft substitutes, including demineralized bone matrix, platelet extracts, and bone matrix protein; and biophysical stimulation, such as mechanical strain applied through external fixators or ultrasound (US), and EMFs.

The electrical properties of bone tissue have been extensively investigated. Yasuda in Japan hypothesized that endogenous electrical activity observed in bone was the mediator of repair and adaptive remodeling responses to mechanical loading and that an exogenous electrical signal alone could stimulate the response [32,33]. A seminal report soon followed on bone piezoelectric properties from the pioneering work of Fukada and Yasuda [34]. These authors showed a voltage could be obtained upon deformation of dry bone. Several groups, notably led by Becker at the State University of New York, Bassett at Columbia University, and Brighton at the University of Pennsylvania, soon reported the generation of electrical potentials in wet bone on mechanical deformation [35–39]. Similar observations were subsequently made in collagen and cartilaginous tissues [40–43]. The important conclusion from these studies was the revelation that bone and other tissue could respond to electrical signals in a physiologically useful manner. This ultimately led to the use of EMFs to modulate bone repair.

The development of modern EMF therapeutics was stimulated by the clinical problems associated with nonunion and delayed union bone fractures. It started with the pioneering work of Yasuda, Fukada, Becker, Brighton, and Bassett, mentioned above, who responded to the fundamental orthopedic question of how bone adaptively and structurally responds to mechanical input by suggesting that an electrical signal may be involved in the transduction of the mechanical signal to cellular activity. This naturally led to the suggestion that superimposing an exogenous EMF upon the endogenous fields accompanying normal cellular activity could help in the treatment of difficult fractures. The first animal studies employed microampere level direct currents delivered via implanted electrodes. Remarkably, this resulted in new bone formation, particularly around the cathode [44]. As these studies progressed, it became clear that the new bone growth resulted from the chemical changes around the electrodes is caused by electrolysis [45]. However, it has been shown that a mechanical stimulus also plays a role in dc bone stimulation [46]. The first therapeutic devices were based on these early animal studies and used implanted and semiinvasive electrodes delivering dc to the fracture site [47,48]. This was followed by the development of clinically preferable externally applied EMF modalities [49–52]. Subsequent studies concentrated on the direct effects of EMFs leading to modalities that provided a noninvasive, no-touch means of applying an electrical or mechanical signal to a cell or tissue target. Therapeutic uses of these technologies in

orthopedics have led to clinical applications, approved by regulatory bodies worldwide, for treatment of recalcitrant fractures and spine fusion [53–59] and recently for osteoarthritis of the knee [60–62]. Additional clinical indications for EMF have been reported in double-blind studies for the treatment of avascular necrosis [63,64] and tendinitis [65]. This spectrum of applications clearly demonstrates the potential of this biophysical modality to enhance musculoskeletal tissue healing.

At present, the clinical modalities in use for bone repair consist of electrodes implanted directly into the repair site or noninvasive capacitive or inductive coupling. Direct current is applied via one electrode (cathode) placed in the tissue target at the site of bone repair and the anode placed in soft tissue. Direct currents of 5–100  $\mu\text{A}$  are sufficient to stimulate osteogenesis [45]. The capacitive coupling (CC) technique utilizes external skin electrodes placed on opposite sides of the fracture site [66]. This requires openings in the cast or brace to allow skin access. Sinusoidal waves of 20–200 kHz are typically employed to induce 1–100 mV/cm electric fields in the repair site [67]. The inductive coupling (PEMF) technique induces a time-varying electric field at the repair site by applying a time-varying magnetic field via one or two electrical coils. The induced electric field parameters are determined by frequency characteristics of the applied magnetic field and the electrical properties of the tissue target [15,30,50,51]. Several waveform configurations have been shown to be physiologically effective. Peak time-varying magnetic fields of 0.1–20 G, (0.01–2 mT in SI units), inducing 1–150 mV/cm peak electric fields in a 3-cm diameter target, have been used [50,68]. The relationship between inductively coupled waveform characteristics and their ability to produce physiologically significant bioeffects will be considered in detail below. One version of the inductive technique utilizes a specific combination of dc and ac magnetic fields (CMFs) that are believed to tune specifically to ion transport processes [17,341].

### **11.2.1.1 Cellular Studies**

The cellular studies have addressed effects of EMFs on both signal transduction pathways and growth factor synthesis. The important overall result from these studies is that EMF can stimulate the secretion of growth factors (e.g., insulin-like growth factor-II, IGF-II) after a short-duration trigger stimulus. The clinical benefit to bone repair is enhanced production of growth factors upregulated as a result of the fracture trauma. The induced electric field thus acts as a triggering mechanism, which modulates the normal process of molecular regulation of bone repair mediated by growth factors.

Studies underlying this working model have shown effects on calcium ion transport [69], a 28% increase in cell proliferation [70], a fivefold increase in IGF-II release [71], and increased IGF-II receptor expression in osteoblasts [72]. Increases of 53 and 93% on IGF-I and IGF-II, respectively, have also been demonstrated in rat fracture callus [73]. Stimulation of TGF- $\beta$  mRNA by threefold with PEMF in a bone induction model in the rat has been reported [74]. This study also suggests the increase in growth factor production by PEMF may be related to the induction of cartilage differentiation [75]. It also suggests the responsive cell population is most likely mesenchymal cells [76], which are recruited early in the duration of PEMF stimulus to enhance cartilage formation. Upregulation of TGF- $\beta$  mRNA by 100%, as well as collagen, and osteocalcin synthesis by PEMF has been reported in the human osteoblast-like cell line MG-63 [77,78]. PEMF stimulated a 130% increase in TGF- $\beta$ 1 in bone nonunion cells [79]. That the upregulation of growth factor production may be a common denominator in the tissue level mechanisms underlying electromagnetic stimulation is supported by studies from the Brighton [80,81], Stevens [82], and Aaron [83] groups.

Using specific inhibitors, Brighton et al. suggest that EMF acts through a calmodulin-dependent pathway [81]. This follows reports by the Pilla group [84–90] that specific PEMF and PRF signals, as well as weak static magnetic fields, modulate  $\text{Ca}^{2+}$  binding to

calmodulin (CaM) by a twofold acceleration in  $\text{Ca}^{2+}$  binding kinetics in a cell-free enzyme preparation. The Stevens group has shown upregulation of mRNA for BMP2 and BMP4 with PEMF in osteoblast cultures. The Aaron group has reported extensively on upregulation of TGF- $\beta$  in bone and cartilage with PEMF. All of these studies have utilized EMF signals identical to those that have demonstrated clinical success. The ion-binding target pathway has recently been confirmed in other studies using static magnetic fields [91,92]. PEMF has been reported to increase angiogenesis by threefold in an endothelial cell culture [93]. A recent study confirms this and suggests PEMF increases *in vitro* and *in vivo* angiogenesis through a sevenfold increase in endothelial release of FGF-2 [94].

### 11.2.1.2 Animal Studies

Bassett et al. [95,96] were the first to report a PEMF signal could accelerate bone repair by 150% in a canine tibial osteotomy model. A bilateral cortical hole defect model in the metacarpal bones in horses showed PEMF treated holes produced a statistically significant increase in amount of new bone formation and mineral apposition rate [97,98]. A capacitively coupled signal was shown to prevent osteopenia due to both sciatic-denervation and castration in rat osteopenia models [99,100]. PEMF inhibited bone loss in an ovariectomized canine model [101]. Combined magnetic fields reversed osteopenia in ovariectomized rats [102]. An avian ulna disuse model showed a significant increase in bone formation when treated with PEMF [103]. The frequency dependence of EMF effects was also studied in this model. The results showed maximal response was observed with a 15 Hz sinusoidal waveform producing 10- $\mu\text{V}/\text{cm}$  peak electric field in tissue. Experimental models of bone repair show enhanced cell proliferation, calcification, and increased mechanical strength with direct currents [104,105]. Capacitive coupled fields have been reported to improve the mechanical strength of experimental fractures and healing osteotomies [67]. Several studies with PEMF showed increased calcification and enhanced mechanical strength in healing bone [106,107]. Exposure time studies report a linear effect of daily exposure with a 6 hour stimulation being most effective [68]. A series of animal studies reported that dc, CC, and PEMF techniques enhance the formation of bone and improve fusion rates in spinal arthrodeses [108–110]. Direct currents of 10  $\mu\text{A}$  per cm of cathode length showed the best acceleration of spinal fusion [111]. The mechanical strength of late phase osteotomy gap healing in the dog was 35% stronger in PEMF treated limbs [112]. PEMF increased bone ingrowth into hydroxyapatite implants in cancellous bone by 50% [113]. PEMF produced a 10% increase in the diameter of arteriolar microvessels in rat muscle from which the authors suggested increased local blood flow could play a role in the PEMF acceleration of bone repair [114]. The use of *in vivo* micro-computed tomography showed PEMF reduced bone loss in a nonunion fibular model in the rat by threefold [362]. In a related study the effect of PEMF waveform configuration was examined. The results showed callus stiffness in a rat fibular osteotomy was increased twofold by a PEMF signal routinely employed for clinical bone repair, whereas a second PEMF waveform with much higher frequency content was ineffective [368].

### 11.2.1.3 Clinical Studies

Electromagnetic stimulation modalities have been used clinically to treat fresh fractures, osteotomies, spine fusions, and delayed and nonunion fractures. The efficacy of EMF stimulation on bone repair has been studied in a formal meta-analysis [12]. Twenty randomized control trials were identified. Fifteen trials supported EMF effectiveness and five failed to show effectiveness. Most studies used PEMF. In all cases, the primary outcome measure was bone healing assessed by radiographs and clinical stability test.



Results from pooled trials of 765 cases supported the effectiveness of PEMF stimulation of bone repair. However, because of the inability to pool data from all studies, conclusions regarding PEMF efficacy in bone repair were only suggestive. PEMF significantly accelerated union of femoral and tibial osteotomies in randomized, placebo controlled studies by approximately 50% [115–117].

PEMF, CC, and dc have been used to promote healing of spine fusions for the treatment of chronic back pain from worn or damaged intervertebral discs. This is measured by the increase in successful fusions from 50% to approximately 80% using EMF as adjunctive treatment. This application has also been subjected to meta-analysis [13]. Five randomized, controlled trials and five nonrandomized case controlled studies showed positive results for the enhancement (by 60%) of spine fusion by electrical and electromagnetic stimulation. There are many studies and reviews that show electrical and electromagnetic stimulation is effective in promoting spinal arthrodesis [118–122].

The effectiveness of EMF in promoting healing of recalcitrant fractures has been reviewed [123]. Twenty-eight studies of ununited tibial fractures treated with PMF were compared with 14 studies of similar fractures treated with bone graft with or without internal fixation. The overall success rate for the surgical treatment of 569 ununited tibial fractures was 82%, while that for PMF treatment of 1718 ununited tibial fractures was 81%, suggesting it is significantly more advantageous for the patient to use PEMF rather than submit to invasive surgery for the first bone graft. There are several observational studies suggesting the efficacy of dc, CC, or PEMF techniques in stimulating healing of delayed unions and nonunions [124–132]. Interestingly, and of huge clinical significance, studies comparing PEMF with bone graft show their equivalence in promoting union of delayed union or nonunion fractures [123,133–135]. Finally, there is a promising study on the effects of PEMF on distraction osteogenesis for the correction of bone length discrepancies [136].

Thus, several physical modalities have been shown to effectively manage nonunions and delayed unions of bone. Implantable direct current stimulation is effective as an adjunct in achieving spinal fusion. PEMFs induce weak nonthermal time-varying currents at the fracture site. Inductively and capacitively coupled EMFs appear to be as effective as surgery in managing extremity nonunions and lumbar and cervical fusions. Low-intensity US has also been used to speed normal fracture healing and manage delayed unions. Although these modalities seem vastly different, there appears to be a common mechanism of action. This will be discussed below.

All of the modalities discussed above now constitute the standard armamentarium of orthopedic clinical practice. Since the success rate for these modalities has been reported equivalent to that for the first bone graft, a huge advantage to the patient ensues because PEMF therapy is noninvasive and is performed on an outpatient basis. PEMF therapy also provides significant reductions in the cost of health care since no operative procedures or hospital stays are involved. This also applies for the increased success rate of spinal fusions with EMF. Thus, the clinical effects of EMF on hard tissue repair are physiologically significant and often constitute the method of choice when standard of care has failed to produce adequate clinical results. It is interesting to note that EMF may be the best modulator of the release of the growth factors specific to each stage of bone repair, certainly more so than the exogenous application of the same growth factors [398].

### **11.2.2 Soft Tissue Applications**

Chronic wounds and their treatment are an enormous burden on the healthcare system, both in terms of their cost (\$5 billion to \$9 billion annually) and the intensity of care

required [137]. There is even more cost to society from attendant human suffering and reduced productivity. More than 2 million people suffer from pressure ulcers and as many as 600,000 to 2.5 million more have chronic leg and foot wounds [137]. Diabetic foot ulcers are the most common chronic wounds in western industrialized countries. Of the millions who have diabetes mellitus, 15% will suffer foot ulceration that often leads to amputation (100,000 per annum in the United States alone).

There is an emerging and substantial clinical application of EMF in wound healing. Soft tissue healing has been reported by the use of direct electrode coupled devices delivering waveforms similar to those produced by several transcutaneous electric nerve stimulation (TENS) devices currently approved by the Food and Drug Administration (FDA) [5]. Regulatory and reimbursement issues have prevented more widespread use of PEMF modalities. However, the clear clinical effectiveness of PEMF signals has resulted in significantly increased use [12]. In fact, the Center for Medicare Services (CMS) has now determined PEMF produces sufficient clinical outcome to permit, and reimburse for, use in the off-label application of healing chronic wounds, such as pressure sores and diabetic leg and foot ulcers [138]. In addition, PRF devices have been cleared by the FDA for the relief of acute and chronic pain and the reduction of edema, all symptoms of wounds from postsurgical procedures, musculoskeletal injuries, muscle and joint overuse, as well as chronic wounds.

As for bone repair, application of EMF to soft tissue repair appears to have begun with observations of the electrical events associated with wound repair [139–143]. Injury currents, which develop in the presence of dermal wounds, are postulated to play an important role in the healing process [144,145]. These currents are, however, at least two orders of magnitude larger than the endogenous currents from SGP and are dc, or near dc, currents. Cells involved in wound repair are electrically charged and endogenous direct currents may facilitate cellular migration to the wound area [146,147]. In a manner similar to that for bone repair, the original working hypothesis was that exogenous EMF signals may enhance the endogenous electrical signals to accelerate wound repair. It has also been suggested that externally applied EMF may interact with the current of injury or trigger a relevant growth factor cascade [148,149]. Wound healing can be accelerated 2.5 fold with 200–800  $\mu\text{A}$  direct current [150]. Both dc and PEMF have been reported to reduce edema, increase blood flow, modulate upregulated growth factor receptors, enhance neutrophil and macrophage attraction and epidermal cell migration, and increase fibroblast and granulation tissue proliferation [147,149]. Most wound studies involve arterial or venous skin ulcers, diabetic ulcers, pressure ulcers, and surgical and burn wounds.

### **11.2.2.1 Cellular and Animal Studies**

The PEMF signal currently utilized for bone repair (see Figure 11.2, top) accelerates vascularization by several fold using cells from human umbilical vein and bovine aorta [151]. Studies on human umbilical vein cells showed that endothelial cell migration to a wounded area is accelerated by about 14% if cell cultures are exposed to an induced electrical field similar to the pulse burst currently used for bone repair (2 mT peak, 25 Hz repetition rate) [151]. Chronic stimulation of rat muscles increased blood vessel density by 14–30%, possibly through angiotensin and vascular endothelial growth factor pathways [152]. PEMF produced a significant increase in the rate of growth of the vascular tissue in the rabbit ear chamber [153] showing a dependence on signal configuration (repetitive pulse burst significantly better than repetitive single pulse). Sinusoidal signals (300 Hz) improved microcirculation and stimulated proliferation and differentiation of fibroblasts [154]. Amplitude, frequency, and orientation dependence of EMF modulation of fibroblast

protein synthesis has been reported [155]. Inductively coupled sinusoidal fields (0.06–0.7 mT; 50, 60, and 100 Hz) increased chick embryo fibroblast proliferation up to 64% [156]. Human fibroblasts exposed to 20 or 500 mT, 50 Hz sinusoidal signals exhibited no effect on fibroblast proliferation [157]. Fibroblasts exposed to a PRF signal consisting of a 65  $\mu$ s burst of 27.12 MHz sinusoidal waves repeating at 600/s (1G peak amplitude) showed enhanced cell proliferation by 130–220% [158,159]. Tissue cultures of human foreskin fibroblasts, when exposed to high 2 V/cm induced electric fields at either 1 or 10 Hz, demonstrated a sixfold increase in internal calcium, but excitation at 100 Hz had no significant effect [160]. Recent animal studies have reported that PRF signals produced a statistically significant several fold increase in neovascularization in an arterial loop model, suggesting an important clinical application for angiogenesis [161,162]. PRF signals, configured *a priori* assuming a Ca–CaM transduction pathway, accelerated wound repair in a rat cutaneous wound model by approximately 60% as measured by tensile strength [163]. However, treatment of identical wounds in the rat with PEMF of the type and intensity used for bone healing (see Figure 11.2, top) failed to produce significant increases in soft tissue fibroblast counts or improvement in wound closure [164]. PEMF increased the degree of endothelial cell tubulization and proliferation (threefold) *in vitro* [94]. In the same study PEMF increased fibroblast growth factor  $\beta$ -2 by fivefold from which the authors conclude that PEMF augments angiogenesis primarily by stimulating endothelial release of fibroblast growth factor-2 (FGF-2).

### 11.2.2.2 Clinical Studies

Nonthermal PRF signals were originally utilized for the treatment of infections in the preantibiotic era [165] and are now widely employed for the reduction of post-traumatic and postoperative pain and edema. Double-blind clinical studies have been reported for chronic wound repair, wherein PRF treated pressure ulcers closed by 84 vs. 40% closure in untreated wounds in one study [166] and 60% closure vs. no closure in the control group in another study [167]; acute ankle sprains, wherein edema decrease was sevenfold vs. the control group [168,169]; and acute whiplash injuries, wherein pain decreased by 50% and range of motion increased by 75% in the treated vs. control patients [170,171]. PRF signals have been reported to enhance skin microvascular blood flow by about 30% in both healthy [172] and diabetic [173] individuals. PRF reduced postmastectomy lymphedema by 56% and increased skin blood flow by fourfold [174]. The PEMF at 600 and 800 Hz, 25  $\mu$ T mean amplitude, significantly reduced the size of venous ulcers by 63%, and decreased pain by 72%, in a randomized control study [175]. A modulated EMF signal at 10 and 100 Hz relieved the main clinical symptoms of diabetic peripheral neuropathy, improved peripheral nerve conduction by about 40% and the reflex excitability of functionally diverse motoneurons in the spinal cord [176].

A meta-analysis was performed on randomized clinical trials using PEMF on soft tissues and joints [12]. The results showed that both PEMF and PRF were effective in accelerating healing of skin wounds [177–183], soft tissue injury [168–171,184], and hair regrowth [185–187], as well as providing symptomatic relief in patients with osteoarthritis and other joint conditions [61,62]. PEMF has been successfully used in the treatment of chronic pain associated with connective tissue (cartilage, tendon, ligaments, and bone) injury and joint-associated soft tissue injury [188,189].

As for bone repair, EMF clinical effects on soft tissue repair are substantial and often constitute the method of choice when standard of care has failed to produce adequate clinical results. This is particularly true for chronic wounds, which often do not respond to standard of care and can be life-threatening if not resolved. It is interesting to note that

EMF can increase angiogenesis several fold in chronic wounds, significantly more than that achieved to date with growth factors such as vascular endothelial growth factor (VEGF), for which there have been generally disappointing clinical results [397]. This may be the primary reason that EMF is so effective with problem wounds wherein increased blood supply is always one of the primary clinical objectives. It is also interesting to note that EMF can provide an alternative to nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., ibuprofen, cox-2 inhibitors, etc.) and other pharmacological analgesics for the relief of chronic and acute pain.

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## 11.3 Biophysical Considerations of EMF Therapeutics

### 11.3.1 Introduction

The above sections have provided an overview of the abundance of *in vitro*, *in vivo*, and clinical evidence that suggest that time-varying magnetic fields of various configurations can produce physiologically beneficial effects for conditions as varied as chronic and acute pain, chronic wounds, and recalcitrant bone fractures. This has all been achieved with low-intensity, nonthermal, noninvasive time-varying EMFs, having many configurations over a very broad frequency range. The reader should be aware that pulsing US and intermittent mechanical loading have also been shown to modulate bone repair and remodeling [190–198]. It has been suggested [199–201] that an ion-binding transduction pathway is common to both mechanical and EMF modalities, which provides useful US signal dosimetry information. This will be further discussed below. There is also compelling evidence that weak static magnetic fields can provide physiologically useful musculoskeletal pain relief [202–209]. Here also, ion binding may be involved in the transduction pathway via the effect of weak magnetic fields on the motion dynamics of the bound ion [19–26,210–216]. This can modulate binding kinetics by accelerating bound ions to preferred active orientations within the binding site or channel. The static magnetic fields as low as 10  $\mu\text{T}$  can be detected in this pathway if the ion remains bound on the order of a second. One model, based upon Larmor precession of the bound ion, predicts static magnetic field effects as well as windows for certain combinations of ac and dc magnetic fields, similar to the ion cyclotron and parametric resonance models, as will be shown below [211–213].

Clearly, many EMF signals appear to have the capacity to achieve a physiologically meaningful bioeffect. Why should such seemingly different doses be effective? Are any signal parameters better than others? Is it the magnetic or electric field, or both? Does the state of the tissue target play a role? Despite the understandable impression that many EMF signals have been chosen in some arbitrary manner, the following sections will attempt to show that EMF dosimetry can have a rigorous quantitative basis based upon relatively simple mechanisms.

The biophysical mechanisms of interaction of weak electric and magnetic fields on biological tissues as well as the biological transductive mechanisms have been vigorously studied. One of the first models was created using a linear physicochemical approach [14,15,29,30,49,50,221,222,223,224,233,256] in which an electrochemical model of the cell membrane was employed to predict a range of EMF waveform parameters for which bioeffects might be expected. This approach was based on the assumption that voltage-dependent processes, such as ion or ligand binding and ion transport at and across the electrified interface of the cell membrane, were the most likely EMF targets. Several

elegant studies further quantified this approach using Lorentz force considerations [16–19], including ion resonance and the Zeeman–Stark effect [20]. These suggested combined low-frequency ac and dc magnetic fields could modulate ion or ligand movement in a molecular cleft (binding site) and thereby affect binding kinetics [19–26,210–216,286]. Direct action of the Lorentz force on free electrons in macromolecules such as DNA has also been proposed [217–219].

At present, the most generally accepted biophysical transduction step is ion or ligand binding at cell surfaces and junctions which modulate a cascade of biochemical processes resulting in the observed physiological effect [81,83,220,225]. A unifying biophysical mechanism that could explain the vast range of reported results and allows predictions of which EMF signals and exposures are likely to induce a clinically meaningful physiological effect has been proposed [29,30].

Electromagnetic bioeffects from relatively weak (below heating and excitation thresholds) signals can be produced with a time-varying electric field,  $E(t)$ , induced from an applied time-varying magnetic field,  $B(t)$ . A large number of electromagnetic clinical devices in present use (particularly for bone and wound repair) induce 1–100 mV/cm peak  $E$  at the treatment site [12,13,68]. A myriad of waveforms have been employed and the fundamental question becomes one of dosimetry. In other words, the relation of waveform configuration to detectability (dose) at the supposed target must be established. The first step is evaluation of the amplitude and spatial dosimetry of the induced EMF within the target site for each exposure system and condition. This has been rigorously carried out for the laboratory dish with coils oriented vertically or horizontally [226–228]. Models have been created for the distribution of induced voltage and current in human limbs [229] and joints [230]. Three-dimensional visualizations of clinical PEMF signals have been reported [361].

### 11.3.2 Inductively Coupled Clinical EMF Waveforms

The electric field induced via a time-varying magnetic field waveform is directly related to the electrical characteristics of the coil employed and the current waveform applied to the coil. Induced electromotive force (emf) is proportional to the rate of change of current in the coil ( $dI_{\text{coil}}/dt$ ), which produces the shape of the induced electric field. The following is applicable to coils that have been utilized for clinical bone fracture repair.

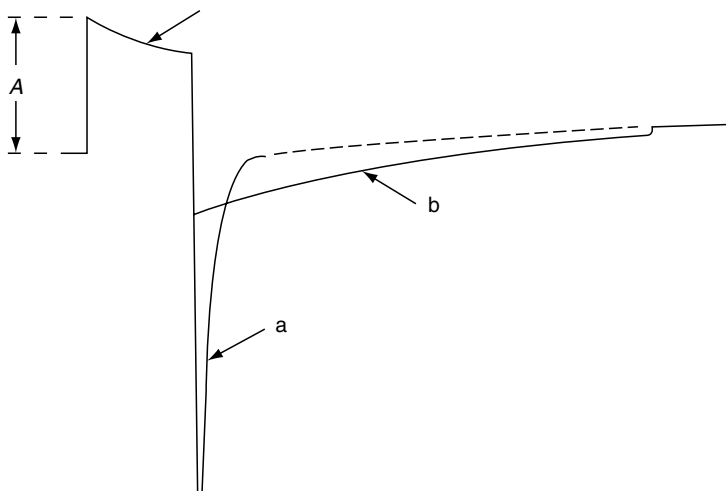
Coil current  $I_{\text{coil}}(t)$  for an air-core inductor driven with a voltage step  $V_o$  rises exponentially to the limiting current defined by the coil resistance as

$$I_{\text{coil}}(t) = (V_o/R_{\text{coil}})[1 - \exp(-t R_{\text{coil}}/L)] \quad (11.1)$$

where  $L$  is the coil inductance and  $R_{\text{coil}}$  is the effective coil resistance, including all connecting cable and driving circuit resistances. The waveform of the induced voltage is a direct function of the time derivative of  $I_{\text{coil}}(t)$ :

$$dI_{\text{coil}}/dt = V_o/L \exp(-t R_{\text{coil}}/L) \quad (11.2)$$

The above equation clearly shows that a rectangular-type induced waveform is achieved for a linear rise in coil current if  $\tau_{\text{coil}} (L/R_{\text{coil}})$  is sufficiently greater (e.g., by 10 times) than the rise time of current in the coil. This can be achieved by the proper choice of  $L$  and  $R_{\text{coil}}$ . One modality is to keep  $L$  relatively small so that safe driving voltages (<25 V) can be employed. Note that, as given by Equation 11.2, the maximum induced voltage (as  $t \rightarrow 0$ ) is inversely proportional to coil inductance for a given  $V_o$ . Effective coil resistance can be



**FIGURE 11.1**

Schematic illustration of an inductively coupled electric field waveform used for bone and wound repair. The coil time constant ( $L/R_{\text{coil}}$ ) determines the shape of this signal during rise of current in the coil. Collapse of coil current can be diode-limited (b) or determined by the impedance of the electronic driving circuitry (a). (From Pilla, A.A., Sechaud, P., and McLeod, B.R., *J. Biol. Phys.*, 11, 51, 1983. With permission.)

kept small by utilizing heavy magnet wire and connecting cable (e.g., 14–16 B&S gauge). With the above taken into account, it is easy to see that, for a given  $R_{\text{coil}}$ , the voltage step ( $V_0$ ) can be applied to the coil for as long as the following relation is approximately valid:

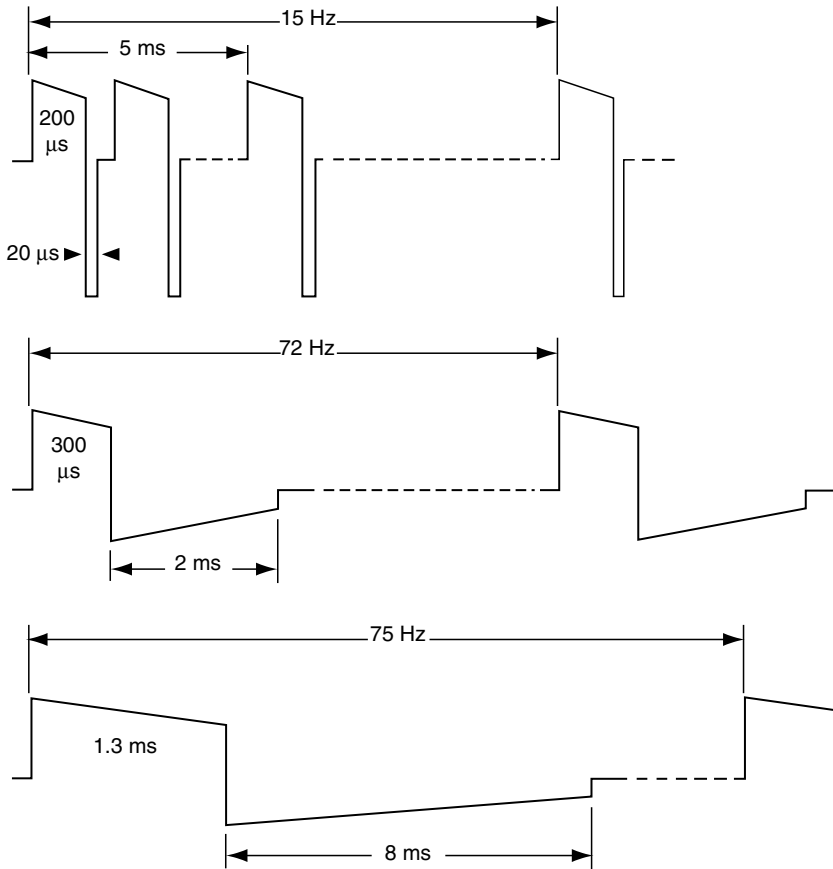
$$(dI_{\text{coil}}/dt)_{t \rightarrow 0} = V_0/L[1 - (t R_{\text{coil}}/L)] \quad (11.3)$$

The above equation shows coil current will rise approximately linearly resulting in an induced voltage waveform in the form of a step having some negative slope, as shown in Figure 11.1.

At the time of coil turn-off, coil current must collapse back to zero. This can be accomplished in either of the two manners. In the first, the coil current is allowed to decay at a rate determined only by the coil and driving circuit impedance (a, Figure 11.1). In the second, the rate of current collapse is controlled by an amplitude-limiting diode (b, Figure 11.1). By using either of these modes, induced waveform patterns having similar or different opposite polarity durations and amplitudes can be achieved.

The waveform shown in Figure 11.1 represents the electric field induced in the cell or tissue target via a coil placed external to the skin surface. Some of the pulse-type–induced electric field waveforms in common clinical use are shown in Figure 11.2. The rationale behind the configuration of these waveforms was based on the assumption that the induced electric field (and associated induced current density) is the primary stimulus. In other words, the magnetic component was considered to be the carrier or coupler, not significantly contributing to the biological effect. That this is correct for many PEMF clinical signals will become evident below. Clinical EMF signals for bone repair are inductively coupled, except for one capacitive coupled 60 kHz sinusoidal signal, for which  $E$  dosimetry, estimated from the geometry and dielectric properties of the target [121], is in the mV/cm peak range. There is also the continued and anomalous use of invasive direct currents, despite the accompanying electrolytic effects, which are known to cause bone formation via an inflammatory response [39–41].

The waveforms shown in Figure 11.1 and Figure 11.2 represent the time variation of the electric field signal induced in the extracellular fluid, cell, or tissue complex in a human,



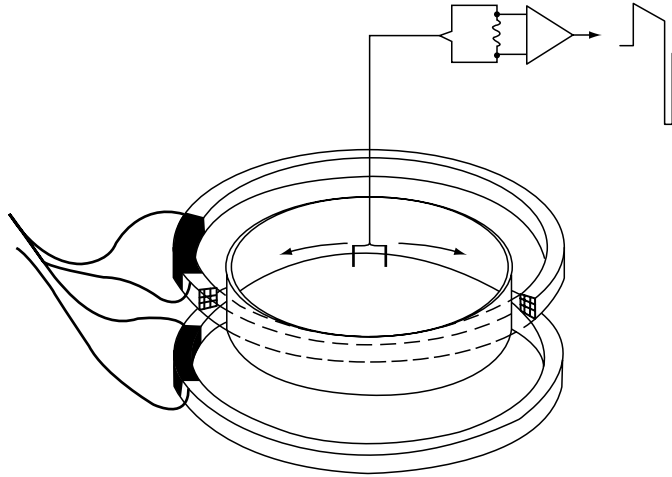
**FIGURE 11.2**

Induced electric field,  $E(t)$ , in tissue from the time-varying magnetic fields utilized in EMF devices for clinical applications. The top waveform consists of bursts of asymmetrical pulses; the others are wide asymmetrical single pulses. For all signals peak  $E$  is 1–10 mV/cm in a 2 cm target. All are detectable by some tissue targets. Positive clinical effects have been reported for all signals.

animal, or plant target. The distribution of current flow depends upon the geometry of coil and target. The basic rule is that the voltage induced will be defined by the distribution of magnetic flux within the tissue and the electrical properties of the target. To illustrate, consider a pair of Helmholtz aiding circular coils. A plastic culture dish, having cylindrical geometry, can be placed between the coils such that the symmetry ( $z$ ) axis is aligned with that of the coils (see Figure 11.3). The  $z = 0$  plane passes through the midplane of a saline solution, of conductivity  $\sigma$ , placed in the dish. Simple EMF theory predicts, as there is total symmetry in the angular ( $\phi$ ) direction, that the angular component of the induced electric field,  $E_\phi$ , varies with radius,  $r$ , as

$$E_\phi(t) = -\frac{dB}{dt} \frac{r}{2} \quad (11.4)$$

which states the instantaneous amplitude of the induced electric field  $E_\phi(t)$ , within the area of a cylindrical target, such as a laboratory dish, penetrated by a uniform magnetic field  $B$ , is proportional to its rate of change with time  $dB/dt$ , and the radius  $r$  of the target. In other words,  $E_\phi(t)$  at any point in space and time is dependent upon the rate of change of  $B$  and its spatial distribution in the target. The actual waveform of  $E_\phi(t)$  depends upon



**FIGURE 11.3**

Schematic illustration of Helmholtz aiding coils with a plastic culture dish placed between the coils in a uniform magnetic field. This is an example of cylindrical geometry for which Equation 11.4 is valid. A special dual electrode probe may be placed at various radii parallel to the induced electric field, allowing  $E_\phi$ , and current density,  $J_\phi$ , vectors to be evaluated. (From Pilla, A.A., Sechaud, P., and McLeod, B.R., *J. Biol. Phys.*, 11, 51, 1983. With permission.)

$dB/dt$ , which defines the applied frequency spectrum. For example, in the case of a clinical PRF signal, which is a sinusoidal wave,  $dB/dt = \omega B$ , where  $\omega = 2\pi f$  ( $f$  = frequency, typically 27.12 MHz for PRF devices in the US). As  $dB/dt$  can be controlled, the induced electric field waveform may be chosen as a function of the electrical properties of the target (impedance, bandpass).

Equation 11.4 shows that the electric field induced in a homogenous cylindrical target is in circular loops, i.e., is rotatory, orthogonal to the magnetic field lines. In addition, the induced  $E$  field will be greater when the magnetic field intercepts a greater cross-sectional area of the sample, i.e., maximum  $E$  field in the target depends upon target size. Peak  $E$  field and associated current density,  $J$ , at a radius of 2 cm is often utilized for dosimetry comparisons. It is also convenient to use  $dB/dt$  as a measure of the peak induced electric field, assuming identical target size, for a given EMF signal. For example, a common clinical bone repair signal produces 20 G peak magnetic field in 20  $\mu$ s. Thus  $dB/dt = 10^6$  G/s for which peak  $E_\phi(t) = 1$  V/cm = 10 mV/cm at a radius of 2 cm in the target, a typical dose metric for EMF bone growth stimulators. Note, however, that  $dB/dt$  alone is not sufficient to evaluate as a dose metric for a specific ion-binding target pathway. This will be discussed in Section 11.3.9.

Of course, Equation 11.4 is not valid for complex geometry and nonhomogenous targets; but it is always true, from Faraday's law of induction, that the induced voltage  $V(t)$ , along the line boundary of the surface  $S$  through which the flux  $\phi$  penetrates, is

$$V(t) = -d\phi(t)/dt = -d/dt \int B(t) d\bar{S} \quad (11.5)$$

This expression indicates that, for any geometry, the time variation of induced  $V$  will also be identical to that of  $E$  in air and any other homogeneous nonmagnetic medium contained within the boundaries defined by  $S$ .

For most PEMF clinical devices the induced electric field and associated induced currents are small enough such that back emfs (due to the magnetic field from the induced or eddy current itself) are negligible. Thus, measurements of induced fields in air accurately



reflect those at the target site for the PEMF devices utilized for bone repair. On the other hand, the induced electric field from PRF devices is orders of magnitude larger at carrier frequencies between 10 and 40 MHz. Therefore, the amplitude of the incident magnetic field (in air) is always perturbed by a tissue or saline load due to the secondary field from the induced currents, which act to cancel the primary magnetic field. Induced field levels and distribution have been evaluated in the presence of a tissue or saline load for PRF signals [249].

### 11.3.3 Electrochemistry at Cell Surfaces

For a living cell or tissue to respond functionally to an exogenous electric field, it is necessary that the field reaches and be detected at the appropriate molecular, cellular, or tissue site. In contrast to electrical potentials (and associated currents) that are applied directly across the cell's plasma membrane, the situation considered in this section involves the induction of electric fields within the cell or tissue target via electrical pathways originating at the external skin surface. As cells are surrounded by a highly conducting ionic medium, it is clear that an electric field containing relatively low frequencies (<100 MHz) can affect the cell only if current flows. This places certain constraints upon the relationship of the electrical characteristics of both the cell or tissue complex and the applied electric signal.

An important step, therefore, is the characterization of the electrical properties of cells and tissues. There are many such studies and the reader is referred to excellent reviews [231–233] (see also chapter 3 of *BBA*). However, it has also been proposed that a complete description of the electrical properties of cells and tissues should include the electrical equivalents of the electrochemical processes, which could be involved in the signal transduction pathway [50]. The electrical equivalents of electrochemical processes at cell surfaces and junctions and their relevance to EMF therapeutics have been described [14,15,30,49,50,234]. For the purposes of this review it is simply necessary to recall that the bilayer lipid structure of the plasma membrane and the electrical double layer at its inner and outer surfaces make the cell membrane a real capacitor, i.e., a charge storage system [240]. The structure of this capacitor is determined primarily by the interactions of water dipoles and hydrated ions with the charged chemical groups associated with the various lipid, protein, and carbohydrate components of the membrane. In addition, this capacitor is leaky because transmembrane ion transport can occur. These general properties can be described using an electrochemical approach to characterize the passage of current at and across the cell membrane by combining the dielectric and electrochemical properties of the membrane. In this manner not only the passive, but also the functional electrical response of the cell or tissue target may be taken into account.

Accordingly, induced current can affect cell surfaces and junctions via a complex, but readily discernible, set of electrochemical steps that are representative of the cell's real-time response to perturbations in its charged environment for any given functional state. Impedance measurements have reported the relative magnitudes of the time constants (relaxation times) of these processes [235–238]. As expected, dielectric and ion-binding time constants are somewhat smaller (1–100  $\mu$ s) than those involving membrane transport (1–100 ms). In addition, the steady-state (dc) current pathway across the plasma membrane exhibits a specific resistivity several orders of magnitude above that of the extracellular fluid [239,241]. This means exogenous EMF signals need to contain frequencies well above dc to produce detectable electric field levels in the electrochemical pathways of ion binding and membrane transport. Exogenous direct currents will be mostly extracellular and generally need to be significantly higher than the peak induced current density from typical PEMF therapeutic signals to affect the distribution of charges (receptors) on the cell surface via electrokinetic mechanisms [242].

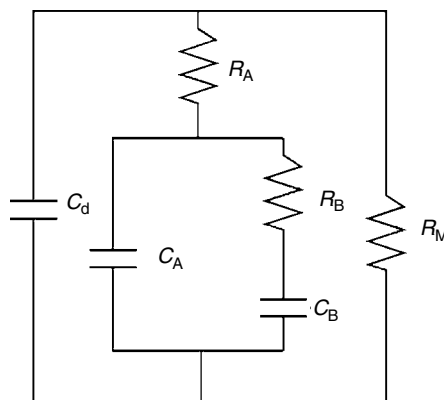
The electrochemical pathways involved in the transduction of an exogenous EMF signal into a physiologically significant endpoint appear to be operationally similar to the initial gating process involved in the production of the action potential via membrane depolarization [231]. It is therefore appropriate to consider the configuration of EMF waveforms in terms of an informational approach, or trigger, in contrast to one designed to supply energy to drive the biochemical cascade. Examples of the latter would be the use of direct currents large enough to cause cells to move along the electric field in wound repair applications and electroporation, wherein short voltage pulses are applied with sufficient electric field to temporarily cause the cell membrane to become permeable to macromolecules such as DNA or chemotherapeutic agents.

### 11.3.4 Electrochemical Information Transfer Model

It was proposed by Pilla in 1972 that nonthermal, subthreshold EMFs may directly affect ion binding and transport and possibly alter the cascade of biological processes related to tissue growth and repair [14]. This electrochemical information transfer (EIT) hypothesis postulated the cell membrane as the site of interaction of low level EMFs through modulation of the rate of binding of, e.g., calcium ion to receptor sites as a first step in a biochemical cascade relevant to the desired clinical outcome.

Ionic interactions at electrically charged interfaces of a cell are generally voltage-dependent (electrochemical) processes. Several distinct types of electrochemical interactions can occur at cell surfaces. One includes all of the nonspecific electrostatic interactions involving water dipoles and hydrated (or partially hydrated) ions at the bilayer lipid–aqueous interface of a cell membrane, which make all cell membranes a capacitor [231–233]. The other involves voltage-dependent ion or ligand binding. Here an ion or dipole can effectively compete with water dipoles and hydrated ions for specific membrane sites, which, in turn, can modulate a downstream cascade.

Equivalent electrical circuit models representing these electrochemical processes at cell surfaces and junctions have been derived [14,15,50,234]. Typically, most calculations consider a membrane model that consists of a capacitance,  $C_d$ , in parallel with an ionic leak pathway,  $R_M$  (see Figure 11.4). Although all membranes exhibit these properties, this simple model does not completely describe the dielectric properties of a functioning



**FIGURE 11.4**

Electrical equivalent circuit of a cell membrane that exhibits a dielectric membrane capacitance,  $C_d$ , a leak resistance,  $R_M$ , an ion-binding pathway having an equivalent resistance,  $R_A$ , and capacitance,  $C_A$ , and a time constant,  $R_B C_B$ , representing a coupled surface step, e.g., conformation change. This circuit requires ion binding to occur prior to the follow-up step.

membrane, particularly with respect to the EMF transduction pathway. Impedance measurements on isolated cells have revealed the existence of relaxation processes which appear to reflect the kinetics of ion or ligand binding, as well as follow-up biochemical reactions [235–238]. Thus, a more general description of membrane dielectric properties, which takes into account electrochemical processes relevant to EMF sensitivity, considers an ion-binding step that precedes and possibly triggers a subsequent chemical reaction at the membrane surface. The current,  $i_b(\omega)$  that flows into this pathway can be written

$$i_b(s) = q_a s \Gamma_a \Delta \Gamma_a(s) \quad (11.6)$$

where  $q_a$  is a coefficient representing the dependence of interfacial charge upon the surface concentration of the bound ion,  $\Gamma_a$ , and  $s (= \sigma + j \omega)$  is the complex frequency variable of the Laplace transform [243,244]. A similar analysis could, of course, be carried out for  $s = j \omega$ , i.e., a classical Fourier analysis [240,243].

Equation 11.6 shows the current in this pathway is a function of the change in surface concentration of the binding ion with time,  $\Delta \Gamma_a(s)$ , which, in turn, is voltage dependent and a function of the change in surface concentration of the product of the follow-up biochemical reaction,  $\Delta \beta_b(s)$ . In order to derive an expression for the impedance,  $Z_A(s)$ , of this pathway, it is necessary to define relationships between  $\Delta \Gamma_a$  and the change in transmembrane voltage ( $V_M$ ), and  $\Delta \beta_b(s)$ . This may be written, for first-order linear kinetics as [14,234]

$$\Delta \Gamma_a(s) = \frac{\nu_a}{\Gamma_a s} [-\Delta \Gamma_a(s) + \alpha V_M - \Delta \beta_b] \quad (11.7)$$

where  $\nu_a$  is the binding rate constant and  $\alpha$  is proportional to the potential dependence of binding ( $\cong \partial \Gamma / \partial V_M$ ). The change in surface concentrations of the ion and the biochemical product can also be described by first-order kinetics

$$\Delta \beta_b(s) = \frac{\nu_b}{\beta_b s} [\Delta \Gamma_a(s) - \Delta \beta_b(s)] \quad (11.8)$$

where  $\nu_b$  is the rate constant for the follow-up chemical reaction (defined as for  $\nu_a$ ) governing the rate of formation (decomposition) of the bound biochemical product after ion binding in a molecular cleft has occurred.

Equation 11.6 through Equation 11.8 allow the electrical impedance of the proposed transduction pathway at the cell membrane,  $Z_A(s)$ , to be written as

$$Z_A(s) = \frac{1}{\alpha q_a} \left[ \frac{1 + \Gamma_a s / \nu_a}{\Gamma_a s} + \frac{1}{\Gamma_a s (1 + \beta_b s / \nu_b)} \right] \quad (11.9)$$

Inspection of Equation 11.9 reveals the existence of two time constants, the parameters of which are identifiable in terms of the rate constant and change in surface concentration of each reaction step. Thus, the equivalent resistance of binding,  $R_A$  is

$$R_A = \frac{1}{\alpha q_a \nu_a} \quad (11.10)$$

which shows the equivalent resistance of binding kinetics is, as expected, inversely proportional to the rate constant,  $\nu_a$ . Correspondingly, the equivalent capacitance of binding,  $C_A$ , is directly proportional to the surface concentration of the binding entity,  $\Gamma_a$  as

$$C_A = \alpha q_a \Gamma_a \quad (11.11)$$

Note that the product  $\alpha q_a$  ( $\alpha \approx \partial \Gamma / \partial V_M$ ,  $q_a = \partial q / \partial \Gamma$ ) in Equation 11.10 and Equation 11.11 has the dimensions of capacitance, the expected proportionality constant to enable equivalent electric circuit parameters to be related to ion-binding kinetics.

The second time constant,  $\tau_B$ , in Equation 11.9 relates to the follow-up biochemical reaction

$$\tau_B = R_B C_B = \frac{\beta_b}{\nu_b} \quad (11.12)$$

where  $R_B$  and  $C_B$  are the equivalent resistance (inversely proportional to reaction rate, as for  $R_A$ ) and capacitance (proportional to the surface concentration,  $\beta_b$ , of the reaction product) of the follow-up biochemical reaction, respectively. Note the follow-up reaction can be a conformational change as happens when  $\text{Ca}^{2+}$  is bound to at least three of the four available binding sites in calmodulin [288].

An electrical equivalent circuit,  $Z_A$ , which requires ion binding to occur prior to the follow-up reaction, is given in Figure 11.4. The membrane capacitance,  $C_d$ , and leak resistance,  $R_M$ , are also shown, as it is necessary to include these pathways in the total membrane impedance,  $Z_M$ , to fully characterize frequency dependence.

The impedance,  $Z_d(s)$ , of the  $C_d/R_M$  pathway is given by

$$Z_d(s) = R_M + \frac{1}{C_d s} \quad (11.13)$$

The most common representation of subexcitation threshold membrane impedance is via Equation 11.13. However, the electrochemical analysis given above shows this is not complete. Indeed, the pathways depicted in the ion-binding impedance given in Equation 11.9 have proven to be necessary to complete the EIT model. Time constants associated with electrochemical membrane processes have been reported [235–238]. One model system studied was the toad urinary bladder membrane having a single-cell thick epithelial layer with tight junctional electrical contact between cells, thereby affording high-resolution impedance values [235]. Isolated cell impedance studies utilized artificial epithelial layers created by deforming living cells under physiologic hydrostatic pressure into well-defined polycarbonate membrane filters. This technique was applied to melanoma, fibroblast, and osteoblast cells [238]. In all cases the results showed, as expected, a first time constant or relaxation process due to the ubiquitous dielectric capacitance of the lipid–protein bilayer. The time constant for this process is similar for all mammalian cells, in the 1–10  $\mu\text{s}$  range. However, all cells exhibited at least a second time constant, which was characteristic of an ion-binding pathway. The time constant for this pathway was significantly different for each cell type, ranging from 20  $\mu\text{s}$  for human erythrocytes to 200  $\mu\text{s}$  for fibroblasts and osteoblasts. In addition, a longer time constant was often present related to passive ion transport across the cell membrane that could be coupled to the ion-binding step [50,234].

The above summarizes the EIT model that strongly guided the creation of the first clinically effective PEMF signal for recalcitrant fracture repair [51,52]. According to the EIT model, the requirements for an effective waveform could be met if it contained frequency components of sufficient amplitude within the time constant of the proposed target pathway [14,15,50]. Transmembrane ion transport, for which kinetics is in the millisecond range [235], was chosen as the target pathway for bone repair. This, coupled with practical restrictions on the size of the coil for patient use, led to the pulse burst

waveform shown in [Figure 11.2](#), top. It was supposed that the cell would ignore the short opposite polarity pulse and respond only to the envelope of the burst that had a duration of 5 ms, enough to induce sufficient amplitude in the kilohertz frequency range. Although the reasoning behind the asymmetric pulse in this waveform was erroneous because the EIT model was not yet complete, requiring a thermal noise analysis as will become apparent below, this signal is nonetheless effective for bone repair. It continues to be part of the standard armamentarium of the orthopedist for the nonsurgical noninvasive treatment of recalcitrant bone fractures.

It is important to note that the role of ions as transducers of information in the regulation of cell structure and function gained widespread acceptance well after the introduction of the EIT model. Ionic regulation mechanisms have now been described in: growth factor activation of the Na K-ATPase enzyme in fibroblasts [246,250];  $\text{Ca}^{2+}$  regulation, via CaM, of the cell cycle [251,252]; differential  $\text{Ca}^{2+}$  requirements of neoplastic vs. nonneoplastic cells [245,253];  $\text{Ca}^{2+}$ -dependent adenylate cyclase activation in macrophages [251]; Ca-CaM regulation of growth factor and other cytokine release [247,248,254,255]. EMF could also modulate the distribution of protein and lipid domains in the membrane bilayer, as well as conformational changes in lipid-protein associations by altering the kinetics of binding. Ion or ligand binding represents a coupling or transduction mechanism for exogenous EMFs at biological surfaces and junctions, which can be used to configure quantitatively and predictively the bioeffective EMF waveforms.

### 11.3.5 Magnetic Field Effects

As stated previously, the first PEMF signals utilized for tissue growth and repair were configured assuming that the induced electric field was the source of the stimulus (information). There is ample evidence that electric field is the dose metric for *in situ* PEMF signals such as those depicted in [Figure 11.2](#). One study showed PEMF enhanced cellular proliferation and extracellular matrix synthesis for bovine articular chondrocytes only when a pair of Helmholtz aiding coils was horizontal to the culture dish. There was no effect when the same coils inducing the same pulsing magnetic field were oriented vertical to the culture dish [262]. Although the magnetic field was exactly the same for both coil orientations, the maximum electric field within the culture dish was more than a factor of 10 lower for vertical vs. horizontal coils, as expected from Faraday's law of induction ([Equation 11.5](#)). This is because the height of the medium in a typical culture dish is approximately 2 mm vs. a dish diameter of 35–60 mm. (Refer to [Figure 11.3](#) for a schematic illustration of the horizontal coil configuration.) When coils are oriented vertically current flow is at right angles to that shown in [Figure 11.3](#) and the path is limited to liquid height, not dish diameter.

Liburdy [263] used specially constructed annular culture dishes of differing diameter to show that calcium transport in mitogen stimulated thymic lymphocytes scaled with the induced electric field from a 22 mT, 60 Hz time-varying magnetic field. The magnetic field was identical for each loop diameter suggesting the dose metric in this case followed [Equation 11.4](#), which shows induced electric field amplitude is directly proportional to the target loop radius for the cylindrical geometry of this experiment. The electric field dose metric was also demonstrated *in vivo* by exposure of fibular osteotomies in the rabbit to a clinically effective pulse burst PEMF signal (see [Figure 11.2](#)) applied via an external coil or via implanted electrodes [264]. The results showed the biomechanical acceleration of bone repair depended only on the *in situ* electric field and not on the magnetic component produced in the external coil to inductively couple the electric field to the repair site. Another study reported optimization of induced electric field parameters from

time-varying magnetic fields to control bone remodeling in an avian model of disuse osteoporosis [265]. Finally, a recent study reported remarkable sensitivity of neutrophil metabolism to induced electric fields as low as  $1 \mu\text{V}/\text{cm}$  [266]. The electric vs. magnetic field dose metric was specifically addressed in this study, and it was established that the observed results were indeed due to the induced electric field.

In contrast to the above-mentioned studies, Liboff et al. [267] reported in 1984 that human fibroblasts in culture exhibited enhanced DNA synthesis when exposed to sinusoidally varying magnetic fields over a wide range of frequencies (15–4 kHz) and amplitudes (2–600  $\mu\text{T}$ ). The effect appeared to be independent of the time derivative of the magnetic field, suggesting magnetic field was the dose metric for these sinusoidal waveforms. These results were somewhat controversial because the induced electric field at frequencies in the kilohertz range was of the same order as that reported effective for PEMF signals in cellular and clinical studies, and could clearly have contributed to the dose metric. However, this study also reported very low-frequency magnetic fields in the microtesla range could produce a similar effect on DNA synthesis. For these signals the induced electric field was well below that of the clinically effective PEMF signals, suggesting a direct magnetic field effect. Thus began the ion cyclotron and parametric resonance era. These models are covered in [chapter 9 BBA](#) [372], however a short summary will be useful.

Ion cyclotron resonance (ICR) [19,268–269] described frequency specific combinations of dc and ac magnetic fields, which can increase ion mobility near receptor sites and through ion channels. The Lorentz force equation was used to relate individual influences of both ac and dc magnetic fields to ligand receptor binding and motions of ions or other charged molecules [268–270]. The main objection to ICR is thermal noise [23,24,214,216,271]. Bianco and Chiabrera [272] have provided an elegant explanation of the inclusion of thermal noise in the Lorentz–Langevin model which clearly shows the force applied by a magnetic field on a charge moving outside the binding site is negligible compared to background Brownian motion and therefore has no significant effect on binding or transport at a cell surface or junction.

Because of thermal noise problems in the ICR model Lednev [20] and others [21,22,26,276–278] formulated an ion parametric resonance (IPR) quantum approach that modeled the ion in the binding site of a macromolecule (e.g., calmodulin, CaM) as a charged harmonic oscillator. It was proposed that the presence of a static magnetic field could split the energy level of the bound ion into two sublevels with amplitudes corresponding to electromagnetic frequencies in the infrared band. The difference between these two energy levels is the Larmor frequency (=cyclotron frequency/2). The IPR model, as for the ICR model, requires parallel ac and dc magnetic fields, and that they both be present.

In addition to difficulties with the experimental verification of the IPR model [279,350,351], the main fundamental objection to the IPR model relates to excited-state lifetime for the low-frequency EMF signals involved [280,281]. Indeed, the acceleration of the bound ion oscillating at frequencies of the order of  $10^{12}$  Hz obviously cannot be affected by the negligible perturbations of the ion orbit generated by weak magnetic fields at  $10^{10}$  lower frequencies. Therefore, the transition rate to the ground state cannot be affected by ELF fields and IPR cannot occur [282]. However, the axis of vibration of bound ligands can be affected by weak ELF, as well as dc, the magnetic fields in a classical manner, e.g., Larmor precession, and some orientations may cause enhanced biological effects [23,24,28,30,213,282].

Notwithstanding all of the above, a clinical device was created on the basis of the ICR model that is currently used for recalcitrant bone fractures. Clinical results from this device appear to be equivalent to those from other inductive and capacitive coupled devices [31,59,68]. The signal is applied using an external pair of coils oriented parallel to

one another. The alternating 40  $\mu\text{T}$  sinusoidal magnetic field is at 76.6 Hz (a combination of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  resonance frequencies). The static (dc) parallel magnetic field is at 20  $\mu\text{T}$ . Since there are no published clinical studies with either the ac or dc component of the magnetic field alone, there is no solid evidence that this combination of ac and dc fields is unique, or, e.g., that either the ac or dc component alone would not have produced the same clinical results. The fact remains, however, that a clinical device, which produces an electrical field too weak to be detected by the tissue target, has demonstrated clinical success. This can only have been achieved via a magnetic, not electric, field.

There is enough additional significant evidence showing both low-frequency sinusoidal magnetic fields, which induce electric fields well below the thermal noise threshold, and weak static magnetic fields, for which there is no induced electric field, can have biologically and clinically significant effects [84–92,202–209,273,283,290,291,360]. In these cases also, the stimulus must clearly be the magnetic field. This was unexpected, particularly for weak dc magnetic fields. There is, however, a promising, and largely overlooked, model, remarkably unhindered by thermal noise, which considers the Lorentz force on a moving charge in a binding site in terms of Larmor precession and its possible effect on reactivity [24,28,210–216]. The Larmor precession model (LPM) is summarized in the following section.

### 11.3.6 Larmor Precession Model

Larmor precession, which describes the effects of exogenous magnetic fields on the dynamics of one state of ion binding, i.e., ions already bound, has been suggested as a possible mechanism for observed bioeffects due to weak static and alternating magnetic field exposures [210–216,271,288]. A bound ionic oscillator in a static magnetic field will precess at the Larmor frequency in the plane perpendicular to the applied field. This motion will persist in superposition with thermal forces, until thermal forces eventually eject the oscillator from a binding site. For a magnetic field oriented along the  $z$ -axis, the precessional motion will be confined to the  $x$ - $y$  plane. The LPM proposes that the biochemical reactivity of a bound ion complex may be affected by changes in the spatial orientation of the bound ionic oscillator.

The effect of weak dc and ac magnetic fields according to the LPM can be summarized as follows. The Lorentz–Langevin equation written to describe the motion of an ion bound in a potential well (molecular cleft) subject to a magnetic field oriented along the  $z$ -axis in the presence of thermal noise forces is

$$\frac{d^2\mathbf{r}}{dt^2} = -\beta \frac{d\mathbf{r}}{dt} + \gamma \frac{d\mathbf{r}}{dt} \times B_0 \mathbf{k} - \omega^2 \mathbf{r} + \mathbf{n} \quad (11.14)$$

where  $\mathbf{r}$  is the position vector of the ion;  $\beta$  is the viscous damping coefficient per unit mass (due to molecular collisions in the thermal bath);  $\gamma$  is the ion charge to mass ratio;  $B_0$  is the magnitude of the magnetic field vector;  $\mathbf{k}$  is the unit vector along the  $z$ -axis;  $\omega$  is the angular frequency of the oscillator; and  $\mathbf{n}$  is the random thermal noise force per unit mass [271,288]. It has also been shown that precession is not limited to the case of a linear isotropic oscillator potential but will occur for any central restorative potential [211].

Equation 11.14 describes the motion of an oscillator (ion) in a molecular cleft due to an exogenous magnetic field in the presence of thermal noise. The solution may be written [212]

$$\mathbf{r}(t) \rightarrow \mathbf{e}^{i\omega t} \mathbf{r}(t) = \mathbf{e}^{i\omega t} [C(t) + \Psi(t)] \quad (11.15)$$

where  $\mathbf{r}(t)$  is the position vector of the bound ion;  $C(t)$  is the coherent oscillation of the bound oscillator, and  $\Psi(t)$  is the contribution due to thermal noise forces. The ion trajectory thus consists of a coherent part given by

$$C(t) = -\frac{C_0}{\omega} e^{-\frac{\beta}{2}t} e^{-i\omega_L t} \sin(\omega t) \quad (11.16)$$

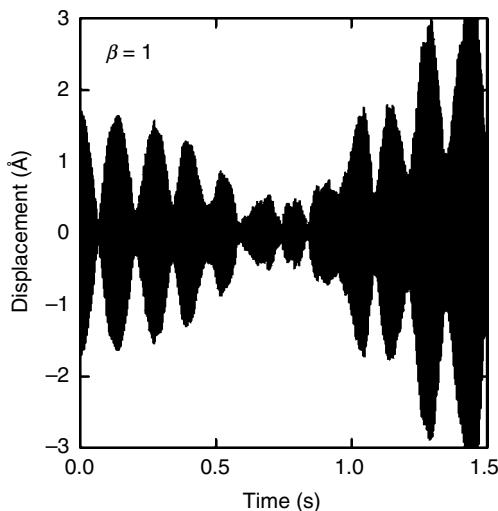
where  $C_0$  is determined by initial conditions and  $\omega_L (= \gamma B/2)$  is the Larmor frequency; and a component due to thermal noise

$$\Psi_n(t) = \frac{k_0}{\lambda_2 - \lambda_1} e^{-\frac{\beta}{2}t} e^{-\frac{i\omega_L}{2}t} [N(t)] \quad (11.17)$$

where  $k_0$  is determined by initial conditions;  $N(t)$  is the accumulation of the thermal component with time; and  $\lambda_1$  and  $\lambda_2$  are the roots and contain the ac and dc magnetic field terms [212].

The thermal component  $\Psi(t)$  of the ion trajectory itself thus consists of an harmonic oscillator driven by thermal noise, subject to viscous damping and undergoing precessional motion at the Larmor frequency about the axis defined by the magnetic field. It oscillates at the fundamental frequency of the oscillator potential with amplitude increasing over time, ultimately resulting in ejection from the binding site after a bound lifetime determined by the magnitude of thermal forces.

Both the coherent and thermal components of an ion at a binding site exhibit Larmor precession in the presence of an applied magnetic field. As the amplitude of the thermal component grows the oscillator orientation still precesses at the Larmor frequency in the plane perpendicular to the applied magnetic field direction. This is illustrated in Figure 11.5, which shows the manner in which the amplitude of the oscillator vibration



**FIGURE 11.5**

Overall effect of thermal white noise on precessional motion in a 10  $\mu\text{T}$  static magnetic field. Note that, for this value of the viscous damping coefficient ( $\beta$ ), precession is coherent and decays exponentially for approximately 0.5 s. Thermal noise then begins to add to the oscillator vibration amplitude, ultimately leading to ejection of the bound ion from the molecular cleft after a bound time of approximately 1.2 s. Until ejection, the oscillator is still precessing. (From Pilla, A.A., Muehsam, D.J., Markov, M.S., *Bioelectrochem. Bioenerg.*, 43, 241, 1997. With permission.)



(at infrared frequencies) is affected by thermal noise. Note that, even though thermal noise is progressively adding to the amplitude of oscillator vibration, the bound ion continues to precess at the original Larmor frequency until ejected from the binding site. Thus, precession frequency is unaffected by thermal noise while the oscillator is bound. The threshold for LPM is, therefore, determined only by the bound lifetime of the charged oscillator, allowing extremely weak magnetic fields to affect its dynamics.

Although thermal forces will in general be distributed throughout the spherical solid angle available in the binding site, it is important to bear in mind that the bound ion or ligand is not executing random motions in an isotropic region. Rather, it is strongly bound in an oscillator potential, with oscillator frequency in the infrared [211,212]. It is also important to emphasize that an ion bound in a molecular cleft exhibits vibrational and rotational, but not translational, degrees of freedom [393–395]. This means the bound oscillator can precess, but will not retain the ability to move in the random directions permitted in its unbound trajectory.

Larmor precession converts the exogenous magnetic field amplitude into a frequency determined by the gyromagnetic ratio of the target. Thus, for an ion oscillating along the z-axis the Larmor frequency  $\omega_L$  is

$$\omega_L = -\Gamma B_{x,y} \quad (11.18)$$

where  $\Gamma = q/2m$  for a simple unhydrated ion. Equation 11.18 illustrates that precession frequency scales with magnetic field provided the magnetic field has components perpendicular to the axis of oscillation. Note that cyclotron frequency =  $2\omega_L$ . Of course, the minimum detectable magnetic field is determined by the contribution of thermal energy to the bound oscillator amplitude as described above.

According to LPM, each Larmor precession frequency determines the minimum time for the bound oscillator to reach reactive orientations at the binding interface. LPM predicts that a bound oscillator will accelerate faster to preferred orientations in the binding site with increasing static magnetic field strength. This can increase binding rate with a resultant acceleration in the downstream biochemical cascade. According to LPM, static magnetic fields in the 0.1–1  $\mu\text{T}$  range can be detected if the oscillator remains bound for the order of a second [271]. To illustrate, the Larmor frequency for calcium in a 50- $\mu\text{T}$  static magnetic field is approximately 18 Hz, suggesting a value for the damping coefficient,  $\beta$ , of about 35 or less is necessary in order for the oscillator to maintain a substantial amplitude over the period of one or more precessional orbits. The geometry of the binding site can create a locally hydrophobic region, from which dipolar molecules such as water are repelled [285], although at least one water dipole remains in a Ca–CaM binding site [287]. Thus, the binding site is a region in which a bound ion would experience very few collisions, resulting in a viscosity significantly below that of bulk water, accounting for the long bound times reported for Ca–CaM.

Weak static magnetic fields have been reported to accelerate Ca–CaM-dependent myosin light chain kinase (MLCK) and protein kinase C (PKC)-dependent processes up to twofold [84–92,273], although one report failed to show effects in the MLCK system [274] and another, which examined  $\text{Ca}^{2+}$  binding to CaM directly using a fluorescence technique, also failed to show effects [275]. Two studies reported the rate of  $\text{Ca}^{2+}$  binding to CaM was increased twofold with 2 G static magnetic field [90,92]. Static magnetic fields as low as 6 G increased cell survival by reducing stress-induced apoptosis threefold via a twofold increase of  $\text{Ca}^{2+}$  influx [352]. Weak dc alone (1 G) caused conformational changes in chromatin of *E. coli* bacteria similar in magnitude to ac–dc combinations chosen according to ICR or IPR models [353,354].

### 11.3.7 Resonance in Larmor Precession Model

There are credible reports of *in vitro* studies that demonstrate resonance behavior for certain combinations of weak ac and dc magnetic fields [19,278,283,349,350]. However, these do not unequivocally support the predictions of either the ICR or the IPR models [350]. Thus, it is interesting to consider ion resonance in terms of LPM. In order to assess the combined effect of simultaneous weak ac and dc magnetic fields on Larmor precession, it is necessary to recall that the bound charged oscillator will precess when only a dc field is present according to Equation 11.18. Addition of an ac magnetic field to an oscillator already precessing in a binding site will modulate oscillator motion for both perpendicular and parallel orientations with peak effects at multiples of the Larmor frequency.

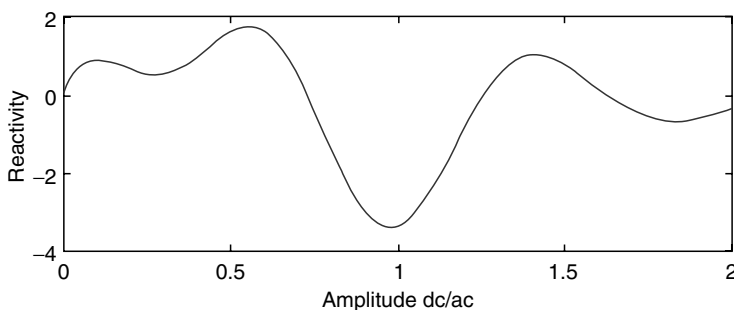
The addition of an ac frequency in either a perpendicular or parallel direction to the axis of a precessing oscillator will modulate the axis of precession with a resultant effect on the time to reach a reactive orientation. This may be quantified by evaluation of the total excursion of the oscillator,  $A(t)$ , for a given binding time,  $t$ , and for various ac–dc combinations

$$A(t) = C_o \int_0^t \omega_L dt = \frac{C_o}{2m} \int_0^t [B_o + B_1 \cos(\omega_{ac}t)] dt = \frac{C_o}{2m} \left[ B_o t + \frac{B_1}{\omega_{ac}} \sin(\omega_{ac}t) \right] \quad (11.19)$$

where  $\omega_L$  is the Larmor frequency;  $B_o$  is the amplitude of the dc magnetic field;  $B_1$  and  $\omega_{ac}$  are the peak amplitude and the frequency of the ac magnetic field, respectively;  $m$  is the mass of the bound oscillator; and  $C_o$  is a proportionality constant.

Equation 11.19 may be evaluated for any ion or ligand, any combination of ac and dc magnetic fields with any relative orientation. An example is shown in Figure 11.6 in which dc magnetic field amplitude is varied for ac fixed at  $\omega_L$  for  $\text{Ca}^{2+}$ . It may be seen that addition of an ac magnetic field to the precessing oscillator either further accelerates or inhibits its time to reach a reactive orientation. This behavior is remarkably similar to reported experimental verifications of IPR [278,350], and supports Larmor precession as a viable alternative mechanism for weak dc and ac magnetic field bioeffects.

It is interesting to speculate whether the parameters of the ac and dc magnetic field components could be chosen to actually prohibit the bound oscillator from reaching a reactive position in the binding site. If this possibility is verified by experiment, a non-invasive treatment for pathologies such as ectopic bone formation (wherein bone grows



**FIGURE 11.6**

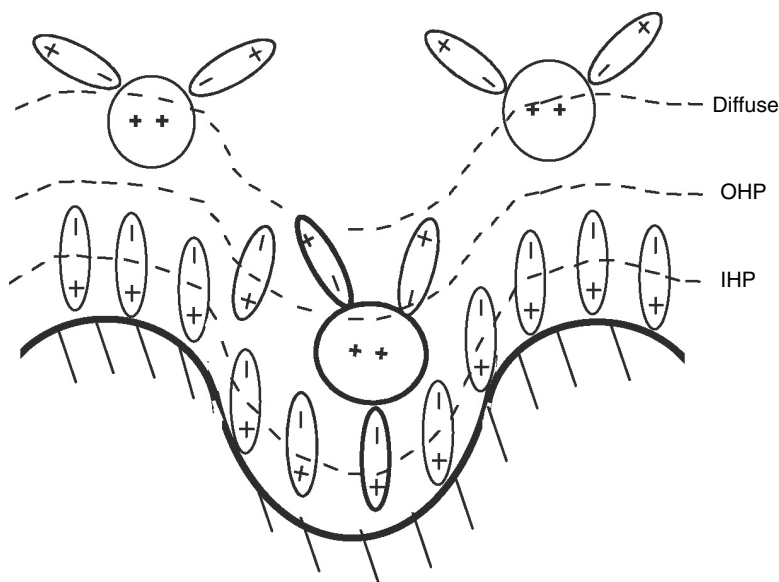
Effect of scanning dc magnetic field amplitude on a bound oscillator precessing at the Larmor frequency. Reactivity, e.g., Ca–CaM-dependent enzyme activity, can be either increased (vs. dc field alone) or decreased, meaning addition of ac can either further accelerate or inhibit the oscillator excursion to a reactive orientation within the binding site.

where it should not) and malignancies without side effects could emerge. This possibility does not appear to exist for PEMF modalities.

### 11.3.8 Dynamical Systems Model

Magnetic fields couple with binding kinetics via the Larmor frequency, while the induced electric field couples to the binding process via its potential dependence (see Equation 11.7). This may be modeled by considering the binding process as a dynamical system wherein the particle has two energetically (double potential well), stable points separated by a few kilotesla either bound in the molecular cleft, or unbound in the plane of closest approach to the hydrated surface (Helmholtz plane) at the electrified interface between the molecular cleft and its aqueous environment [288], as shown in Figure 11.7. Ion binding or dissociation is then treated as the process of hopping between these two states driven by thermal noise and EMF effects are measured by modulation of the ratio of time bound (in the molecular cleft) to time unbound (in the Helmholtz plane). This model does not require the ion to move in a trajectory far from the binding site, i.e., the ion is still in a prebound state while in the Helmholtz plane.

According to this model, modulation of the kinetics of binding is considered as either a change in the height or in the bias of the energy barrier between unbound and bound states. This is thus a dynamical system wherein thermal white noise is taken as the driving force for ion binding and dissociation. The reaction coordinate  $q(t)$  is subject to inertia, a damping force proportional to  $dq/dt$  and a potential energy function  $V(q, E_{ind})$



**FIGURE 11.7**

Cartoon of a molecular cleft illustrating an ion in a bound state that resides in the inner Helmholtz plane, IHP. Kinetics of binding is determined by ratio of time bound to unbound (outer Helmholtz plane, OHP). Bound ion hops between IHP and OHP driven by thermal noise and statistically does not drift into the diffuse double layer where it is subject to mechanical (electrokinetic) perturbation. While bound, the ion possesses rotational degrees of freedom and can precess. Note that water dipoles may remain resident in the binding site while the ion is in the bound state. (From Pilla, A.A., Muehsam, D.J., Markov, M.S., *Bioelectrochem. Bioenerg.*, 43, 241, 1997. With permission.)

dependent upon the induced electric field  $E_{\text{ind}}$ . The system can be described by the following differential equation

$$\frac{d^2q(t)}{dt^2} + \eta \frac{dq(t)}{dt} + \frac{dV(q, E_{\text{ind}})}{dq} = F_{\text{noise}} \quad (11.20)$$

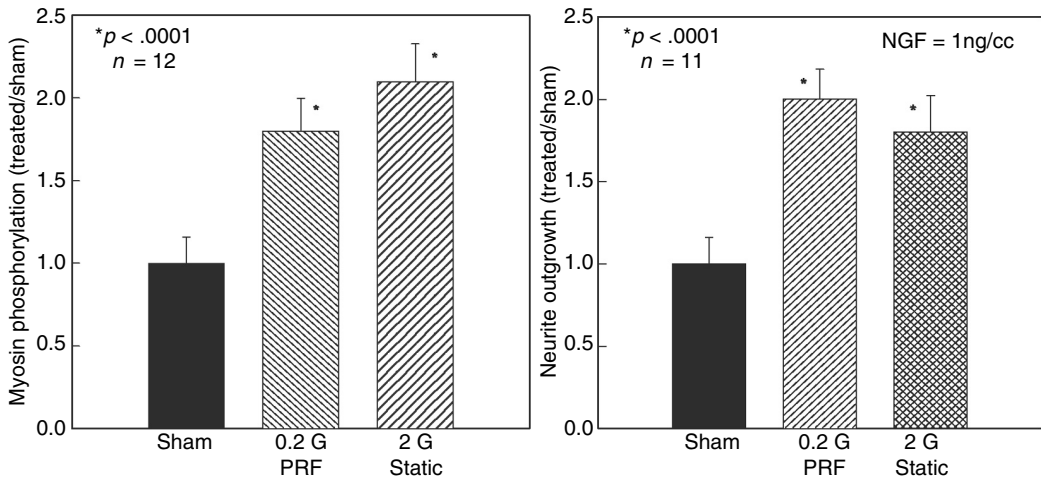
where  $\eta$  is the coefficient of damping and  $F_{\text{noise}}$  is the force due to thermal noise on the bound particle. The force imparted on a charged ion by the induced electric field may be expressed as a perturbation of the potential energy function

$$V(q, E_{\text{ind}}) = \alpha_1 \frac{q^4}{4} - \alpha_2 \frac{q^2}{2} + \varepsilon q E_{\text{ind}} \quad (11.21)$$

where the nonnegative coefficients,  $\alpha_1$  and  $\alpha_2$ , are characteristic of the receptor molecule-hydration environment and  $\varepsilon$  is the effective (hydrated) ion charge.

Equation 11.20 describes a double well potential wherein the potential energy wells correspond to the bound and unbound phases of the binding process. The dynamical system describes binding in a statistical sense. In terms of this model the force due to an induced electric field can modulate the relative depth of the wells thereby affecting the ratio of time bound to time unbound and thus the kinetics of the binding process. A weak magnetic field can indirectly affect the double well potential via Larmor precession by changing the dwell time of the bound vibrating oscillator, which, in turn, modulates the ratio of time bound to time unbound and therefore reaction rate.

The dynamical system model provides a rationale for linking observed static magnetic (LPM) and electric (EIT) field effects in identical molecular and cellular systems. For example,  $\text{Ca}^{2+}$ -CaM-dependent myosin phosphorylation has been extensively studied with both static magnetic fields [30,90], and with several PRF signals [29]. A comparison of typical results is shown in Figure 11.8, left, which shows a 2-G static field, and a 0.2 G,



**FIGURE 11.8**

Comparison of the effect of a 0.2-G PRF signal having a 500- $\mu\text{s}$  burst of 27.12-MHz sinusoidal waves repeating at 1 per s, and a 2-G static magnetic field on  $\text{Ca}^{2+}$ -CaM-dependent myosin phosphorylation (left), and neurite length from embryonic chick ganglia explants (right). Both signals elicit the same response for identical exposure times, however, the PRF signal couples to the target pathway via induced electric field, whereas the magnet couples via magnetic field.

500  $\mu\text{s}$  burst of 27.12 MHz sinusoidal waves at 1-burst/s PRF signal, both accelerate phosphorylation nearly twofold in the presence of suboptimal free  $\text{Ca}^{2+}$  concentration. Similar behavior has been obtained for dendrite outgrowth from embryonic chick ganglia in the presence of suboptimal nerve growth factor (NGF) concentration (Figure 11.8, right). Dendrite length was also increased approximately twofold with both static and pulsing magnetic fields.

In both of these systems, the PRF signal induced a time-varying electric field and negligible magnetic field (vs. ambient), and the magnet produced only a static magnetic field, approximately 7X ambient. Dosimetry, therefore, depended upon the characteristics of the induced electric field for the PRF signal and on the magnetic field amplitude for the magnet. The common target pathway for both weak electric and magnetic fields, as proposed in the dynamical systems model, is ion or ligand binding. The common target pathway in both cases has been proposed to be the kinetics of  $\text{Ca}^{2+}$  binding, for which signal detection (SNR) can be estimated, as shown below, for the PRF signal, or for which the LPM can be employed to make predictions of effective, as well as saturation, static magnetic field strengths.

### 11.3.8.1 Calcium–Calmodulin-Dependent Myosin Phosphorylation as a Dynamical System

The EMF effects on  $\text{Ca}^{2+}$ –calmodulin-dependent myosin phosphorylation have been reported to occur only for  $\text{Ca}^{2+}$ -depleted conditions and during the nonequilibrium phase of the reaction [84–92]. For these depleted conditions, enzyme kinetics favor the bound state according to  $k_{\text{on}}/k_{\text{off}} \approx 10^2\text{--}10^3$  [288], the instantaneous exchange reaction rate  $v(t)$  is dependent upon the instantaneous free  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}(t)]$ , and myosin phosphorylation increases for increasing  $[\text{Ca}^{2+}(t)]$ . Enzyme kinetics are interpreted here in a statistical sense wherein  $[\text{Ca}^{2+}(t)]$  is taken to be proportional to the ratio of the mean time the ion is free to the mean time bound

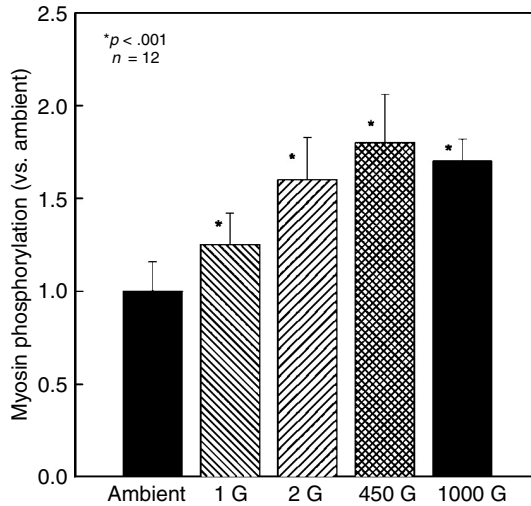
$$[\text{Ca}^{2+}(t)] = \rho \frac{t_{\text{free}}}{t_{\text{bound}}} \quad (11.22)$$

where  $\rho$  is a proportionality constant.

The instantaneous exchange reaction rate  $v(t)$  for myosin phosphorylation is proportional to the concentrations of free ions and CaM in the linear phase of the reaction and the reaction rate is thus a function of ion-binding dynamics

$$v(t) \propto [\text{Ca}^{2+}\text{CaM}] \leftrightarrow \rho \frac{t_{\text{free}}}{t_{\text{bound}}} + [\text{CaM}] \quad (11.23)$$

Ion-binding kinetics determines the sensitivity of this system to both induced electric field and weak magnetic fields. Dosimetry for electric fields is determined by detectability in the binding pathway. This requires sufficient voltage in the binding pathway to increase net  $[\text{Ca}^{2+}]$  above that due to thermal noise. The requirement is to configure the induced electric field waveform to produce sufficient amplitude in the binding pathway within the frequency range defined by binding kinetics. This will be described in detail in the next section. Direct magnetic field effects are linked via Larmor precession to ion-binding kinetics. In fact, the mean residence time of  $\text{Ca}^{2+}$  in the CaM binding site must be a multiple of the binding time constant. Reported values for this time constant range between  $10^{-2}$  and  $10^{-3}$  s [289]. Assuming a factor of 3, for simple exponential behavior, the static magnetic field effect on binding should be detectable at approximately 10  $\mu\text{T}$



**FIGURE 11.9** Effect of static magnetic field amplitude on Ca–CaM-dependent myosin phosphorylation. Acceleration of  $\text{Ca}^{2+}$  binding appears to saturate at approximately 200  $\mu\text{T}$ , as predicted by the Larmor precession model.

above ambient ( $\approx 50 \mu\text{T}$ ) and saturate at 200–300  $\mu\text{T}$ . Results reported to date [90,271] support this prediction. See also Figure 11.9.

It should also be noted that  $\text{Ca}^{2+}$  has been implicated in the EMF transduction pathway independent of its interactions with CaM. The most commonly reported are those related to  $\text{Ca}^{2+}$  influx, efflux, or oscillations [27,257–261].

### 11.3.9 Dosimetry for Induced Electric Fields

The biophysical lore prevailing until the late 1980s and lingering to this day is that, unless the amplitude and frequencies of an applied electric field were sufficient to trigger an excitable membrane (e.g., heart pacemaker), produce tissue heating, or move an ion along a field gradient there could be no effect. This was a formidable obstacle in the quest for therapeutic applications of weak EMF signals. However, this position had to be changed as the evidence for weak (nonthermal) EMF bioeffects became overwhelming. The clinical evidence offered by many double-blind clinical studies coupled with the database of hundreds of thousands of successful treatments of delayed and nonunion bone fractures registered with the FDA simply could no longer be ignored. Noninvasive PEMF treatment is actually as successful as the first bone graft to the huge benefit of the patient. The task now was to provide solid testable models for the biophysical mechanism of weak electric field bioeffects.

The underlying problem for any model, which claims to describe the biophysical mechanism of weak EMF bioeffects, relates to signal detection at the molecular, cellular, or tissue target in the presence of thermal noise, i.e., SNR. Considering the cell membrane as the target, the burden of proof is to show the induced voltage is not buried in thermal and other voltage noise, i.e., that the applied signal is detectable. One of the first simple models assumed the EMF target to be a spherical cell of approximately 10  $\mu\text{m}$  radius. The dielectric properties of the cell membrane in this model were limited to a simple membrane capacitance, with no attempt to take specific ion-binding pathways into account. These calculations often lead to an unfavorable SNR for many EMF signals,

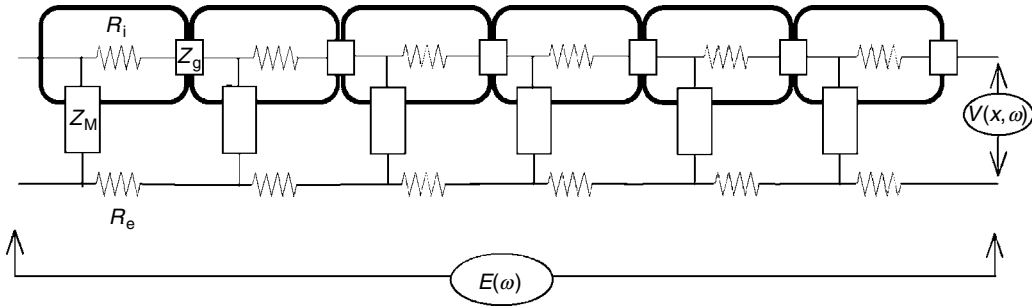
which have otherwise demonstrated biological effect [292–294]. Other attempts to account for the thermal noise problem assume unsubstantiated signal processing, such as signal averaging or rectification [295], by the target pathway. Stochastic resonance in which increasing noise strength can increase SNR for a signal having frequency components at a characteristic frequency (e.g., Larmor frequency) has also been proposed [296]. Finally, metabolic amplification via the out of equilibrium state of the ligand–receptor system due to basal cell metabolism has been proposed [297]. None of these models have been proven experimentally.

Without resorting to signal processing or metabolic amplification, it is still necessary to attempt to understand the remarkable sensitivity of biological systems to weak electric fields. In terms of target geometry, certainly the spherical cell model is oversimplified and cannot represent the geometric complexity of cellular and tissue EMF targets. For example, the successful outcome of a healing fracture, wherein bone tissue differentiates both functionally and spatially, is a clinically relevant illustration of cell–cell communication [298]. This suggests that the target for the PEMF signals used to affect nonunions and delayed unions of bone is a highly organized ensemble of cells. In fact, all organized tissue is developed and maintained by an ensemble of complex geometry cells, which have coordinated activity [299]. The most prevalent cell shape in living system tissue is elliptical and flattened, with processes extending in at least two directions. For example, human fibroblasts can typically exceed 100  $\mu\text{m}$  when attached to a substrate (connective tissue) [300]. In addition, nerve axons can be tens of centimeters in length [364]. Gap junctions provide pathways for ions and molecular intercellular communication [301]. They are present in all tissues including bone. The role of cooperative organization in the EMF sensitivity of biological systems has been qualitatively considered [363]. Gap junctions provide ionic coupling and metabolic cooperation, without which disorders in growth control and tissue repair, as well as neoplastic transformations, could occur [301]. Functional modification of gap junctions by modulated microwave fields, as well as EMF signals has been reported [303,304]. There have been several recent reports of modulation of gap junction activity by PEMF signals [305–309].

As shown in [Sections 11.3.4](#) and [Section 11.3.5](#), EMF dosimetry requires knowledge of the electrical properties of the cell or tissue target. Several reports have emerged showing how induced electric field dosimetry can be altered by the presence of gap junctions [310–317,363–367]. A distributed-parameter electrical analog (cable) is often employed to represent the electrical properties of an ensemble of cells in gap junction contact. Most of the cable models represent the cell by a simple membrane capacitance and leak resistance [318,367,368]. However proper dosimetry requires the complete electrochemical state of a functioning cell membrane to be taken into account. A summary of this approach, which incorporates electrochemical processes in the electrical equivalent circuits of the cell membrane, is presented below.

### 11.3.10 Cell Array Model

Gap junctions allow a group of organized cells, as in a tissue, to present a larger antenna to detect the induced electric field from an exogenous EMF. This increases EMF sensitivity well beyond that due to a molecule or single cell, with a resultant increase in target sensitivity. A useful model is the distributed-parameter linear electrical analog (transmission line) allowing induced transmembrane voltage,  $V_M$ , to be evaluated as a function of frequency and position along the array. This is similar to the electrophysiological models which have been proposed for current spread in electrotonically coupled tissues, the dc model proposed to account for tissue sensitivity to the weak electric currents commonly



**FIGURE 11.10**

Distributed-parameter cell array model showing cells in gap junction contact via impedance  $Z_g$ . This model behaves electrically identical to a transmission line wherein the applied electric field  $E(\omega)$  propagates throughout the array causing progressively higher changes in induced transmembrane voltage  $V(x, \omega)$ .  $Z_M$ ,  $R_i$ , and  $R_e$  are the membrane impedance, and intracellular and extracellular resistances per unit length, respectively.  $Z_g$  represents the gap junction impedance. (From Pilla, A.A., Nasser, P.R., Kaufman, J.J., *Bioelectrochem. Bioenerg.*, 35, 63, 1994. With permission.)

found in developing and regenerating tissues [314,315], for direct currents in wound repair [364] and for nonlinear electroporation [365]. Obviously the complete model should be three-dimensional, however the linear model presented here serves as a good first approximation to illustrate the array effect on EMF sensitivity.

The transmission line model is a lossy ladder network, or cable, of finite length, having different internal and external conductance. The steps of this ladder are constituted by the membrane impedance per unit length, which provides a pathway for the internal current to exit the cell. Details are available elsewhere [312], however it is useful to show how the presence of gap junctions affects the induced electric field detected in the cell array when electrochemical processes that represent the EMF transduction pathway are taken into account. The electrical equivalent circuit is shown in Figure 11.10.

Induced transmembrane voltage  $V_M(x, s)$  at any position  $x$  in the cable shown in Figure 11.10 representing the cell array and for any induced electric field waveform  $E(s)$  is

$$V_M(x, s) = -E(s) \frac{1}{\gamma} \frac{\sinh(\gamma x)}{\cosh(\gamma L)} \quad (11.24)$$

where

$$\gamma = \sqrt{\frac{R_e + R_i + R_g}{Z_M(s)}} \quad (11.25)$$

and  $R_e$  and  $R_i$  are, respectively, the extracellular and intracellular resistances per unit length along the  $x$ -axis,  $R_g$  is the gap junction resistance per unit length, and  $Z_M(s)$  is the membrane impedance per unit length.

Evaluation of  $V_M(x, s)$  has been reported for realistic values of the specific electrical parameters related to the cell array. Typical values for  $R_e$  and  $R_i$  are  $10^{10} \Omega/\text{m}$ . These values would be expected to be of the same order, given the cell volume percentage for a typical tissue (50%). The values for  $R_g$  range from  $10^{10}$  to  $10^{15} \Omega/\text{m}$ , representing the limiting electrical conditions of a completely open or completely closed gap junction, respectively [312]. The exact form of  $Z_M$  depends upon the impedance assumed. In the most simple form a membrane consists of a capacitance,  $C_d$ , representing the real capacitor made up of the lipid bilayer and its electrified interfaces, in parallel with an ionic leak pathway,  $R_M$ , a real resistor, as discussed in Section 11.3.5.

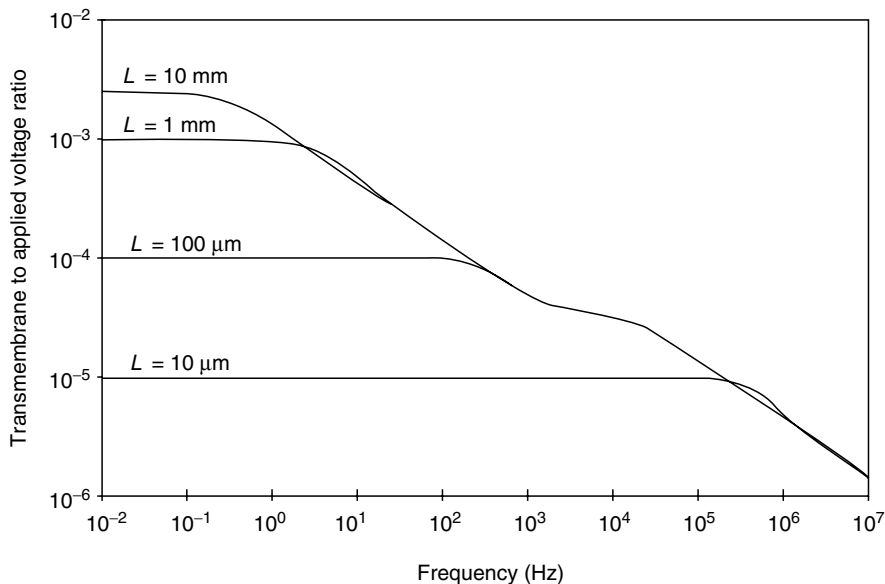


When an ion-binding pathway is added (Figure 11.4) the membrane admittance per unit length is

$$Y_M(\omega) = \frac{1}{R_M} + C_d j \omega + \frac{1}{\left[ R_A + \frac{1}{C_A j \omega} \right]} \quad (11.26)$$

where  $R_A$ , the equivalent binding resistance, ranges between  $10$  to  $10^3 \Omega\text{-m}$ , and  $C_A$ , the equivalent binding capacitance, ranges from  $10^{-6}$  to  $10^{-5} \text{ F/m}$ . Typical values for  $R_M$  range from  $10^3$  to  $10^5 \Omega\text{-m}$ , and for  $C_d$  from  $10^{-7}$  to  $10^{-6} \text{ F/m}$ . Equation 11.22 describes two time constants, the first due to the membrane capacitance  $C_d$  and the membrane leak resistance  $R_M$ . The second represents the ion or ligand binding process, the parameters of which are identifiable in terms of rate constants and changes in surface concentration, as shown in Section 11.3.4 and Section 11.3.5.

Equation 11.22 and Equation 11.24 have been employed to evaluate the effect of signal frequency and array length,  $L$ , on induced transmembrane voltage. The example shown in Figure 11.11, for a membrane at which ion binding occurs, is typical of models of the effect of gap junctions or long cells on induced transmembrane voltage [310–313]. As shown, there is a substantial increase in  $V_M(L, \omega)$  as  $L$  increases which can render this target more sensitive to exogenous EMF signals. This is simply due to the increased size of the target. However, overall frequency response is also significantly affected. The frequency response for a single cell ( $L = 10 \mu\text{m}$ ) indicates that  $V_M$  is maximum between  $10^5$  and  $10^6$  Hz, as expected. In contrast, for a 1 mm cell array,  $V_M$  is about  $10^2$  higher than for a single cell, but only at frequencies below 100 Hz. Note the presence of the second time constant due to ion binding between  $10^3$  and  $10^4$  Hz. Interestingly, the ion-binding time constant does not contribute substantially to the overall frequency response of the single isolated cell. The significance of this will become apparent below.



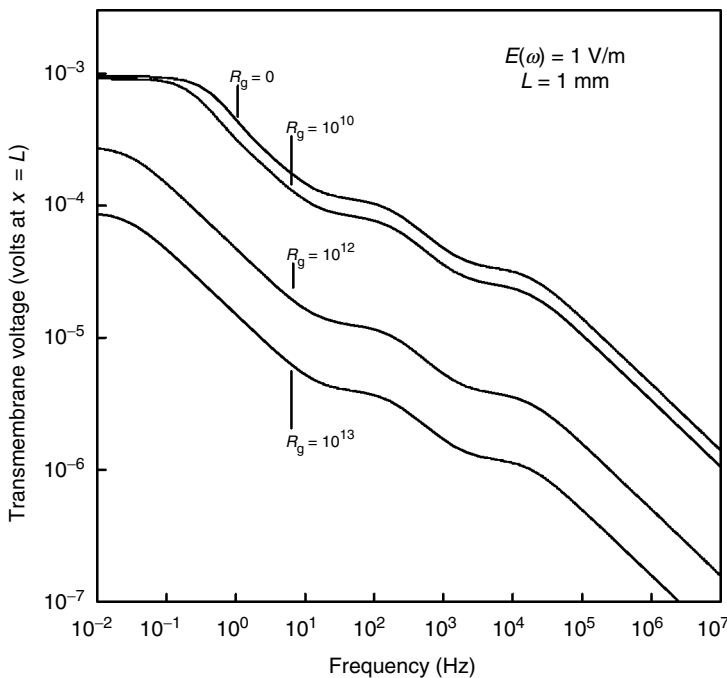
**FIGURE 11.11**

Frequency dependence of induced transmembrane voltage  $V_M$  for various cell array lengths. As predicted by the cell array model, there is a substantial increase in transmembrane voltage as array length  $L$  increases, but at significantly lower frequencies vs. that for a single molecule or cell, reflecting the increased propagation time (low pass filter behavior) for longer array lengths.

The presence of gap junctions in the cells of an organized or organizing (repairing) tissue causes the induced transmembrane voltage to be substantially higher than that for the same cell in isolation for the same applied EMF. The frequency range in which increased  $V_M$  occurs vs. that for a single isolated cell is shifted toward a substantially lower range. This places different frequency requirements on the induced electric field waveform dependent upon whether the target is a macromolecule, single cell, or tissue. As array length increases beyond 1 mm, the rate of increase in  $V_M$  diminishes because of the dissipation of intracellular current via  $R_M$ . In the case of myelinated nerve axons,  $R_M$  is substantially higher and array lengths above 1 cm can provide further significant increases in  $V_M$  [363].

The effect of gap junction impedance on signal amplification resulting from the cell array structure may be assessed by choosing physically meaningful values for  $R_g$  corresponding to the state of conductivity of the gap junction. When the gap junction allows macromolecules, as well as ions to pass freely ( $R_g \rightarrow 0$ ), its resistance is negligible compared to  $R_i$  and  $R_M$  [299]. This represents a realistic lower limit for  $R_g$ . When the gap junction is completely closed, its resistance can increase to that of an insulating membrane ( $R_g \rightarrow 10^{15} \Omega/\text{m}$  for an artificial lipid bilayer, nearly a perfect insulator). Under these conditions, all cells in the array are still physically connected, i.e., the long antenna still exists, but has much higher dc resistance. The maximum value of  $R_g$  for a cell with a typical membrane resistance of  $R_M = 10^5 \Omega\text{-m}^2$  is of the order of  $10^{13} \Omega/\text{m}$ .

Quantitatively, the effect of  $R_g$  is assessed using Equation 11.24. The results are given in Figure 11.12, wherein  $Z_M (= 1/Y_M)$  is defined by Equation 11.26, for a 1 mm array. As may be seen, signal amplification decreases as  $R_g$  increases. However, it is important to note



**FIGURE 11.12**

Effect of gap junction impedance,  $R_g$ , on spatial amplification for cells at the end of a 1 mm array. An increase of many orders of magnitude in  $R_g$  has relatively little effect on the induced transmembrane voltage. When the value of  $R_g$  is equivalent to that of the transmembrane leak, a physiologically realistic limit, spatial amplification is reduced by only 10X. (From Pilla, A.A., Nasser, P.R., Kaufman, J.J., *Bioelectrochem. Bioenerg.*, 35, 63, 1994. With permission.)

that, even when  $R_g$  approaches the value of the membrane leak resistance ( $R_M$ ), i.e., increases by a factor of  $10^{13}$ ,  $V_M$  decreases only by approximately one order. In fact, the array effect is negated, i.e.,  $V_M$  is reduced to that of a single cell, only when  $R_g$  is significantly larger than the membrane leak resistance, a physiologically unrealistic limiting condition. These results strongly suggest that tissue structures, in which the cellular junctions are physically intact may always be significantly more sensitive than single cells to weak EMF, even though the resistance of the gap junction may change by several orders of magnitude throughout the functional cycles of the living cell.

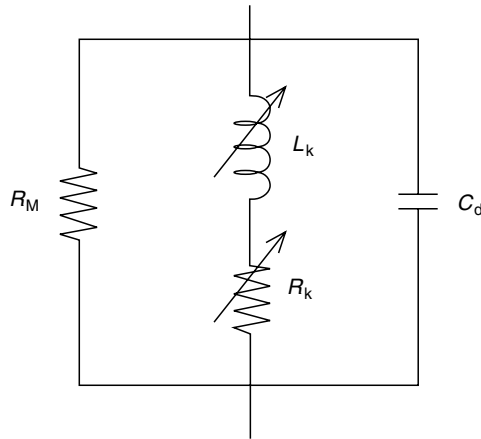
Examination of Equation 11.24 shows reasoning similar to that given above will hold for the effects of increasing intracellular resistance,  $R_i$ . It is also interesting to consider the effect of increasing transmembrane leak resistance,  $R_M$ . This is equivalent to increasing the insulating properties of the membrane, an increase of which would allow the signal to propagate a further distance along the array before substantial leakage into the extracellular compartment. An example of such an array is the nerve axon. The myelin sheath that covers the outer cell membrane surface has very high resistance, which allows electrical signals to propagate tens of centimeters before the advantage of array length becomes negligible [363].

The effect of gap junctions upon EMF response for cells in a culture dish has been reported [318]. This study showed alkaline phosphatase activity increased in an osteosarcoma cell line only when cells were electrically connected via gap junctions. The signal employed was 30 Hz sinusoidal at 1.8 mT peak-to-peak amplitude. Interestingly, in the absence of gap junctions, cell division was inhibited for the same signal suggesting the magnetic component of this signal had a different biological effect. Another study [319] found that magnetic fields over a frequency range from 30 to 120 Hz and field intensities up to 12.5 G decreased gap junction intercellular communication in preosteoblastic cells during their proliferative phase of development in a dose-dependent manner. Identical exposure conditions did not affect gap junction communication in well-differentiated osteoblastic cell line and when the preosteoblastic cells were more differentiated. The authors conclude this signal may affect only less differentiated or preosteoblasts and not fully differentiated osteoblasts. Consequently, EMFs may aid in the repair of bone by effects exerted only on osteoprogenitor or preosteoblasts. This is supported by the vast clinical experience that fully differentiated and remodeled bone is not affected in a physiologically significant manner by the same EMF signal that accelerates a repairing fracture, as well as by many cellular studies. This will be reviewed in a later section.

### 11.3.11 Resonance with Electric Field Signals

As for magnetic field bioeffects, resonance is one manner by which a cellular target can have increased sensitivity to an induced electric field over a restricted frequency range. This will depend upon the structure of the membrane impedance, as can be illustrated by adding a voltage-dependent Hodgkin–Huxley  $K^+$ -conduction pathway [320–324] to the simple membrane. Because the conductance is related to the  $K^+$  pathway resistance,  $R_n$ , by  $g_n = 1/R_n$  and the time constant  $\tau_n$  has the properties of an inductance, i.e.,  $\tau_n = L_n/R_n$ , the  $K^+$ -conduction pathway may be described via the electric circuit analog shown in Figure 11.13. The corresponding voltage-dependent admittance,  $YK(V)$ , written in terms of the analogous electrical circuit components for the membrane and the  $K^+$  pathway, is thus

$$YK(V) = \frac{1}{R_M} + i\omega C_d + \frac{g_n}{1 + i\omega\tau_n} \quad (11.27)$$



**FIGURE 11.13**

Potassium conductance is described in Hodgkin–Huxley formulation via addition of resistance–inductance branch to the simple membrane model.  $R_k$  and  $L_k$  are the equivalent voltage-dependent (arrows) resistance and inductance representing the voltage-dependent kinetics of  $K^+$  transport across the cell membrane. The characteristics of the time constant  $\tau_n = L_k/R_k$  of the inductive branch lead to resonance-type frequency response of cell array in a low-frequency range. (From Muehsam, D.S. and Pilla, A.A., *Bioelectrochem. Bioenerg.*, 48, 35, 1999. With permission.)

where  $g_n$  and  $\tau_n$  are the voltage-dependent  $K^+$  parameters given by Hodgkin and Huxley [320,321] and Fishman et. al. [321–324], and the other parameters represent the base membrane impedance.

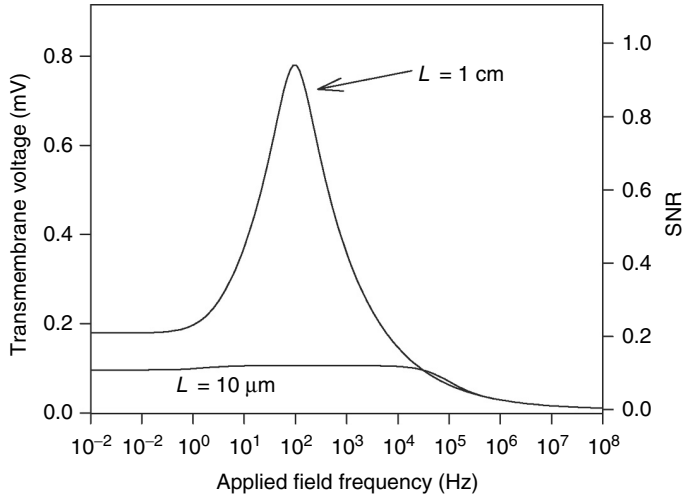
An electric circuit analog for the  $K^+$ -conduction pathway may be formed by associating the conductance  $g_n$  with a pathway resistance  $R_k$  via  $R_k = 1/g_n$  and time constant  $\tau_n$  to an inductance via  $\tau_n = L_k / R_k$  [310]. The corresponding admittance can be written as

$$YK(V) = \frac{1}{R_M} + i\omega C_d + \frac{1}{R_k + i\omega L_k} \quad (11.28)$$

The membrane model, thus, contains a series resistance–inductance pathway in parallel to the membrane resistance and capacitance pathway as shown in Figure 11.13.

The effect of the  $K^+$  conduction membrane pathway on the frequency response of the cell array may be illustrated by holding the membrane-resting potential constant (voltage clamp conditions). Using reported values for  $R_k$  and  $L_k$  in the Hodgkin–Huxley formulation, the addition of a series resistance–inductance pathway to the membrane model results in a local minimum in the admittance at low frequencies. The frequency response is shown in Figure 11.14 wherein  $V_M$  reaches a peak at approximately 16 Hz, for a flat input ( $E(\omega) = 10$  mV/cm, a typical therapeutic PEMF) and with the membrane voltage clamped at 40 mV. The introduction of a linear inductive element, according to the Hodgkin–Huxley formulation, to the membrane model produces a broad resonance response to applied fields and thus increases sensitivity to induced electric fields having frequency components in the low-frequency range of this resonance. Note, however, that resonance is only significant for long cells or cells arrays, not for single cells.

Thus, in addition to resonances that may occur via Larmor precession for applied magnetic fields, there is also the possibility of a resonance due simply to the electrical characteristics of the target pathway. In the example given, the presence of a resonance-type response due to  $K^+$  membrane transport depends upon the initial state of membrane polarization. The stimulus to the membrane under these conditions is well below



**FIGURE 11.14**

Response of cell array with  $K^+$  conductance membrane model to 10 mV/cm electric field at a given transmembrane-resting voltage. Frequency response of array exhibits a wide resonance response for applied field frequencies in the 16 Hz range.  $K^+$  conductance at a given voltage is described in the Hodgkin–Huxley formulation as a series resistance–inductance branch in the membrane model. Note the broad resonance at frequencies in the 10–100 Hz range. SNR was calculated according to the method described in Section 11.3.11. Note that resonance is not significant for a single cell, only for a long cell or cell array.

excitatory threshold and the primary response is  $K^+$  membrane transport for which the equivalent electric circuit contains an inductor. This, coupled with the normal capacitive properties of the cell membrane naturally leads to resonance behavior.

### 11.3.12 Signal to Thermal Noise

In order for an induced electric field to modulate a cellular target, the first requirement is that it be detected by the target. This means the waveform must be configured to produce sufficient voltage at, e.g., a binding site to significantly affect the  $t_{\text{free-to-}t_{\text{bound}}}$  ratio for the binding ligand as defined in Equation 11.18 (Section 11.3.8.1). There are several sources of transmembrane noise in biological membranes. The most common are due to thermal, flicker ( $1/f$ ), shot, and conductance fluctuations [325]. The latter three usually relate to ion transport and their interpretation is model dependent. Thermal noise is present in all voltage-dependent membrane processes and represents the minimum requirement to establish adequate SNR, a front-line measure of detectability. SNR can be evaluated, assuming only the presence of thermal noise (no signal processing or other enhancements which are model dependent) via [326]

$$\text{SNR} = \frac{|V_{\text{target}}(\omega)|}{\text{RMS}_{\text{noise}}} \quad (11.29)$$

where  $|V_{\text{target}}(\omega)|$  is the maximum amplitude of the induced voltage in the target pathway and  $\text{RMS}_{\text{noise}}$  is the root mean square of the noise voltage

$$\text{RMS}_{\text{noise}} = \left[ 4kT \int_{\omega_1}^{\omega_2} \text{Re}Z_{\text{target}}(x, \omega) d\omega \right]^{1/2} \quad (11.30)$$

where represents the real part of the total impedance of the target  $Z_{\text{target}}$  and the limits of integration ( $\omega_1, \omega_2$ ) are determined by the bandpass of the target, typically  $10^{-2}$ – $10^7$  rad/s.

SNR, as defined above, can be a powerful indicator of whether a given EMF signal can be expected to have a physiologically significant effect. To illustrate, consider a target in which the pathway is Ca–CaM-dependent. This includes most growth factors in every stage of bone and wound repair [69–83,146,147,151–153,], as well as nitric oxide synthase (NOS) that modulates NO, a signaling molecule in many neurological and cardiac pathologies and in bone and cartilage repair [327,328,388–392]. To evaluate SNR for this target, the quantity of interest is the effective voltage,  $E_b(\omega)$  induced across the equivalent binding capacitance,  $C_A$  (see Figure 11.4), which is directly proportional to  $[\text{Ca}^{2+}]$  as defined in Equation 11.18, and given by [329]

$$E_b(\omega) = \frac{X_C E(\omega)}{(R_A^2 + X_C^2)^{1/2}} \quad (11.31)$$

where  $X_C = 1/\omega C_A$  and  $R_A$  is the equivalent binding resistance. Equation 11.18 describes the relation between the frequency response of the target,  $E_b(\omega)$ , and applied field waveform  $E(\omega)$ , illustrating clearly EMF response is dependent upon applied waveform parameters.

SNR is evaluated using  $E_b(\omega)/\text{RMS}_{\text{noise}}$ ; however, the binding time constant  $\tau_A = R_A C_A = 1/k_b$ , must be known or be estimable. Consider a  $\text{Ca}^{2+}$ –CaM-dependent process, for which free  $[\text{Ca}^{2+}]$  concentration is the EMF-sensitive rate-limiting factor [90,92]. Linearized Michaelis–Menton kinetics describing  $\text{Ca}^{2+}$  binding to CaM is [90]

$$V_{\text{max}}/v = [1 + K_D/[\text{Ca}^{2+}]] \quad (11.32)$$

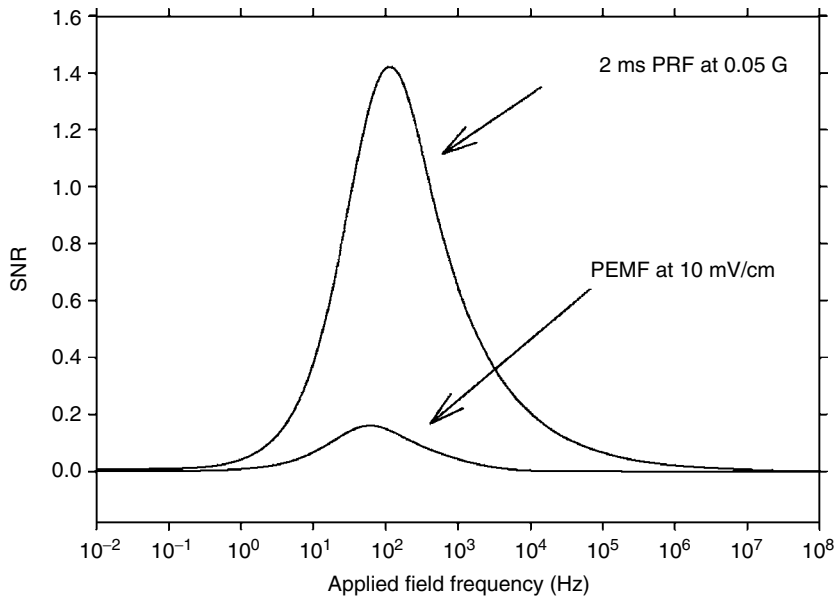
where  $V_{\text{max}}$  is the maximal reaction rate, i.e., the slope of the corresponding Lineweaver–Burke plot, the reaction velocity  $v$  is given by  $v = k_b [\text{Ca}^{2+}\text{CaM}]$  and  $K_D$  is the equilibrium constant.

The Michaelis–Menton relation thus determines  $k_b$ , i.e., defines the binding time constant for use in Equation 11.27

$$k_b = \frac{V_{\text{max}}}{[\text{Ca}^{2+}\text{CaM}](1 + K_D/[\text{Ca}^{2+}])} \quad (11.33)$$

Employing numerical values for which EMF sensitivity has been reported,  $V_{\text{max}} = 10^{-6}$ – $10^{-7}$  per s,  $[\text{Ca}^{2+}] = 1$ – $3 \mu\text{M}$ ,  $K_D = 20$ – $40 \mu\text{M}$ ,  $[\text{Ca}^{2+}\text{CaM}] = K_D([\text{Ca}^{2+}] + [\text{CaM}])$ , yields  $\tau_A$  between 1 and 5 ms [90].

Evaluation of SNR for  $\text{Ca}^{2+}$  binding in the manner outlined above may be performed for molecular, cellular, or tissue targets. An interesting example is wound repair. A common model is the full thickness linear incision performed through the skin down to the fascia on the dorsum of adult Sprague Dawley rats [331]. Acceleration of wound repair is assessed by tensile strength measurements at 21 days postoperative. At this time point untreated (control) strength is approximately one-third of that of the fully healed wound. One study used the PEMF signal commonly employed for bone repair (Figure 11.2, top) and reported no effect [331]. A second, more recent study, used a PRF signal specifically configured to enhance  $\text{Ca}^{2+}$  binding to CaM with the specific goal of enhancing growth factor release and reported a 59% increase in tensile strength vs. controls at 21 days,  $p < .001$  [163,332]. SNR analysis for the signals used in these studies is shown in Figure 11.15. It is clear that the induced electric field produced by the PEMF bone repair



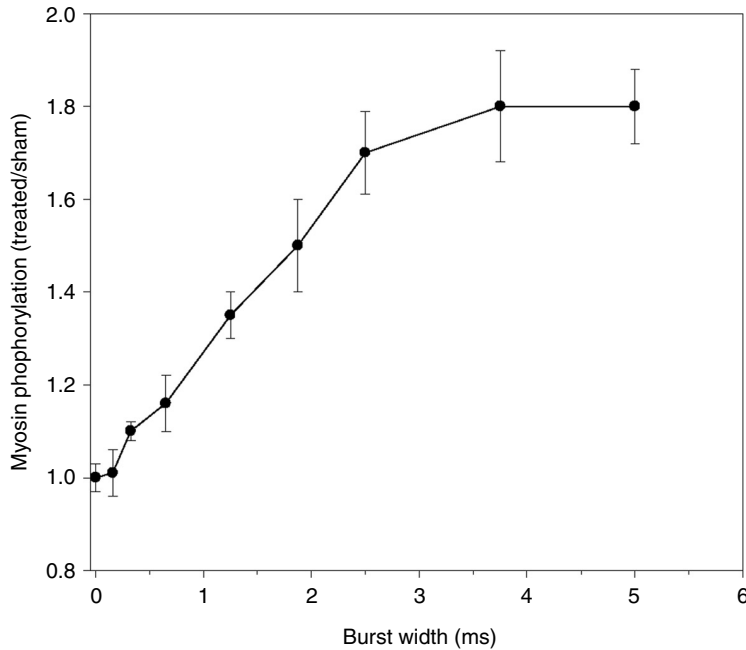
**FIGURE 11.15**

SNR in a Ca–CaM pathway for PEMF and PRF waveforms used in a rat cutaneous wound model. The asymmetrical repetitive pulse burst PEMF bone repair signal produced low (below detectability threshold) SNR and had no effect on wound repair. The 27.12 MHz repetitive sinusoidal burst produced sufficient SNR for detectability in the Ca–CaM pathway and enhanced tensile strength by 59% at 21 days.

signal, which consists of a 5 ms burst of bipolar pulses (200/20  $\mu$ s asymmetrical duration) repeating at 15 per s (see Figure 11.2, top), and inducing a gross peak electric field of 1 mV/cm ( $dB/dt = 10^6$  G/s), produced very low induced voltage across  $C_A$  (Figure 11.4) in the Ca–CaM pathway. The resultant SNR was below the detectability threshold. In contrast, the PRF signal which consisted of a 2 ms burst of 27.12 MHz sinusoidal waves repeating at 1 per s,  $dB/dt = 10^7$  G/s, produced a significantly larger induced voltage across  $C_A$  with a larger effect on  $Ca^{2+}$  binding. It is to be noted that increasing the gross induced electric field produced by the PEMF bone repair signal (Figure 11.2, top) to 50 mV/cm (5X) would have increased SNR by the same factor and allowed this signal to be equally detectable in the  $Ca^{2+}$  binding target pathway. Unfortunately, such an amplitude comparison was not performed in this study.

A recent study compared the effects of the PEMF bone repair signal utilized in the example above ( $dB/dt = 10^6$  G/s) with a 65  $\mu$ s burst of rectangular pulses of 4 and 12  $\mu$ s duration per polarity repeating at 1.5 bursts/s ( $dB/dt = 10^4$  G/s) on bone repair in a rat osteotomy model [369]. In this study the standard clinical bone repair PEMF signal produced a twofold increase in new woven bone and callus stiffness, whereas the 4/12  $\mu$ s signal was ineffective. SNR, calculated as above for each signal, assuming a Ca–CaM target pathway, reveals peak SNR > 1 for the clinical PEMF signal and peak SNR  $\ll$  1 for the 4/12  $\mu$ s signal. Note that modulation of the Ca–CaM pathway requires frequency components of sufficient amplitude in the  $10^2$ – $10^4$  Hz range and neither of these signals was configured accordingly.

Further support for the SNR and Dynamical Systems model for weak electric field bioeffects has been reported wherein bioeffective waveforms based upon the PRF signal were configured *a priori* for Ca–CaM-dependent myosin phosphorylation, neurite outgrowth from embryonic chick dorsal root ganglia and for bone repair in a rabbit model [29,30]. Growth factor production in the latter two pathways is Ca–CaM-dependent



**FIGURE 11.16**

PRF signal modulates  $\text{Ca}^{2+}$  binding kinetics (CaM) with increased effect vs. burst duration at constant amplitude (0.05 G) and repetition rate (1 per s). SNR analysis predicted a dose dependence on burst width, with a plateau at approximately 3 ms, in good agreement with experiment. The number of frequency components having detectable amplitude at the  $\text{Ca}^{2+}$  binding time constant increases with burst duration, thereby increasing SNR.

[81,336]. To illustrate signal prediction, PRF modulation of Ca–CaM-dependent myosin phosphorylation is reviewed here. Specifically, SNR was evaluated for the Ca–CaM pathway for burst durations in the 0.1–5 ms range with constant burst repetition (1 per s) and peak amplitude (0.05 G), and compared to experiment. Recall that the clinical PRF signal consists of repetitive bursts of a 27.12 MHz sinusoidal wave. SNR analysis showed peak SNR > 1 only for burst durations above 0.3 ms, with a plateau at approximately 3 ms. The experimental results shown in Figure 11.16 suggest no significant PRF effect on myosin phosphorylation for burst durations below 0.5 ms and no further significant effect beyond approximately 4 ms, in good agreement with the SNR model.

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## 11.4 Ultrasound for Tissue Repair

As indicated in earlier sections, connective and endothelial tissues, particularly bone, adapt to the mechanical environment by remodeling to accommodate the magnitude and direction of the applied stress [373,374]. Mechanical signals exist in functionally loaded bone [375] and represent strong regulatory signals to skeletal tissue [378], even during fracture healing [377]. Indeed, controlled movement during fracture repair has been shown to enhance healing [378]. There is also considerable evidence that low-intensity US (<100 mW/cm<sup>2</sup> Spatial Average Temporal Average, SATA) can accelerate healing of



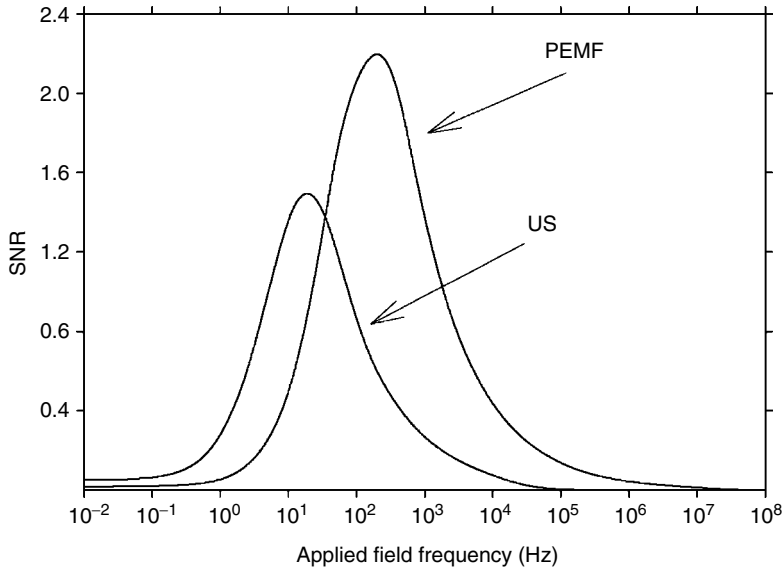
fresh fractures and established nonunions of bone [379]. Indeed, US stimulation is now part of the standard armamentarium of the orthopedist. In addition, basic studies have demonstrated that US can modulate each of the three key stages of the healing process (inflammation, repair, and remodeling) because it can enhance angiogenic [382], chondrogenic [381], and osteogenic [382] activity as for PEMF signals.

Although it is well known that many cells have mechanoreceptors allowing direct response to a mechanical signal [383], a direct electrical effect, linking US and PEMF signals, has been proposed [199–201]. The absorption of US in a tissue target gives rise to the phenomenon of microstreaming, i.e., the movement of fluid across surfaces. If the fluid contains ions and the surfaces are charged (cell membranes), streaming causes ions in the diffuse electrical double layer to be displaced from their equilibrium or resting positions and the electrokinetic phenomenon of streaming potentials occurs. This could be termed a mechanically induced electrophoretic effect. US and other mechanical inputs such as controlled weight bearing during fracture repair, walking, jumping, hitting a tennis ball, etc., cause rapid flow of fluids past cell surfaces thereby generating a time-varying, mechanically induced, electric field. Should this electric field have sufficient SNR in a proposed transduction pathway, it could act in a similar manner to exogenous PEMF.

The low-intensity US signal most commonly employed clinically is a 500  $\mu$  burst of 1.5 MHz sinusoidal acoustic waves repeating at 200 Hz and at an amplitude of 30–50 mW/cm<sup>2</sup> SATA. The sound pressure wave causes fluid flow during each burst, which, in turn, produces a repetitive time-varying voltage. This streaming potential has the form of a distorted trapezoid-like waveform with millisecond rise times and longer relaxation times, similar to the waveforms observed when bone is rapidly deformed. It has been reported that the clinical US signal produces 1–10 mV/cm peak electric field amplitude in tissue fluid [199]. This waveform may be analyzed using the SNR model given above, but it is necessary to define the target pathway.

Low-intensity pulsed US appears to act at the cellular level via biochemical pathways which are remarkably similar to those reported for EMF. Thus, US has been reported to induce a threefold increase in prostaglandin E2 (PGE2) production in murine osteoblasts through the upregulation of cyclooxygenase-2 (COX-2). COX-2 and PGE2 are known mediators in a bone forming response to external stimuli [384]. Pulsed US induced the transient expression of the early response gene *c-fos* and elevated mRNA levels for IGF-I in bone-marrow-derived stromal cells. Osteocalcin, bone sialoprotein, and bone matrix proteins were also modulated [385,386]. The US stimulation of primary rat chondrocytes elevated the intracellular concentration of Ca<sup>2+</sup>. Chelating or removing Ca<sup>2+</sup> from the medium inhibited the stimulatory effects of US on proteoglycan synthesis, suggesting US-stimulated synthesis of cell matrix proteoglycan, associated with accelerated fracture healing, is mediated by intracellular calcium signaling [387]. The US significantly increased VEGF mRNA. Early inhibition of nitric oxide (NO) production, but not calcium or PGE2, significantly reduced US-enhanced VEGF levels. Osteoblasts responded to US treatment by increasing NO production and NOS catalytic activities. Inhibition of NOS activity by *N*-nitro-*L*-arginine methyl ester (L-NAME) reduced VEGF levels [388].

The latter report is strong evidence that pulsed US acts via a pathway that is remarkably similar to that for PEMF. Indeed, PEMF has been reported to enhance proteoglycan synthesis in human cartilage cells via early stimulation of NO production [391]. NO is regulated by NOS, which, in turn, is activated by Ca–CaM or even by Ca<sup>2+</sup> binding directly to NOS [392]. This provides strong support for the validity of comparing SNR for the US and PEMF signals in the Ca<sup>2+</sup> binding pathway of CaM. The results are shown in [Figure 11.17](#), wherein it may be seen the PEMF clinical signal (see [Figure 11.2](#), top), as well as the streaming potential induced by the US signal, has sufficient amplitude to be detectable in the frequency range corresponding to Ca<sup>2+</sup> binding kinetics.



**FIGURE 11.17**

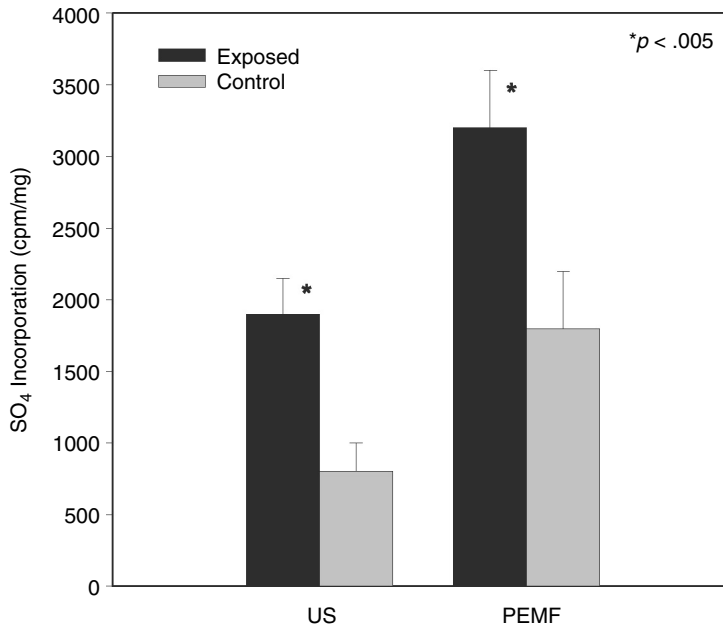
SNR in a Ca–CaM transduction pathway for a standard bone repair PEMF (see Figure 11.2, top) and the streaming potential from a pulsing US signal, also in use for bone repair (see text for details). These curves show there is sufficient SNR produced by both signals in a transduction pathway which may be common to both signals. SNR peak for the US signal is shifted toward the lower frequency range reflecting the lower frequency content of the mechanically induced time-varying electric field.

This analysis may be tested by examination of results reported in the literature for the effects of PEMF and US signals on identical cellular targets. This is illustrated in Figure 11.18, which shows both signals accelerated proteoglycan synthesis approximately twofold for chondrocytes in culture.

It is clear that both mechanical and EMF stimuli can modulate bone repair and affect a variety of cellular processes. The proposal that both modalities act through the EIT mechanism is reviewed here using the tools of the SNR and Dynamical Systems model. This may further help the reader to assess the myriad of signals and treatment modalities reported in the rapidly expanding literature in this area.

## 11.5 Effect of Initial Cell or Tissue State on EMF Sensitivity

Clinical experience, particularly in bone and wound repair, as well as numerous *in vitro* studies, suggests that the initial conditions of the EMF-sensitive target pathway determine whether a physiologically meaningful bioeffect can be achieved. Thus, surrounding normal bone tissue, which receives the same PEMF dose as a fracture site, does not respond in a physiologically significant manner, whereas fracture repair may be accelerated [123]. Local peripheral blood flow is not affected by EMF in a healthy subject, but is increased when a musculoskeletal injury or other exogenous stimulus is present [172]. Ca<sup>2+</sup> binding to CaM is modulated by EMF only under depleted Ca<sup>2+</sup> conditions [90]. Human fibroblasts in culture exhibit maximum increase in DNA synthesis with sinusoidal EMF during the S phase of the cell cycle [267]. Dendritic outgrowth in a nerve



**FIGURE 11.18**

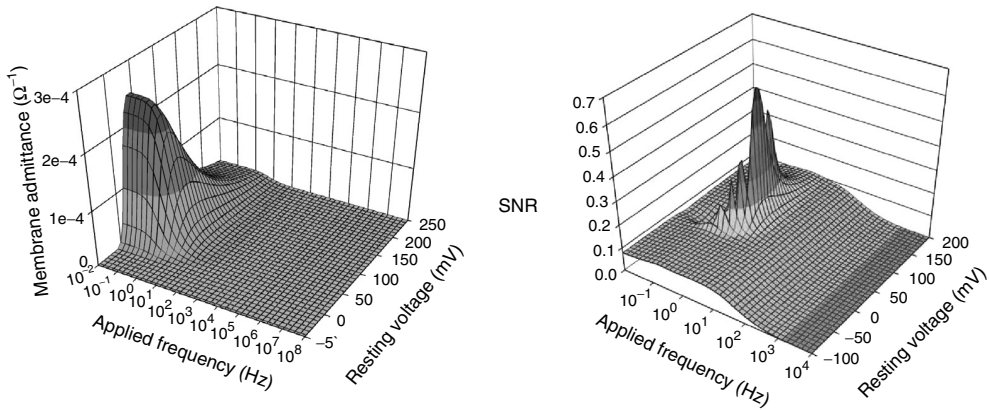
Comparison of the effects of US and PEMF signals employed clinically for bone repair on chondrocytes in culture. Both signals accelerated proteoglycan synthesis approximately twofold suggesting the transduction pathway may be similar or even identical.

regeneration model is modulated by EMF only in the presence of subsaturation concentrations of NGF [333–335]. PEMF effects on osteoblasts depend upon their maturation stage [337].

This behavior is consistent with the SNR and Dynamical Systems model because it is known that the electrical properties of a tissue target depend upon its initial state. Indeed, SNR calculations depend upon the electrical characteristics of the EMF-sensitive target which can be substantially different when the target is at rest or when reacting to a sudden change in its environment such as a fracture or other musculoskeletal injury [338]. Thus, for an identical EMF stimulus, the impedance of the EMF-sensitive pathway may alter sufficiently in the presence of an injury or pathology to enable a signal, which has otherwise been ignored (not detected), to be detected.

An extension of the SNR and Dynamical System model that examines the variation of the electrical characteristics of the voltage-dependent  $K^+$  conduction model (see Section 11.3.12) with membrane polarization has been proposed [339]. Both conductance  $g_n$  and time constant  $\tau_n$  (see Equation 11.24) are voltage dependent and resting or baseline transmembrane voltage can affect membrane impedance. The electrical characteristics of the target pathway can depend upon the membrane-resting potential, which, in turn, is defined by the baseline activity of the cell via, e.g., up- and down-regulation of receptors involved in the biochemical cascades relevant to baseline activity or response to injury.

The voltage-dependent characteristics of the Hodgkin–Huxley  $K^+$  conduction conductance  $g_n$  and time constant  $\tau_n$  produce the dependence of admittance upon membrane-resting potential and frequency shown in Figure 11.19, left-hand plot. For this example, the Hodgkin–Huxley values for the  $K^+$  conduction pathway are employed and the time constant  $\tau_n$  reaches a maximum at a membrane-resting potential of about 12 mV, corresponding to the  $K^+$  activation voltage. The admittance drops rapidly with increasing frequency, reaching its lowest value at  $\approx 100$  Hz.



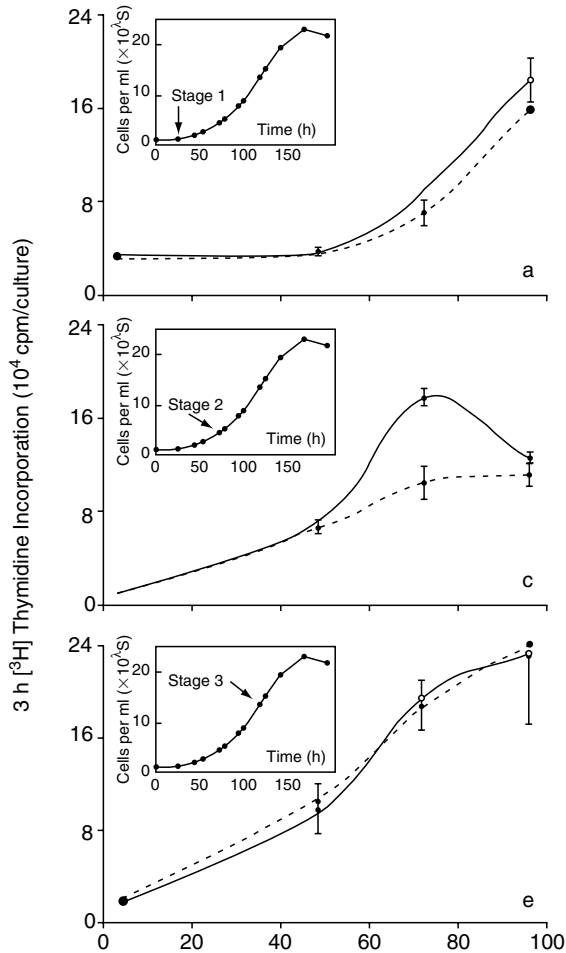
**FIGURE 11.19** (See color insert following page 412)

(Left) Membrane admittance depends upon frequency and membrane potential for Hodgkin–Huxley  $K^+$ -conduction pathway. Region of higher admittance (reduced impedance) corresponds to greater  $K^+$  flux across membrane and admittance drops rapidly with increasing frequency. (Right) Response of Hodgkin–Huxley  $K^+$ -conduction membrane pathway to exogenous electric field. Voltage induced in  $K^+$  pathway varies with membrane potential and frequency. Thus, EMF sensitivity depends upon the target initial state.

Figure 11.19, right-hand plot, shows the response to a flat input,  $E(\omega) = 10 \text{ mV/cm}$ , of a 1 mm (50–100 cells) cell array is determined by the membrane-resting voltage. In a manner similar to that shown in Figure 11.13 for the fixed-voltage  $K^+$  pathway, the voltage-dependent  $K^+$  pathway also exhibits a resonance-like response to applied field frequencies in the 16 Hz range. However, the width of the resonance frequency region changes with changing membrane-resting potential. For membrane-resting voltages less than approximately  $-10 \text{ mV}$ , the array responds equally to all frequencies below approximately 16 Hz. The admittance approaches zero for the depolarized membrane, so that for this condition the response of the  $K^+$  pathway is identical to that of the simple membrane ( $R_M, C_d$ ). Thus, as the membrane-resting potential increases to 40 mV or more, the array (target) begins to exhibit a preferential response, producing sufficient SNR only for applied field frequencies in the 16-Hz range.

An *in vitro* example of the effect of cell initial conditions upon EMF sensitivity is shown in Figure 11.20 [339]. Jurkat cells were exposed to the PEMF signal shown in Figure 11.2, top. The results show the PEMF signal could only achieve sufficient SNR to produce physiologically significant results in growth stage II. Cells in early and late log growth phase were insensitive to the identical PEMF signal. The authors also report PEMF amplified the antiproliferative effects of anti-CD3 (antibody to the T cell receptor). This was interpreted as a potential clinically significant anti-inflammatory property stemming from PEMF down-regulation of T cells that are activated at the T cell receptor during inflammation. In the absence of inflammation, T cell receptors are most likely not up-regulated, i.e., not EMF-sensitive and the identical PEMF signal could not achieve sufficient SNR to be detected in this pathway. In terms of the cell array model this translates to a substantially different electrical impedance at this pathway, dependent upon whether the T cell receptor is up- or down-regulated.

It may thus be seen the complete dosimetry picture for weak EMF modulation of tissue growth and repair clearly involves the changing electrical properties of the target with functional state. Realization of this may provide an explanation for the sometimes-elusive repeatability of EMF studies.



**FIGURE 11.20**

Illustration of the effect of cell growth stage (initial condition) upon EMF sensitivity. This study was performed on a lymphocyte cell line (Jurkat) and clearly demonstrates how an identical signal can have a large or no effect dependent upon the initial state of the cell. Pilla [50] interpreted these results as down-regulation, by EMF, of the T cell receptor that is apparently not upregulated in growth stages 1 or 3. (From Nindl, G., Swezb, J.A., Millera, J.M., Balcavage, W.X., *FEBS Lett.*, 414, 501, 1997. With permission.)

## 11.6 The Future

This chapter has attempted to provide the reader with enough information to show there is an abundance of experimental and clinical data demonstrating that exogenous EMFs of surprisingly low levels can have a profound effect on a large variety of biological systems. Both electrical and electromagnetic devices have been demonstrated to positively affect the healing process in fresh fractures, delayed and nonunions, osteotomies, and spine fusion in orthopedics and for chronic and acute wound repair. These clinical results have been validated by well-designed and statistically powered double-blind clinical trials and have survived meta-analyses. The FDA has approved labeling for these biophysical devices, limited at present to these indications. EMF stimulation technologies provide an additional arm to current treatment management strategies for these pathologies. However, the potential clinical applications of EMF therapeutics extend far beyond

those considered here and the clinical rewards are certain to be huge. Great strides have been made in the use of PEMF for chronic and acute wound repair. It is often the only effective treatment for chronic wounds and has been chosen as the treatment of choice for postoperative pain and edema reduction in plastic and reconstructive surgery. The advent of new more effective signals may even expand applications in bone repair. There is a significant emerging application for the treatment of osteoarthritis [61,356–358] and rheumatoid arthritis [369,370].

The state of knowledge in EMF therapeutics has significantly advanced in the past decade. The mechanism of action is much better understood, so much so it is often possible to configure, *a priori*, pulsing waveforms for an expected bioeffect. The example is PEMF and PRF signals have been successfully configured for a Ca–CaM ion-binding transduction pathway, which the biologists have established as a primary regulatory pathway. As we continue to learn to properly match dosimetry to pathology, the dreams that many of us had more than 35 years ago may well be realized. Cancer, cardiac muscle regeneration, diabetes, arthritis, and neurological disorders are just some of the pathologies that have already been shown to be responsive to EMF therapy. Successful applications of low-frequency EMFs have been reported for treatment of bronchial asthma, myocardial infarction, and venous and varicose ulcers. There is emerging research on EMF effects on angiogenesis and the manner in which this may increase stem cell survival in the treatment of Alzheimer's and Parkinson's diseases. There are also many studies that point to the possibility of the use of EMF for peripheral nerve regeneration [333–336,360].

There are numerous reports suggesting a role for EMF in the treatment of cancer. In 1979–1981 Larry Norton studied the effect of PEMF (using a 50 ms burst version of the PEMF signal in [Figure 11.2](#), top) on transplanted tumors in mice. Initial results suggested PEMF significantly increased survival time and led to preliminary clinical trials [341,342]. More recent studies on animals provide data that are reliable, and this certainly suggests that clinical applications on humans are possible in the near future [343–348,351,352,355].

EMF therapy modalities are simple, safe, and significantly less costly to the health care system. They offer the ability to treat the underlying pathology rather than simply the symptoms. The time is particularly opportune given the increased incidence of side effects from the use of pharmacological agents. EMF therapeutics will have a profound impact upon health and wellness and their costs worldwide.

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# 12

## *Therapeutic Heating Applications of Radio Frequency Energy*

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## 12.1 Introduction

The Bioelectromagnetics Society recognizes distinguished scientists in the field of bioelectromagnetics with its highest award, the d'Arsonval Award. This award honors a French scientist Jacques-Arsène d'Arsonval who pioneered research on the beneficial effects of radio frequency (RF) energy in the late-19th century and the beginning of the 20th century. Since the 1940s, diathermy ("through heat") has been used in rehabilitation medicine to relieve pain from sprains and strains. RF and ultrasound energy have been used for achieving deep-tissue heating to increase blood flow and collagen tissue extensibility, decrease joint stiffness, and muscle spasm [1]. In the mid-1970s, scientists and engineers started applying hyperthermia (40–45°C heating) in combination with ionizing radiation or chemotherapy to treat localized or metastasized cancer [2,3]. In the 1980s, RF ablation (50–100°C heating) was first used for cardiac arrhythmia and recently in the last ten years, for inducing local tumor necrosis [4,5]. Diathermy, hyperthermia, and ablation all use the same RF energy-heating characteristics to achieve different temperatures to treat various clinical conditions and diseases. Other applications using the propagating characteristics of the RF energy for medical purposes, such as RF telemetry to couple sound signals to implanted hearing devices (cochlear implant or middle-ear hearing device), are not discussed here [6,7].

In this chapter, RF heating mechanisms are first explained, followed by a discussion of the relationship between RF heating and temperature rise. Then, RF hyperthermia and ablation are emphasized and some other heating applications are briefly discussed. Clinical applications published since 1995 are selected as examples, but animal and laboratory studies as well as engineering developments are not included because of page limitation. Also, the use of RF energy frequency current less than 100 kHz that can be used for bone and wound healing, nerve regeneration, and other nonheating applications (wave propagation) are not in the scope of this chapter.

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## 12.2 RF Heating Mechanisms

Most of us have experienced shocks from touching leaky 50–60 Hz household appliances. The human body, although not a good conductor like metals, is not an insulator like plastic or rubber. As the frequency increases to 100 kHz, the electro-stimulatory effect gradually turns into a thermal sensation. Between 100 kHz and 5 MHz, it is still possible to sense the electro-stimulation effect if the RF energy is pulsed. At higher frequencies, many RF engineers have experienced RF burns. To understand how RF energy causes tissue heating, it is necessary to understand how the RF energy interacts with tissues. When RF energy is impinging on a human body, some energy is reflected at the body surface and some penetrates into the body, similar to sunlight shining on a lake. The amount of reflection and penetration depend on a property of the medium called the dielectric property as discussed in one of the previous chapters.

In general, biological tissues can be classified into two major categories. High water-content tissues, such as muscle, skin, kidney, and liver, have higher dielectric constants and conductivity than tissues with low water content, such as fat or bone. Brain, lung, and bone marrow tissues contain intermediate amounts of water and have dielectric constants and conductivities that fall between the values of the other two groups. In the RF range, tissues with high water content conduct electrical current well, because of the ions and the

polar water molecules. Tissues with low water content, such as fat and bone, have mostly bound charges that do not easily support conduction currents. Dielectric properties are a function of frequency. With increasing frequency, the dielectric constant decreases whereas the conductivity increases. Camelia Gabriel and colleagues [8] have published extensive dielectric property data for 43 tissues. A calculator for obtaining tissue dielectric constant and conductivity for 10–6000 MHz can be found on the following website from the FCC: <http://www.fcc.gov/fcc-bin/dielec.sh>.

The action of RF fields on tissues produces two types of effects: (1) the oscillation of free charges on ions and (2) the rotation of polar molecules at the frequency of the applied field [9]. Free charge motion loss is due to the electrical resistance of the medium. At radio frequencies below approximately 10 MHz, the alternating field induces a net movement of ions. Resistive (ohmic) losses associated with the induced current causes “joule heating.” This is the mechanism of RF ablation heating using <500 kHz RF current. At frequencies above about 100 MHz, the radiative mode of electromagnetic propagation and dielectric losses in tissue predominate over the conduction current losses. Under these conditions, heating results primarily from friction caused by mechanical interactions between adjacent polar water molecules, which are oscillating in an attempt to maintain alignment with the time-varying electric field. This is the primary heating mechanism of microwave applicators used in hyperthermia treatment of cancer as well as microwave ovens used to cook food. The resistive and friction losses are the basis of RF energy absorption, which produces heating in tissue [10,11].

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### 12.3 Temperature Rise Due to RF Heating

The rate of temperature rise (heating rate, HR) in tissue heated with RF energy is related to the rate of energy absorption (specific absorption rate, SAR) by Equation 12.1. The SAR is associated with the electrical field in the tissue and tissue conductivity as shown by Equation 12.2. The final tissue temperature is a function of the deposited energy, metabolic heating rate  $Q_m$ , power dissipation by thermal conduction  $k\nabla^2T$ , and blood flow  $c_b w(T - T_a)$ , as expressed in the Pennes equation (Equation 12.3).

$$HR = \frac{SAR}{69.77C_H(^{\circ}C/min)} \quad (12.1)$$

$$SAR = \frac{\sigma}{\rho} E^2 (W/kg) \quad (12.2)$$

$$\rho c_H \frac{dT}{dt} = k\nabla^2T + SAR + Q_m - c_b w(T - T_a) \quad (12.3)$$

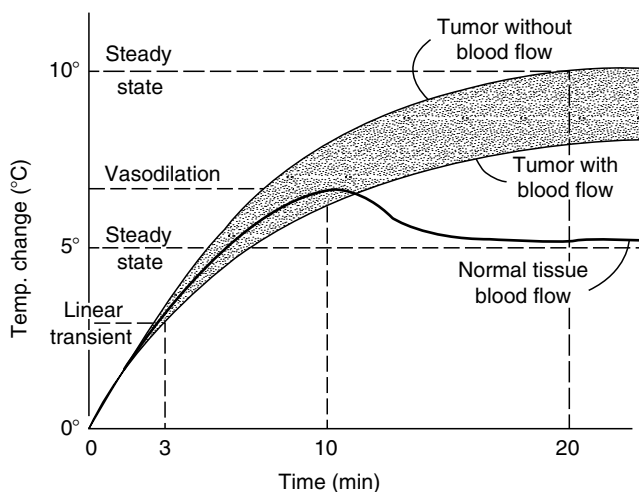
where HR is heating rate in tissue ( $^{\circ}C/min$ ); SAR is specific absorption rate (W/kg);  $c_H$  is the specific heat capacity of tissue (kcal/kg  $^{\circ}C$ );  $E$  is the root-mean-square value of the induced electric field strength in tissue (V/m);  $\rho$  is the density of tissue ( $kg/m^3$ );  $\sigma$  is the dielectric conductivity of tissue (siemens/m);  $T$  is the tissue temperature ( $^{\circ}C$ );  $k$  is the thermal conductivity of tissue (W/m  $^{\circ}C$ );  $Q_m$  is the metabolic heat generation (W/kg);  $c_b$  is the blood heat capacity (kcal/kg  $^{\circ}C$ );  $w$  is the blood perfusion rate ( $kg/m^3$  sec); and  $T_a$  is the temperature of incoming arterial blood ( $^{\circ}C$ ).

In a typical clinical treatment of soft-tissue tumors using about 50 W output power of an external applicator, the temperature,  $T$ , will increase as shown in Figure 12.1. Initially,

there is a linear increase lasting approximately 3 min. In normal tissue, after this linear increase in temperature, there is a period of nonlinear temperature rise usually lasting another 7–10 min, where  $T$  becomes high in dissipating the absorbed energy through heat diffusion. In tissues with a low or negligible blood flow, the temperature will monotonically approach a steady state value dictated by the magnitude of SAR, as shown on the upper curve in Figure 12.1, with equilibrium reached when  $SAR = -k\nabla^2 T$  (heat diffusion). For vascular tissues, a marked increase in blood flow will occur due to vasodilatation when the temperature reaches the range of 42 to 44°C, as shown in Figure 12.1, when  $SAR = -k\nabla^2 T + c_b w(T - T_a)$  (heat diffusion and blood flow cooling). This characteristic is used in diathermy treatment to increase blood flow to the treated area.

During hyperthermia treatment, the blood flow in many tumors is vigorous at the periphery and sluggish in the center [12]. Because the blood vessels in tumors are often fully open during normal conditions, there is, in many cases, no vasodilatation during the heat treatment. After steady state conditions are reached, the final temperature of the tumor is higher than that of the surrounding normal tissue. The shaded area in Figure 12.1 indicates the range of temperature rise in tumors. The lower boundary is for the periphery of the tumor and the upper curve is for the center, where no blood flow exists.

The rate of energy absorption, SAR, must be sufficiently high so that a therapeutic temperature level can be maintained over the major portion of the treatment period. If too little power is applied, the period or the level of temperature elevation will not be long enough or high enough for any benefit, whereas with too high a level, the temperature of the normal tissue may not be maintained at a safe level by vasodilatation. For diathermy treatment, the pain sensors are a reliable and sensitive means for alerting the patient of an unsafe temperature. If the applied power level is set so that only mild pain or discomfort is experienced by the patient, vasodilatation in normally vascular tissue will be sufficient to limit or even lower the temperature to a level that is both tolerable and therapeutically effective. For RF ablation, SAR is very high to cause a large temperature rise (15–60°C) in a short time (<6 min) to destroy tissues. The short-duration heating makes the temperature rise directly proportional to SAR, before significant heat diffusion can take place.



**FIGURE 12.1**

Temperature change in tumor and normal tissues following application of RF energy. (Reprinted from Guy, A.W. and Chou, C.K., Physical aspects of localized heating by radio frequency waves. In *Hyperthermia in Cancer Therapy*, Storm K., Ed., Boston, G.K. Hall Publisher, 1982, p. 279. With permission.)

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## 12.4 Hyperthermia for Cancer Treatment

Cancer hyperthermia (HT) is a treatment in which the temperature of either local tissue or whole body is raised to a therapeutic level to eradicate tumors. Over the last three decades, much has been learned about the effects of heat on cells and the interactions between heat and ionizing radiation therapy (RT) and chemotherapeutic agents [2,3]. The scientific rationale for its use either alone or combined with other methods is multifactorial. Heat may be directly cytotoxic to tumor cells or complementary to the effects of ionizing radiation with regard to inhibition of potentially lethal damage and sublethal damage repair, cell-cycle sensitivity, and effects of hypoxia and nutrient deprivation. The synergism of RT and HT is accomplished by the thermal killing of hypoxic and S phase (DNA syntheses) cells, which are resistive to ionizing radiation alone. HT has been used in combination with chemotherapy because heating increases membrane permeability and the potency of some drugs. Increasing drug uptake into cells enhances the amount of DNA damage, inhibits repair of DNA damage, and can reverse drug resistance. Recent developments in gene therapy may also apply HT for targeted, localized induction of gene therapy using the heat shock promoter [14–17].

Although cell and animal studies provide strong biologic rationale for the clinical application of HT, implementation remains difficult. Numerous factors affect the results of HT. The foremost problem in HT, however, is the generation and control of heat in tumors. Current heating methods for whole body heating use hot wax, hot air, hot water suits, or infrared radiation, and for partial body heating, utilize ultrasound, heated blood, fluid perfusion, RF fields, or microwaves. The effective temperature range of HT is very narrow, only 42–45°C. At lower temperatures, the effect is minimal. At temperatures higher than 45°C, normal cells are damaged. Because of this narrow temperature range, the response is highly dependent on to what extent is the tumor tissue heated to a therapeutic level. The clinical use of HT has been hampered by a lack of adequate equipment to effectively deliver heat to deep-seated and even large superficial lesions and by a lack of thermometry that provides reliable information on heat distribution in the target tissues. In RF hyperthermia, the final temperature of tumors is mainly dependent on energy deposition. When RF heating methods are used, the energy deposition is a complex function of the frequency, intensity, and polarization of the applied fields, the applicator's size and geometry, as well as the size, depth, geometry, and dielectric property of the tumor [18]. The material, thickness, and construction of a cooling bolus also influence the amount of energy deposition [19]. Although there are tremendous technical difficulties, there are now eight randomized trials of HT in human cancer patients. The majority demonstrate a survival advantage with the addition of HT to RT. Dewhirst et al. [3] pointed out that as technology improves, the benefits of this form of therapy will only become more visible.

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## 12.5 Methods of RF Hyperthermia

The electromagnetic energy used in HT is usually classified by frequency as either RF or microwave energy. Strictly speaking, the RF spectrum is between 3 kHz and 300 GHz, but for hyperthermia it generally refers to frequencies below 300 MHz. Common RF frequencies are 13.56 and 27.12 MHz, which have been widely used in diathermy. Microwaves occupy the frequency band between 300 MHz and 300 GHz. The most commonly used

microwave frequencies in HT are 433, 915, and 2450 MHz, which are designated ISM (industrial, scientific, and medical) frequencies in the United States and Europe (433 MHz in Europe only). Frequencies higher than 2450 MHz have no practical value for HT due to their limited penetrations. At lower frequencies, field penetration is deeper but the applicator must be larger and focusing is difficult.

For superficial (2–5 cm deep) and localized heating, 915 MHz is suitable. To keep the skin below 43°C and minimize pain, a deionized-water cooling bolus is generally placed between the applicator and patient body, although air-cooled method can also be used. For the treatment of tumors deeper than 5 cm, more penetrating lower frequencies must be used to deliver RF energy to the tumor. Applying 5–30 MHz capacitively coupled RF current, heating can be induced in tumors deep inside the body by varying the electrode sizes to control the current density and therefore the heating pattern in patients. Because the highest current-density occurs at the electrodes, cooling boluses must be used to protect the skin. Since fat heats significantly because of its low specific heat and low dielectric constant, RF capacitive heating is applicable only for thin patients. To produce deep-focused heating, a phased array method has been used (Figure 12.2). The system consists of a ring array of antennas to treat tumors inside torsos or legs. Utilizing antennas with RF fields of varying amplitude and phase, due to constructive or destructive interferences, heating can be steered to the desired locations. Noninvasive temperature monitoring of this hyperthermia and magnetic resonance hybrid system has been validated in a heterogeneous phantom [20]. Details of RF heating methods for local, regional, and whole body hyperthermia are available [2,3,13,18–23].

It is impossible for a single piece of equipment to fulfill all of the clinical requirements for patient treatments. Depending on the location and vascularity of the tumor and adjacent tissues and the general physical condition of the patient, the HT practitioners should have the option of choosing the most appropriate equipment. HT is a complicated technique and should be applied only by well-trained individuals. Due to the complexity of RF energy coupling to human tumors, careful heating pattern studies should be performed on all exposure geometries and contingencies prior to treatment to assure the best treatment conditions for the patient. The clinical situation is complicated by the reality that HT in combination with high-energy RT cannot be repeated after the tumor receives a maximal dosage of ionizing radiation. Accurate thermometry is particularly important in all phases of clinical hyperthermia, especially when the patient is anesthetized.



**FIGURE 12.2**

BSD-2000/3D/MR Hyperthermia System, Combining 3D treatment therapy and MRI treatment monitoring, provides the capability to monitor changes in perfusion, temperature, necrosis, and chemistry. (Courtesy of BDS Medical, Salt Lake City, Utah.)

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## 12.6 Hyperthermia Clinical Phase III Trials

Dewhurst et al. [3] recently reviewed the results of major published Phase III trials on advanced disease and primarily superficial malignancies. The following sections are condensed from that review.

### 12.6.1 Breast Cancer

Five Phase III trials of breast cancer chest wall recurrence were combined in an international collaborative study [24]. Patients were randomized to either RT alone or RT with HT. In some respects, HT techniques differed between institutions but are well documented, including information concerning temperature distributions and thermal dosimetry. The five trials demonstrated a significant overall improvement in the complete response rate for patients receiving HT + RT compared with RT alone (59% in the former group and 41% in the latter with an odds ratio of 2.3 ([95% Confidence Interval, 1.4 to 3.8]). The greatest effect was observed in patients with recurrent lesions in previously irradiated areas where further irradiation was limited to low doses; however, survival advantages were not apparent.

### 12.6.2 Other Superficial Malignancies

In a Radiation Therapy Oncology Group (RTOG) study published in 1991 [25], 307 patients with tumors were treated with RT alone or with RT + HT. Approximately, half of the cases comprised head and neck tumors, one third were breast carcinoma (chest wall recurrences), and the remaining patients were a variety of superficial malignancies. Complete response rates were not significantly different between the two groups (RT + HT = 32%; RT alone = 30%); however, subgroup analysis revealed significant improvements in duration of local control in patients with tumors smaller than 3 cm and in those with breast or chest wall recurrences. For lesions larger than 3 cm there was no difference in outcome, which is reasonable since large tumors are more difficult to heat. This trial was also characterized by highly variable heating techniques and thermal dosimetry that led to the development of subsequent RTOG guidelines for performing hyperthermia [26].

### 12.6.3 Head and Neck Cancer

There are two randomized studies for this category. The first study randomized 65 patients to RT alone versus RT and HT [27]. Patients in stage III who received the combined treatment had a 58% complete response (CR) as compared with 20% in the RT-alone group. Similarly, patients in stage IV receiving combined treatment of RT and HT achieved a CR of 38% as compared with 7% for those receiving RT alone. There was no benefit for more than 90% of patients in stages I and II of the disease; these patients achieving a CR with either treatment. A second trial from Italy restricted evaluation to metastatic cervical lymph nodes [28]. This study randomized 41 patients with advanced local regional squamous cell carcinoma of the head and neck to treatment with either RT alone or RT combined with HT. The CR rate was 41% in the RT-alone arm versus 83% in the combined arm. Five-year survival was 0 in RT-alone patients and 53% in the RT + HT group. These statistically significant differences clearly indicate that HT is beneficial.



#### **12.6.4 Esophagus**

Two randomized studies demonstrated an advantage for the addition of HT to chemoradiotherapy or chemotherapy in the treatment of esophageal cancer [29,30]. In the first study, 53 patients with squamous cell carcinoma of the thoracic esophagus were randomized to preoperative HT, RT, and chemotherapy, compared with only chemoradiotherapy. Clinical CR and pathologic responses were significantly improved in the trimodality arm—pathologic CR of 26% combined in the trimodality group versus 8% in the only-chemoradiotherapy group. In a follow-up study, an additional 40 patients were treated with chemotherapy only (bleomycin and cisplatin) or combined with HT. Again, an improvement in histopathologic response was noted favoring the HT group (41% versus 19%).

#### **12.6.5 Malignant Melanoma**

Seventy patients with 134 metastatic or recurrent malignant melanoma lesions were randomized to receive RT or RT followed by HT [31]. There was a significant overall benefit for the addition of HT, with a two-year local control of 46% in the combined group compared with 28% for those receiving RT alone. Quality assurance was an issue in this trial, with only 14% of treatments achieving the protocol objective of 43°C for 60 min.

#### **12.6.6 Glioblastoma Multiforme**

Seventy-nine patients with glioblastoma multiforme were randomized following external beam RT and chemotherapy to receive brachytherapy alone or combined with HT [32]. Both time-to-tumor progression and survival were significantly improved for the HT patients as compared with those treated with brachytherapy alone. Two-year survivals in the two groups were 31 and 15%, respectively. Toxicity was slightly greater in the HT patients, with seven grade 3 toxicities reported as compared with one reported grade 3 toxicity in the brachytherapy alone group. Good heating was achieved in most patients, but no good correlation was evident between thermal dose and response.

#### **12.6.7 Carcinoma of the Cervix**

This study randomized 361 patients with locally advanced, previously untreated, pelvic tumors to receive RT alone or RT + HT [33]. The patients were approximately equally divided between rectal, bladder, and cervix carcinoma. Complete response rates were 39% after RT alone and 55% after RT + HT ( $p = <0.001$ ). The duration of local control was also significantly improved with RT + HT as compared with RT only. Most of the benefit appeared to occur in patients with cervical carcinoma, the CR rate following RT + HT was 83% compared with 57% after RT only. Three-year survival was 27% in the RT-only group of cervix cancer patients and 51% in the RT + HT group. The difference is statistically significant.

#### **12.6.8 Summary of Clinical Trials**

The results of the above-published Phase III trials are intriguing; apart from the RTOG trials, they all appear to demonstrate substantial benefits from the addition of HT to RT. All of the trials were associated with significant design and implementation problems including relatively small numbers of patients, inadequate thermal dosimetry information, thermal goals that were often not achieved, and control arms that may not have repre-

sented optimal standard therapy. Despite these difficulties, Dewhurst et al. [3] concluded that the generally positive character of these trials suggest the future of HT is quite promising and that technological advances may well lead the way to even more positive results.

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## 12.7 RF Ablation

Radio frequency ablation (RFA) is a modified electrocautery technique that destroys tissue by means of electric current. In surgery, high-frequency electric current is used to cauterize small blood vessels to stop bleeding. Since 1985, there have been numerous reports of the use of RF current as an alternative energy source for cardiac ablation for correcting arrhythmias [34]. RFA has evolved to become a method for creating thermally induced coagulation necrosis in tumors using either a percutaneous approach with image-guided or direct surgical placement of electrodes into tissues. The necrotic tumor becomes smaller and disappears over time to achieve ablation. Goldberg [4] and Rhim et al. [35] reviewed the principles and techniques of tumor ablation with RF energy. A review by Friedman et al. [5] summarized clinical applications for treating a host of malignant diseases throughout the body. Dr. Bradford Wood of the National Institute of Health and Dr. Nahum Goldberg of Harvard University are two leading scientists in this field. Most of the following materials are from their reviews [4,5] with some updates added. Readers who are interested in details can refer to their original articles.

### 12.7.1 RFA Methods

In contrast to hyperthermia, which elevates tumor temperature to less than 45°C, RFA applies RF electric fields (375–500 kHz) to produce ionic currents in tissue and cause resistive heating to at least 50°C. At 50–52°C, cells undergo coagulation necrosis in 4–6 min; for temperatures greater than 60°C, coagulation necrosis is within seconds. However, temperature greater than 100°C is avoided to prevent tissue boiling, vaporization, and carbonization. These phenomena cause impedance increases and result in reduced ablation. Therefore, the goal of RFA is to achieve and maintain a temperature of 50–100°C throughout the target volume.

Rhim et al. [35] collected essential technical tips for a successful RFA from five international experts. To introduce RF energy into tumor, electrodes are inserted at the center of the tumor. For adequate destruction of tumor tissue, the entire volume of a lesion must be subjected to cytotoxic temperatures (50–100°C for 4–6 min). However, the relatively slow thermal conduction from the electrode through the tissues increases the duration of application to 10–30 min. With single monopole electrodes, coagulated tissue diameters are limited to about 1.6 cm. Many investigators have explored and several commercial companies have manufactured new RFA devices that have increased heating efficacy. Goldberg [4] also reviewed a variety of methods for increasing coagulation volume with RFA. The techniques are briefly summarized below. In addition to an increase in the generator power to 150 W, expandable, multitined electrodes permit the deposition of this energy over a larger volume (e.g., see Figure 12.3). Temperature changes are monitored via embedded thermocouples to avoid overheating. Another method to prevent overheating is to directly measure impedance changes for power control. For single electrode application, a cooled electrode design can minimize carbonization and gas formation



**FIGURE 12.3**

The StarBurst Xli—Enhances RFA device incorporates controlled infusion of normal saline to promote complete ablation in soft tissue up to 7 cm in diameter. The device features real-time, multipoint temperature feedback system from the seven active arrays. (Courtesy of RTIA Medical Systems, Mountain View, California.)

around the needle tip by eliminating excess heat near the electrode, and therefore increases the treatment volume. Pulsing of RF energy has been also shown to be useful. For example, a combined approach that involves use of a multiprobe cluster of internally cooled electrodes with pulsing has also been claimed to create larger lesions than those achieved by any method alone [36]. These technological developments can be used to create an ablation lesion with a maximum diameter of 5 cm.

Although there are improvements from engineering and technical manipulations, it is still very difficult to achieve large lesions, because of the low tumor conductivity and the cooling effect from nearby blood vessels. Therefore other methods to modify the biology or tumor characteristics have also been investigated. These include (1) improved heat conduction by injection of saline and other compounds to increase tissue conductivity; (2) saline injection during RFA; (3) reduced blood flow by occlusion; and (4) drug modulated blood flow. Combination with chemotherapy or chemoembolization to improve tumor response has been shown to be more effective than RFA alone. For details of the RFA techniques, please refer to Goldberg [4] and Rhim et al. [35].

## **12.7.2 RFA Clinical Applications**

### **12.7.2.1 Liver Tumors**

Surgical resection is the only curative treatment for liver cancer patients but only 10–20% of patients with liver tumor have resectable disease [37]. For this reason, RFA has become a popular procedure [38]. Since this method was used 15 years ago [39,40], there has been extensive work on liver tumor RFA [41–43]. Studies of over 3000 RFA-treated patients have shown the efficacy of percutaneous RFA for small (<3 cm) liver tumors. Complete local response averaged 70–75% with tumors between 3 and 5 cm, and drops to 25% in larger tumors. With successful ablation, 5-year survival rates of 40–50% have been reported. This pattern of excellent local control for small liver tumors with significant long-term nonlocal recurrence is also true in RFA of hepatic metastases. Currently, there are no randomized prospective controlled studies comparing liver RFA to standard surgical resection in any population. Livraghi et al. [44] concluded that RFA is a relatively low-risk procedure for focal liver tumor treatment based on results with 3554 lesions.

### **12.7.2.2 Kidney**

Standard radical or partial nephrectomy may be excessively invasive for small kidney-tumors, and RFA can offer an alternative, minimally invasive treatment [45]. Similar to its use in treating liver tumor, RFA is most effective in treating kidney tumors less than 3 cm in diameter [46–50]. With increasing size, there is an increased risk of local recurrence. In general, the number of patients treated is relatively small with short follow-up. However, early short-term results suggest a 70–90% small kidney-tumor success rate.

### **12.7.2.3 Bone**

RFA has been used for more than ten years to treat osteoid osteoma, a benign, slow-growing painful lesion [51]. With RFA, a probe is placed through a bone-penetration cannula into the lesion and activated for 4–6 min at 90°C [52,53]. Success rates for a single ablation range from 91 to 94% with long-term follow-up, and most recurrences can be completely ablated in a second procedure [54]. Cioni et al. [55] did a follow-up of 38 patients for 12–66 months. Their results show primary and secondary clinical success rates were 78.9% (30/38 patients) and 97% (35/36 patients), respectively. Treatment of osteomas by RFA is highly effective, safe, and allows early return to function while minimizing morbidity.

### **12.7.2.4 Pain**

RFA may provide rapid pain relief (in hours to days) for patients with cancer pain resistant to conventional forms of palliation in comparison to the radiation therapy, which palliates over the course of 7–21 days [56]. Treatments of low back-pain [57–59], trigeminal neuralgia [60], and other neurolysis have just started. Currently, the National Institute of Health is conducting trials on painful soft-tissue neoplasms and painful bone tumors resistant to traditional techniques.

### **12.7.2.5 Lung**

For patients with inoperable lung cancer, percutaneous RFA under computer tomography (CT) guidance represents an alternative and minimally invasive treatment [61,62]; however, there are few studies [63–65] and the efficacy of lung cancer ablation is yet to be determined.

### **12.7.2.6 Breast**

Breast cancer RFA is also in the early stage of development [66–68]. The largest study to date has 26 patients with T1 or T2 breast tumors [67]. Coagulation necrosis was confirmed by histology in 96% of the patients following surgical lumpectomy. Several breast RFA clinical trials are underway that will help determine whether RFA may be an alternative breast-conserving treatment in the future.

### **12.7.2.7 Other Tumors**

Other potential applications of RFA include treatment of tumors in the adrenal [69] and prostate [70].

### **12.7.2.8 Miscellaneous Applications of RFA**

Li et al. [71] evaluated the effect of RF treatment of 22 patients' palates on speech, swallowing, taste, sleep, and snoring. After a mean follow-up of 14 months, no adverse effect was reported. The success of RF volumetric reduction of the palate diminishes with time, as with other palate surgical procedures. However, the minimal invasiveness of RF provided high patient-retreatment acceptance and snoring relapse improvement.

Hultcrantz and Ericsson [72] compared two techniques for pediatric tonsil surgery with respect to pain and postoperative morbidity. The two methods were the partial tonsil resection using RF technique versus traditional tonsillectomy. They concluded that RF appears to be a safe and reliable method for tonsil surgery with much less postoperative morbidity than regular tonsillectomy.

Islam et al. [73] described a study on six pediatric patients using RF treatment of the lower esophageal sphincter and showed it is a potentially successful modality to treat recurrent gastroesophageal reflux disease in children.

### **12.7.2.9 Summary of RFA Clinical Studies**

RFA has been studied for about ten years and initial results are promising. Multicenter trials, long-term follow-up studies, further technique refinements, and RFA combined with adjuvant therapies are all still in progress.

---

## **12.8 Other Applications**

### **12.8.1 Cosmetics**

Narins and Narins [74] used an RF device to induce tightening of skin via uniform volumetric heating into deep dermis, resulting in a “nonsurgical facelift.” Twenty treatment areas in 17 patients were treated to evaluate the efficacy and safety of RF treatment to the brow and jowls. The technique was reported to produce gradual skin tightening in most patients with no adverse effects.

Fitzpatrick et al. [75] studied 86 subjects who were evaluated for six months after treatment with an RF tissue-tightening device. A single treatment produced objective and subjective reductions in peri-orbital wrinkles and measurable changes in brow position. These changes were indicative of a thermally induced early tissue-tightening effect followed by additional tightening over a time course consistent with thermal wound-healing response.

Sadick and Shaoul [77] treated 40 adult patients (skin phenotypes II–V) with varied facial and nonfacial hair colors with a combination of laser and RF energy. Maximum hair reduction was observed at 6–8 weeks after each treatment. An average clearance of 75% was observed in all body locations at 18 months. No significant adverse sequelae were reported. They concluded that combined laser and RF energy with contact cooling is a safe and effective method of long-term hair reduction in patients of diversified skin types and varied hair colors.

Chess [76] evaluated laser and RF combination therapy on 25 patients with a total of 35 sites (0.3–5.0 mm vessel diameters). At 1 and 6 months after the final treatment, approximately 77% of treatment sites exhibited 75–100% vessel clearance, and 90% had 50–100% vessel clearance.

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## **12.9 Concluding Remarks**

Although there are controversies concerning potential health effects of low-level RF exposure, many beneficial effects using high-level RF energy to heat tissue or destroy tumors are utilized in clinics as shown by the examples in this chapter. Diathermy treatment has a long history as it has been used for more than half a century and millions of patients have benefited from treatment. More recent research on hyperthermia and ablation for cancer treatment has demonstrated the efficacy of RF heating. The literature

includes reports of the use of therapeutic RF heating devices as alternative techniques for surgery on the palate, tonsils, and esophagus. Other applications include cosmetic procedures. Complicated RF dosimetry makes the heat delivery an art. Interdisciplinary team work between medical, engineering, and physical science is vital to successful medical applications of RF technology.

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