Session C. Effects of Microwave Radiation at the Cellular and Molecular Level

Chairman: *E. H. Grant*
Vice-Chairman: *B. Servantie*
Rapporteurs: *K. H. Illinger and S. Szmigielski*

PRINCIPLES OF INTERACTION OF MICROWAVE FIELDS AT THE CELLULAR AND MOLECULAR LEVEL*

H. P. Schwan

Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania U.S.A.; Max Planck Institute of Biophysics, Frankfurt am Main, Federal Republic of Germany

The electrical properties of tissues and other biological systems do not only control the mode of propagation of electromagnetic energy in tissues. They also tell us how electromagnetic fields interact with biological systems at various levels of complexity. I shall first briefly summarize the rather advanced state of our present knowledge of such properties. Next I shall draw some conclusions about possible mechanisms which may or may not cause subtle non-thermal effects. No consideration will be given to magnetic properties since the latter are, for our purposes, identical to those of free space.

ELECTRIC PROPERTIES

The first figure indicates the dielectric behavior of practically all tissues. Three relaxation regions α, β, γ of the dielectric constant exist at low, medium and very high frequencies. The mechanisms responsible for these regions are indicated in Table 1.

Inhomogeneous structure is responsible for the β-dispersion, i.e., the polarization resulting from the charging interfaces, i.e., membranes through intra- and extracellular fluids (Maxwell-Wagner effect).

Rotation of molecules having a permanent dipole moment, such as water and proteins, is responsible for the γ-dispersion (water) and a small addition to the tail of

* This work was supported by NIH Grant HE-01253 and ONR Contract N00014-67-A--0216-0015.

Table 1

Electric relaxation mechanism

Inhomogeneous Structure (Maxwell-Wagner)	β
Permanent Dipole Rotation (Debye)	γ, β tail
Counter-Ion Relaxation Electrophoretic Relaxation	α

the β-dispersion resulting from a corresponding β_1-dispersion of proteins. The tissue proteins only slightly elevate the high frequency tail of the tissue's β dispersion since the addition of the β_1-effect caused by tissue proteins is small as compared to the Maxwell-Wagner effect and since it occurs at somewhat higher frequencies. Another contribution to the β-dispersion is caused by smaller subcellular structures, such as mitochondria, cell nuclei and other subcellular organelles. Since these structures are smaller in size than the surrounding cell, their relaxation frequency is higher, but their total dielectric increment is smaller. They therefore contribute another addition to the tail of the β-dispersion (β_1).

The γ-dispersion is solely due to water and its relaxational behavior at about 20 GHz. A minor additional relaxation (δ) between β and γ-dispersion is caused in part by rotation of amino acids, partial rotation of charged side groups of proteins and the relaxation of protein-bound water which occurs somewhere between 300 and 2000 MHz.

The α-dispersion is presently least clarified. Intracellular structures such as the tubular apparatus in muscle cells, which connect with the outer cell membranes could be responsible in all tissues which contain such cell structures. Relaxation of counter ions about the charged cellular surface is another mechanism suggested by us. Last but not least, relaxational behavior such as reported recently for the giant squid axon membrane can account for it. The relative contribution of the various mechanisms varies no doubt from one case to another and needs further elaboration.

Electrophoretic relaxation is the counterpart of the relaxation due to counter ion movement. It results from the oscillatory movement of charged particles with the alternating field. Its magnitude can be calculated and is sufficiently small to noticeably contribute to the other relaxation mechanism indicated in Table 1.

Gross structure

α — Excited membrane?
Intracellular structure?
β — Tissue structure (Maxwell-Wagner)
γ — Water

Fine strucutre

α_1 — Charge transfer (ion relaxation)
β_1 — Subcellular components, biologic macromolecules)
δ — Bound H_2O, side chain rotation, amino acids

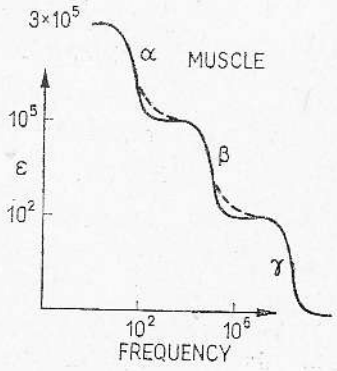

Fig. 1. Gross and fine structural relaxation contributions to the dielectric constant of muscle tissue. Dashed lines indicate fine structural contributions. The data and various structural contributions are typical for all tissues of high water content.

WHO – Biologic effects & health hazards of microwave radiation 1974

No attempt is made to summarize conductivity data. Conductivity increases similarly in several major steps symmetrical to the changes of the dielectric constant. These changes are in accord with the theoretical demand that the ratio of capacitance and conductance changes for each relaxation mechanism is given by its time constant or, in the case of distribution of time constants, by an appropriate average constant.

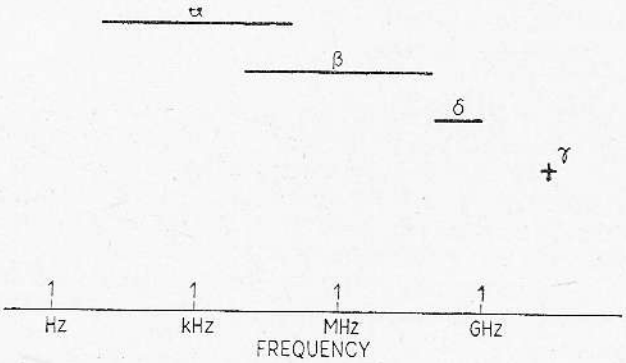

Fig. 2. Ranges of characteristic frequencies for various biological systems.

Figure 2 indicates the variability of the characteristic frequencies for the various mechanisms α, β, γ, and δ from one biological object to another. For example blood cells display a weak α-dispersion centered at about 2 kHz, while muscle displays a very strong one near 0.1 kHz. The β-dispersion of blood is near 3 MHz, that of muscle tissue near 0.1 MHz. Clearly there is considerable variation depending on cellular size and other factors. However the γ-dispersion is always sharply defined at the same frequency range and there may not be as strong a variation in the δ-effect as there is for the α- and β-dispersion frequencies.

Table 2

Dispersion characteristics of certain biologic materials

Electrolytes	γ
Biologic Macromolecules:	
Amino Acids	$\delta + \gamma$
Proteins	$\beta + \delta + \gamma$
Nucleic Acids	$\alpha + \beta + \delta + \gamma$
Cells, free of protein:	$\beta + \gamma$
Charged	$\alpha + \beta + \gamma$
Excited membranes	$\alpha + \beta + \gamma$

Table 2 attempts to summarize at what level of biological complexity the various mechanisms occur. Electrolytes display only the γ-dispersion characteristic of water. Biological macromolecules in water add to the water's γ-dispersion the δ-dispersion caused by bound water and rotating side groups. And proteins and nucleic acids in particular add further dispersions in the β and γ-range as indicated. Suspensions of

WHO – Biologic effects & health hazards of microwave radiation 1974

cells free of protein would display a Maxwell-Wagner β-dispersion and the γ one of water. If they contain protein an additional comparatively weak β-dispersion due to the polarity of protein is added and a δ-dispersion. If the cells carry a net charge an α-mechanism due to counter ion relaxation is added and if their membranes on their own relax, as some excitable membranes do, an additional α-mechanism appears.

SOME CONCLUSIONS

Obviously much is known about mechanisms responsible for the electric properties of biologic materials. In all cases where mathematical tools can be applied to fairly simple shapes, as in the case of spherical cells and even erythrocytes, the theoretic prediction is precisely in agreement with the experimental data.

In cases where the complexity of biologic structure exceeds mathematical power, at least semiquantitative agreement with approximating theories has been achieved. In any case, our present understanding is sufficient to warrant confidence in the various models chosen. This is by no means meant to imply that there are no more unresolved problems. As a matter of fact the following Table 3 attempts to summarize some of the work which has to be done in the future:

Table 3

Unresolved problems

Origin of δ-dispersion
Extendend theory of counter ion relaxation and applications to macromolecules and cells
Protein data vs. concentration (both β and δ); the same for amino acids and nucleic acids
Origin of α-dispersion in membranes (counter ion induction, related to excitation, internal structure or chemical relaxation?)

The precise origin of the δ-dispersion and the relative contribution of bound water and polar side chain rotation requires further clarification.

The theory of counter ion relaxation has so far been developed only under fairly restricting assumptions and further work is required to fully assess the contribution of counter ions to the low-frequency data.

There are still more data to be gathered for amino acids, proteins and nucleic acids, particularly as functions of concentration and field strength. Macromolecular interactions may affect dielectric data at typical biologic concentrations. There is need to better understanding of various relaxations typical of nucleic acids. There is a particular need to gather data as functions of field strength in order to find out where macromolecular dielectric saturation occurs. This is needed to assess the possibility that high field strength levels as may be applied with pulsed fields may go beyond saturation levels and perhaps cause denaturation, a possibility which is judged by the writer to be remote but not entirely impossible.

Last, but not least, the origin of the α-dispersion needs further elaboration. We should learn to assess the relative contributions of counter ion effects, dielectric effects in excitable membranes caused by time-dependent sodium and potassium and due to possible chemical relaxation effects in membranes, and dielectric effects due to intracellular membrane structures.

It is quite possible that such work may provide further insight into the various ways

in which electric fields of whatever frequency and magnitude may reversibly and irreversibly affect biological structures.

In the meantime the past work and the internal consistence achieved can be summarized in some fairly simple conclusions. The electric data are entirely consistent with biologic matter consisting of membranes surrounding and being surrounded by intracellular and extracellular fluids which contain macromolecules. There is no doubt that the large part of the water contained is "free" since both dielectric data and conductivities at microwave frequencies are essentially those of electrolytes containing biopolymers and the characteristic frequencies in the γ-range are identical for tissues and free water. From this well supported picture emerge the following conclusions of interest here:

1. At frequencies above those characteristic of the α-dispersion all biological membranes have a fairly frequency-independent capacity of about 1 μF/cm^2, with the precise value varying between 0.5 and 1.5 μF/cm^2. This capacitance range which is presently unchallenged corresponds, say at 3 GHz, to an impedance of about 50 microohms. For a current density of about 3 mA/cm^2, i.e., a current density which corresponds to a flux of 10 mW/cm^2 in tissues of a typical 3 GHz resistivity of 100 to 200 ohm-cm, an alternating membrane potential of only 0.15 μV is induced. This value is easily 1000-fold, if not 10 000-fold, smaller than potentials which are reported to cause effects on excitable membranes.

2. All the properties reported above are "linear" ones in the range of potentials at which they were studied, i.e., the reported dielectric constants and conductivities are independent of field strength at field strength levels of interest at this meeting. Membrane data change at first at field strength levels which almost compare with those corresponding to a typical resting potential of 70 mV, i.e., at imposed field levels of the order of 100 kV/cm. Biological macromolecules, judging from the restricted body of data available, are linear up to field levels of many kV/cm. This means that the energies which are imparted by thermal collisions are far greater than those which can be applied with fields of the order of several V/cm. The present knowledge therefore does not indicate any possibility of denaturation by the field strength levels of interest here. Moreover, the characteristic frequencies quoted are smaller than those of microwaves, making it thereby unlikely for biopolymers and membranes to respond to microwave frequencies.

3. The electric data known so far are without exception a reflection of relaxation effects, and there is no indication of any resonance behavior. For example the data on biopolymers are consistent with those of polar molecules surrounded by a medium which has the viscosity of water, i.e., a viscosity high enough to preclude any resonance type of behavior. The same is true for the membrane data so far reported. The abundant data therefore do not lend support to the concept that microwaves may induce resonance effects which may somehow denature and cause irreversible damage.

FORCES CAUSED BY EM-FIELDS

While the above-discussed electric properties provide much insight into the mode of interaction of alternating electric fields, they cannot reveal readily whatever mechanic effects may be imparted. It has been known for quite some time that not only DC fields but also alternating ones can evoke forces which may or may not be significant. The study of the manifestations of these forces has been of considerable interest over a number of years in our laboratory. The well-known pearl chain formation effect and the orientation of nonspheric particles are some of the effects which have been studied (Fig. 3).

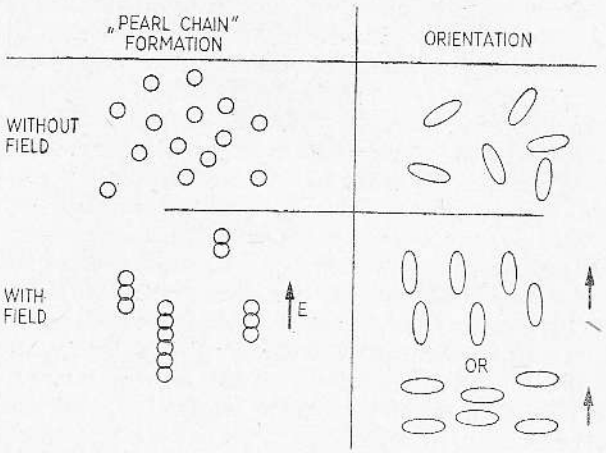

Fig. 3. Schematic presentation of some effects of alternating electric fields on particle and cellular arrangements.

In general it may be said that such forces become significant in comparison with random thermal ones if the ratio of the electric potential energy of the system considered or its change as the observed effect takes place is larger than the thermal energy. This ratio is proportional to the expression given in Figure 4 and the proportionality

$$\frac{L(P)}{L(T)} \sim \left|\frac{\varepsilon_p^* - \varepsilon_a^*}{\varepsilon_p^* + 2\varepsilon_a^*}\right|^2 \frac{R^3 E^2}{KT} \varepsilon_p$$

T ABS. TEMPERATURE
E „DISTANT" FIELD STRENGTH
R RADIUS
K BOLTZMAN CONSTANT

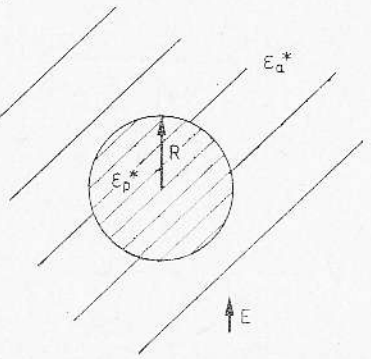

Fig. 4. Ratio of electric potential energy L(P) to thermal energy L(T) of a particle of a complex dielectric constant E_p^* and radius R in a medium of complex dielectric constant E_a^* and exposed to a field E.

constant depends on the particular phenomena considered. For example in the case of the movement of a particle in an inhomogeneous field, termed "dielectrophoresis" by Pohl, the proportionality factor characterizes the inhomogeneity of the field and the force generated is given by

$$F = 2\pi R^3 \varepsilon_p \frac{\varepsilon_p^* - \varepsilon_a^*}{\varepsilon_p^* + 2\varepsilon_a^*} \cdot \text{grad}\,(E_a^2)$$

WHO – Biologic effects & health hazards of microwave radiation 1974

It is apparent from the expressions given that the threshold field strength to evoke such effects as mentioned changes inversely with the square root of the volume of the particle exposed. Indeed, typical cellular dimensions of the order of some μm require field values of several hundred volt/cm, i.e., values which have been reported in the literature for erythrocyte pearl chain formation, *E. coli* orientation, etc. On the other hand large unicellular organisms have recently been subject to investigation in our laboratory and respond dramatically if the field strength is only of the order of 1 V/cm. It is therefore indicated that forces which are caused by alternating fields are not likely to be significant at the molecular and microscopic cellular level unless huge field strength values are assumed, values which are so large that the accompanying thermal effects would be overwhelming. However, on a macroscopic level these effects may well be significant. We have pointed out before on several occasions that the phenomenon of hearing pulsed microwave fields may well be explained by the forces rhythmically applied to the middle ear structures as the field is turned on and off. Since only the strength of the applied field dictates the force generated, the observation by several investigators that the peak power is the important parameter is readily unterstood. This observations is in no contradiction to our statement that for "steady state" effects, such as pearl chain formation or orientation, the effect of a pulsed field can at most equal that of a continuous field of the same average power (see Fig. 5).

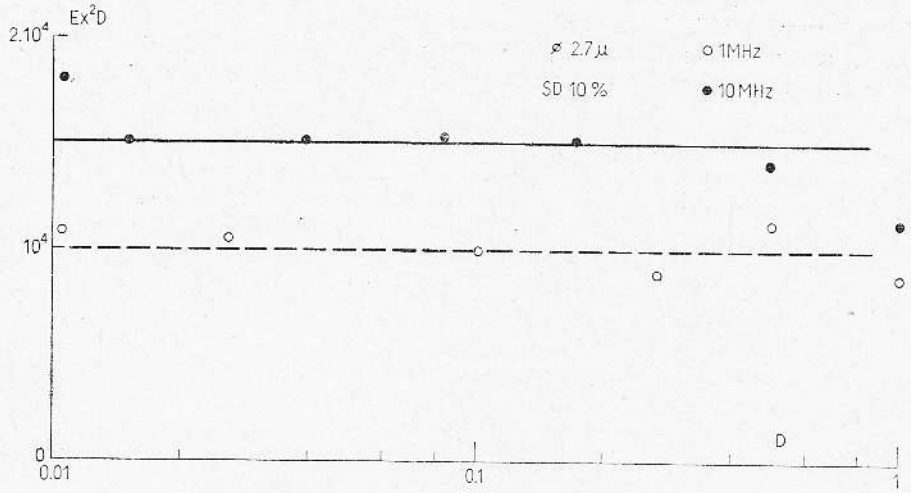

Fig. 5. Threshold of pearl chain formation as function of duty cycle D. The ordinate is given in terms of the product of D with the square of the peak field strength. The data provide experimental evidence that the phenomenon of pearl chain formation cannot respond to a pulsed field more readily than to a continuous field of the same average power (S. Takashima and H. P. Schwan, submitted for publication).

In summary: The electric properties of biologic systems are well known and understood. This understanding does not indicate so far the existence of nonthermal effects at the molecular or cellular level. However, forces which can be generated by the application of alternating fields (field-evoked forces, dielectrophoresis, electromechanical effects as various authors have termed them) deserve consideration and may well be responsible for the phenomenon of "hearing" pulsed fields and, hence, some of the behavioral effects reported in the 1 to 10 mW/cm² range.

REFERENCES

It is not possible to mention all the many hundreds of references which establish the body of facts summarized in the preceding paper. However, the following books, reviews, and book chapters may serve the interested reader further.

Electric properties of cells and tissues:

1. COLE, K. S.: Membrane Capacity. In: *Ions, Impulses and Membranes.* Ed.: C. A. Tobias, University of California Press. Berkeley and Los Angeles. 1968, p. 12.
2. SCHWAN, H. P.: Electrical Properties of Tissue and Cell Suspensions. In: *Advances in Biological and Medical Physics.* Eds.: J. H. Lawrence, and C. A. Tobias, Academic Press. New York. 1957, p. 147.
3. SCHWAN, H. P.: *J. Cell. Comp. Physiol.,* 1965, 66, 5.

Electric properties of macromolecules:

1. BOETTCHER, C. J. F.: *Theory of Electric Polarization.* Elsevier Publishing Co. Amsterdam, 1952.
2. HILL, N. E., VAUGHAN, W. E., PRICE, A. H., DAVIES, M.: *Dielectric Properties and Molecular Behavior.* Van Nostrand. London, 1969.

Field evoked forces:

1. POHL, H. A.: *J. Biol. Phys.,* 1973, 1, 1.
2. SCHWAN, H. P., SHER, L. D.: *J. Electrochem. Soc.,* 1969, 116, 170.
3. SCHWAN, H. P., SHER, L. D.: Electrostatic Field-Induced Forces and Their Biological Implications. In: *Dielectrophoretic and Electrophoretic Deposition. Eds.:* H. A. Pohl, and W. F. Pickard, Electrochemical Society, Inc. New York. 1969, p. 107.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

INTERACTION BETWEEN MICROWAVE AND MILLIMETER-WAVE ELECTROMAGNETIC FIELDS AND BIOLOGIC SYSTEMS: MOLECULAR MECHANISMS

K. H. Illinger

Department of Chemistry, Tufts University, Medford, Massachusetts, U.S.A.

INTRODUCTION

The objective of this discussion is to treat the interaction between electromagnetic radiation and the molecular components of biologic systems, in order to delimit the possible coupling mechanisms with EM fields which may lead to an alteration of the energetics and/or dynamics of the molecular system. The latter is deemed a prerequisite for any consequent biologic effects; possible interactions at the systems level, with a salient coupling mechanism to forms of biologic organization beyond the molecular level, are not considered here.

Salient aspects of the interaction between RF and microwave radiation and biological systems have been summarized *inter alia* by Schwan (43) and Michaelson (34). The subsequent discussion treats some aspects which complement and extend their conclusions regarding primary coupling mechanisms between EM fields and biologic systems. The motivation for the treatment of certain heuristic model systems is to delimit the probability and to define the nature of any biologic effects expected to arise as a result of these physical coupling mechanisms. The current intractability of the theoretical problem, whose physics has been sketched in the above, forces almost complete reliance, in the assessment of possible biologic effects, on empirical evidence obtained from experiment. Analysis of experimental findings in terms of a heuristic model may serve to correlate and generalize the observed behavior.

Although the structural variety of molecular systems of biological importance is myriad, the degree of conformational variety and organization implicit in certain model structures representative of a general biopolymer may elucidate the general problem. One such system is that of the poly-α-amino acids; a model system on which considerable structural, spectroscopic, and dielectric information is available is poly-(α-benzyl L-glutamate) or PBLG. This molecule is a representative poly-amino acid, with polar side-chains attached to the polymer backbone, and capable of undergoing a helix-coil transition. It is of interest to discuss the degrees of freedom of this complicated system, and to represent its interaction with EM fields.

Characteristic of biopolymeric systems whose helical form consists of a concatenation of polar helical turns, with a dipole moment of 1—5 D per unit turn, are very large values of the total dipole moment in the helical conformation, of the order of 10^3 D (23, 24, 26), while the statistical average of the dipole moment in the random coil form is essentially zero. Hence, it is expected that the helical form of molecules such as PBLG will exhibit overall rotational relaxation and couple to an RF field; such a relaxation process has in fact been found (53) in the region near 10 kHz, for PBLG of weight-average molecular weight of 150 000. Furthermore, while the detailed theory (44, 45, 46) of dielectric relaxation induced by chemical reaction is of some complexity,

one may expect that the very large changes in dipole moment for the helix-coil transition will lead to a second absorption mechanism. In fact, PBLG absorbs MW radiation near 1 MHz as originally predicted (44) and subsequently found experimentally (32, 47); this absorption is associated with a correlation time of 5×10^{-7} s for the helix-coil transition (5, 30, 35, 47, 54). It is of interest to mention that, while the electrical susceptibility undergoes large changes in the helix-coil transition, analogous changes in the magnetic susceptibility of this diamagnetic system exhibit an upper limit of less than one percent (9). It is expected that the polar side chains attached to the PBLG backbone will exhibit rotational relaxation in the range 1—10 GHz, since the magnitude of their group-rotational relaxation times lies near 10^{-10} — 10^{-9} s. Although the value of the relaxation time will differ in the two cases, both the helix and the coil conformations should share this relaxation mechanism. For the random-coil form, one may surmise that a rather wide distribution of relaxation times will occur, owing to the variety of the intermolecular environments that the side-chains will experience, tending to broaden the frequency spectrum of this absorption. A summary of the RF and microwave absorption frequencies of PBLG, including those of two mechanisms to be discussed in the following section, is as follows:

mechanism	**frequency**	**correlation time**
rotational diffusion[a]		
overall rotation of helix	10 kHz	10^{-4} s
group-rotation of side chains	1—10 GHz	10^{-10} — 10^{-9}
chemical relaxation	1 MHz	5×10^{-7}
quasilattice vibration[a]	10—1000 GHz	—
libration motions	100—1000 GHz	—
X—H...Y vibration	3000 GHz	—

[a] function of the degree of helicity and molecular weight.

QUASI-LATTICE VIBRATIONS OF BIOPOLYMERS

The two rotational diffusion mechanisms cited above are expected to show the inverse temperature dependence of the relaxation time τ characteristic of relaxation phenomena. There exists another mechanism for MW absorption in an ordered, extended structure such as the α-helix which lies at the borderline of relaxation processes and is perhaps best described as a quasilattice vibration of the helix perturbed by molecular collisions. The complete analysis of the 3N-6 normal modes of vibration, where N is the total number of atoms, of a biopolymer is a formidable task since N is of the order of 10^4 for molecular weights of the order of 10^5. Considerable effort has been expended in the attack on the general theoretical problem of the normal-coordinate analysis of polymers in the unperturbed state (1, 10, 11, 12, 20). The spectrum of normal modes may be divided into three regions, associated with widely different force constants; namely, (a) group vibrations, involving vibrational motion of small numbers of atoms, with force constants of the order of 1—10 mdyn/Å (1 mdyn/Å = 10^5 dyn/cm), and associated frequencies of the order of 10^3 cm^{-1} (1 cm^{-1} = GHz) infrared; (b) vibrations which correspond to the distortion of the unit which comprises the building blocks of the overall polymer, e.g. a single turn of the helix in a helical system, with force constants in the case of hydrogen-bonded helices such as PBLG $\sim$ 0.1 mdyn/Å 34.37 and frequencies $\sim$ 100 cm—1 or 3000 GHz; (c) vibrations which comprise distortion of essentially the entire polymer and which, for a quasi-infinite system, would be the lat-

WHO – Biologic effects & health hazards of microwave radiation 1974

tice vibrations. It is the latter which are of interest in the microwave- and millimeter-wave region.

While the coupling of EM fields E_0 to the rotational degree of freedom of a molecule depends on a nonvanishing value of $\mu E_0 \cos\vartheta$, where μ is the permanent molecular (or segmental) dipole moment and Θ is the angle of orientation of μ in the field, the vibrational degree of freedom will be active in absorption of EM fields if the change of the total dipole moment with vibrational distortion, represented by the normal coordinate Q_k, is nonvanishing, $(\vartheta\mu/\vartheta\, Q_k) \neq 0$. In the case of the quasilattice vibrations of biopolymers in the α-helical form, longitudinal vibrations are expected to produce substantial values of the dipole-moment derivative. If the vibration can be treated as a harmonic oscillator, the associated intensity of absorption of EM fields has the form, $K_k \sim \omega_k\, (\vartheta\mu/\vartheta\, Q_k)^2$, where ω_k is the harmonic frequency, and A_k is independent of the temperature (18). The frequency of the vibration, ω_k, for the isolated molecule is a constant and can be estimated by a simplified normal-coordinate analysis which treats the distortion of one helical segment against the other as the distortion of a two-mass point system, or, in a more refined model, as the distortions of a helical-staircase model, where the helical backbone is connected by rungs parallel to the axis of the helix comprised of the X—H...Y hydrogen bonds. Perturbation of a molecular vibration, even of rather small force constant, by molecular collisions is expected to be considerably smaller than that of an overall or segmental rotation. As a first approximation, one may stipulate the quasi-lattice vibrational frequency ω_k to be independent of the average thermal energy of collisions, i.e. independent of T, but affected by the mean value of the intermolecular potential.

A microwave absorption in the range between 1 and 20 GHz for PBLG of MW $\sim 10^6$, independent of T, has been observed (8), and interpreted in terms of a simple classical model. The α-helix may be approximated classically as a continuous spring with an effective force constant associated with the X—H...Y distortions. The principal longitudinal vibrational frequency can then be estimated by: $\omega_p = (s/L)\ (k/m)^{1/2}$, where s is the pitch of the helix, with $2\pi s$ being the distance between successive turns of the helix, k the effective force constant per unit term determined from the X—H...Y force constant (40), L the length of the helix, and m the effective mass. Although this classical model is a drastic simplification of the quasilattice vibration problem and is applicable only to the simple α-helix, it correlates with the magnitude of the observed MW absorption of PBLG. A more complete analysis of the quasilattice vibrations of ordered biopolymer structures provides the dependence of the frequency ω_k on the molecular weight. Since ω_k will decrease in a monotone fashion with increasing MW, a range extending from the millimeter-wave into the centimeter-wave region is to be expected for this absorption process, depending on the MW of the system. The damping of the vibrational excitation by molecular collisions will be considerably less effective than rotational diffusion in a fluid; accordingly, the predictions of dielectric relaxation theory are not appropriate to this interaction. Although the α-helix is a special case of very high symmetry (helical screw-axis throughout the molecule) and biopolymers, such as proteins (55), have in many cases rather irregular three-dimensional structures, where regularity of three-dimensional structure and nonvanishing dipole-moment derivatives for quasi-lattice vibrational distortion occur, the coupling mechanism discussed here may become operative. Irregularity in structure may be thought of as leading to destructive interference of vibrational modes and a consequent broad, featureless spectrum of lattice modes, in contrast to the discrete spectrum of a highly regular structure. Finally, it is of interest to point out that the distortion of the hydrogen bridges in the α-helix, parallel to the helix axis, which generates the active longitudinal vibrations near 10 GHz, has no direct counterpart in double-helical structures such as that

of DNA since in this case the hydrogen-bonded subunits lie essentially perpendicular to the helix axis. This is not to say that DNA exhibits no longitudinal quasilattice vibrations, but that the hydrogen-bonded subunits are not involved in these vibrations in the same fashion as those of the α-helix (56). In particular, vibrational distortion of the hydrogen-bridged system in DNA is primarily associated with perpendicular quasilattice vibrations, expected to lie above 100 cm^{-1} and essentially independent of the degree of polymerization, whereas distortion in the α-helix system contributes both the far-infrared frequency near 100 cm^{-1} and the microwave frequency range near 1 cm^{-1}, the latter being a function of molecular weight.

DIELECTRIC SATURATION IN BIOPOLYMER SYSTEMS

For simple monomeric molecular systems with permanent dipole moments ~ 1 D $= 10^{-18}$ esu cm and polarizability anisotropies one has for the ratio of the field-interaction energy to the average thermal energy the inequalities for attainable laboratory field strengths E_0 and ordinary temperature T. The average thermal energy dominates

$$|\alpha_{\parallel} - \alpha_{\perp}| \sim 10^{-24}\ cm^3 \qquad [1]$$

over the field interaction, and the ordinary (non-saturation) approximations of dielectric theory hold. For biopolymeric systems with dipole moments $> 10^3$ D and/or polarizability anisotropies $> 10^{-20}$ cm^{-3}, the status of the above-mentioned inequalities is altered to approach unity for attainable field strengths E_0. The theory of dielectric saturation has been discussed by a number of authors (23, 24, 37, 38, 39). Saturation of the electrical birefringence (Kerr effect) at fields of several kV/cm has been studied for PBLG (25, 40) and tobacco mosaic virus (19), and virtually total dielectric saturation of PBLG has been achieved in static (21) and 1 msec pulsed fields (13) with fields up to 60 kV/cm. The shift of the relaxation time for overall rotation of PBLG and of ethyl cellulose has been reported in fields up to 10 kV/cm (4).

$$(kT)^{-1} \langle \mu E \cos\Theta \rangle_{Av} \ll 1, \quad (kT)^{-1} \langle |\alpha_{\parallel} - \alpha_{\perp}|\, E_0^2 \rangle_{Av} \ll 1 \qquad [2]$$

The acquisition of large values of the dipole moment and the polarizability anisotropy in ordered structures of polymers made up of polar monomeric units is a general phenomenon. As a first approximation, the total dipole moment for a polymer consisting of N monomeric units with the z-component of the monomeric moment, μ_z, (parallel to the symmetry axis of the polymer) is simply:

$$\mu_{\parallel} = \sum_i (\mu_z)_i \cong N\mu_z \qquad [3]$$

The polarizability anisotropy at zero frequency may be written as:

$$\alpha_{\parallel} - \alpha_{\perp} = \left(\alpha_{\parallel}^{el} - \alpha_{\perp}^{el}\right) + \left(\alpha_{\parallel}^{vib} - \alpha_{\perp}^{vib}\right) \cong \alpha_{\parallel}^{el} + \sum_{k_{\parallel}} \omega_{k_{\parallel}}^{-2} \left(\partial_{\mu}/\partial Q_{k_{\parallel}}\right)^2 \qquad [4]$$

where α_{II}^{el} and α_{I}^{el} are components of the electronic polarizability, α_{II}^{vib} and α_{I}^{vib} the vibrational components, which may be written in terms of the vibrational dipole-moment derivates and frequencies (56). The superposition of monomer moments longitudinally produces the large value of μ_{II}. The fact that the field along the z axis sees a concatenation of the N electronic chromophores leads to $\alpha_{II}^{el} >> \alpha_{I}^{el}$, and the preponderance of low-frequency vibrational modes in the parallel direction leads to

WHO – Biologic effects & health hazards of microwave radiation 1974

$\alpha_{II}^{vib} >> \alpha_{I}^{vib}$; hence, the polarizability anisotropy at zero (or microwave) frequencies considerably exceeds that just below electronic frequencies.

Certain structures other than highly symmetrical biopolymers may be expected to show pronounced dielectric saturation effects, for reasons similar to those which altered the status of the ratio of thermal-energy to field-interaction energy in the ordered biopolymer cases discussed above. One such situation is the decoupling of Zwitterion molecules from a micelle, whose average dipole moments is essentially zero, to form the non-micellar state with associated large values of μ. Pulsed-field measurements of fields up to 100 kV/cm and duration $\sim$ msec have been carried out (22) on egg lecithin, a phospholipid of the type found in cell membranes. It is found that near 10 kV/cm application of a series of pulses is sufficient to disrupt the micelles to essentially mono--molecular aggregates. In translating measured dielectric saturation effects into expected behavior *in vivo*, attention must be given to the effect of the environment of the system (e.g. solvent in the laboratory measurement) on the intermolecular forces and the dielectric properties.

SUMMARY AND CONCLUSIONS

A basic physical description of the dynamics of a molecular system in the presence of perturbations by an EM field and by intermolecular forces was provided.

Information indicating that the natural EM background in the biosphere at microwave- and RF-frequencies in orders of magnitude below the power levels assiociated with technological and/or scientific applications was provided. In contrast, the natural background at VLF, ELF and zero-frequency (static) fields in the biosphere is of the same order of magnitude as certain technical applications.

A detailed model, at the level of organization representative of the molecular components of biological systems, of mechanisms of interaction between microwave and millimeter-wave EM fields and biological systems was delineated. From these considerations, it would appear that irreversible effects arising from the perturbation of rotational diffusion in biological fluids by external EM fields in the RF and centimeter-wave region, at normal *in vivo* temperatures, have a low probability at field strength below saturation levels. The existence of quasilattice vibrations in extended biopolymetric structures (e.g. the alpha-helix) indicates the possibility of damped resonant interactions between millimeter-wave and far-infrared EM fields and biological systems.

Irreversible saturation effects in biological fluids are expected only at (static) field strengths of the order of $kVcm^{-1}$, in helix-coil transitions of certain biopolymers and in micelle-random-Zwitterion transitions.

The problem of microwave EM field-induced proton shifts in the hydrogen bonds of biopolymers was discussed; such field-induced proton shifts, and hence possible associated genetic-code alterations, are improbable for systems such as DNA.

APPENDIX

DESCRIPTION OF THE MOLECULAR SYSTEM

The time-evolution of the energy of a molecule in the presence of collisional perturbation by other molecules and of perturbation by an electromagnetic field may be characterized by the (Hamiltonian) energy operator H (t):

$$H(t) = H_0 + V(t) + H_f(t) \qquad [5]$$

Here, H_0 represents the (time-independent) energy operator for the isolated molecule, separable to first approximation, into the electronic, vibrational, and rotational degrees of freedom:

$$H_0 \cong H_0(el) + H_0(vib) + H_0(rot) \qquad [6]$$

V(t) is the (time-variant) potential energy due to molecular interaction, and $H_f(t)$ is the interaction operator due to the EM field. If the form of V(t) and $H_f(t)$ is accurately known, H(t) is defined and the energy of the system can be predicted, in principle, by solution of the time-dependent Schroedinger equation:

$$\frac{i}{\hbar}\frac{\delta}{\delta t}\Psi(\underline{r},t) = H(\underline{r},t)\,\Psi(\underline{r},t) \qquad [7]$$

where Ψ (r, t) is the wave function and $\hbar = h/2$, where h is the Planck constant. In Equation [7], the dependence of the energy operator and of the wave function on both the time, t, and all the spatial coordinates of the n-particle system, $r = (r_1, r_2, \ldots r_n)$, has been articulated. In practice, direct solutions of Equation [7], even for simple forms of the intermolecular potential V(t), as well as for arbitrarily large field interactions $H_f(t)$ are not available. Progress toward solving Equation [7] can be made in the limiting case where both V(t) and $H_f(t)$ are small compared to H_0, since it can then be assumed that $V(t) + H_f(t)$ can be treated as a perturbation operator and employed, to varying degrees of approximation, within the framework of perturbation theory and with the solutions of the time-independent Schroedinger equation,

$$H_0(\underline{r})\;\Psi_0^n(\underline{r}) = E_0^n\;\Psi_0^n(\underline{r}) \qquad [8]$$

to obtain the energies of the perturbed system. Here, the eigenvalues E_o^n are the unperturbed energies of the system. For complex molecules, Equation [8] can be solved to fair accuracy, albeit with computational effort which increases rapidly with the total number of electrons in the molecule. In spite of the computational intractability of the theoretical framework traced in the above, it may serve as the basis of a semi-quantitative discussion.

It is of interest to examine first the nature of the molecular interactions represented by the intermolecular potential energy V(t) in Equation [5]. Whereas the total energy of an unperturbed molecule in its ground state is of the order of 100 kcal mole^{-1} per chemical bond, intermolecular forces generate interaction energies V of the order of 0—5 kcal mole^{-1} during collisions at average intermolecular separations near STP. Schematically, the instantaneous intermolecular potential V(r, t′) can be constructed by the superposition of several terms, (15) e.g.,

$$V(\underline{r},t') = V_{rep}(\underline{r}) + V_{dis}(\underline{r}) + V_{es}(\underline{r}) + V_{ind}(r) + V_{hb}(\underline{r}) \cdot \qquad [9]$$

at an instant of time t′. Terms on the rhs of Equation [9] which pertain to any atomic or molecular system are the repulsive term $V_{rep}(r)$ and the so-called dispersion term $V_{dis}(r)$. The former arises from the symmetry constraints imposed upon the electric charge distribution in an n-electron system by the Pauli exclusion principle; in effect, the energy of any atomic or molecular system will increase if the interelectronic distances, r_i—r_j, are decreased below the equillibrium distance. Thus, any two atoms or molecules colliding with sufficient kinetic energy will be repelled by a force whose integral is the term V_{rep}; this interaction potential is short-ranged, i.e. operative only at small interaction distances, and can be approximated by functions of the form $+Ae^{-kR}$

or, less accurately, $+A'/R^{12}$, where R is the average intermolecular distance, and A, A', and k are parameters. The foregoing statement becomes invalid in the case of a chemically-reactive collision, in which case collision may form a new molecule, thereby changing H_0 and altering the energetics of the system by a magnitude commensurate with forming a chemical bond. Chemically-reactive collisions will be excluded in the present discussion. The competition between $V_{rep}(R)$ and attractive terms in Equation [9], which leads to a characteristic value of the interatomic or intermolecular distance S for which $V = 0$, defines a molecular radius. While compression of mean distances between electrons invariably leads to $V(R) > 0$ for short-range interactions, R being of the order of a few angstroms, correlation of electronic motion in colliding atoms or molecules leads concomitantly to V(R) becoming attractive at slightly longer ranges. The latter is represented by $V_{dis}(R)$, a potential term which, owing to its relation to the polarizability, is said to arise from the dispersion force; the leading term in a reasonably accurate expression of $V_{dis}(R)$ has the form $-B/R^6$, for a simple two-atom interaction. For interactions between atoms, $[V_{rep}(R) + V_{dis}(R)]$ represents the total instantaneous potential V(R, t); its variation with time is defined by the values of the distance, R(t), which are consistant with a given collision path.

For molecules, additional terms in Equation [9] become nonvanishing. Whereas all atoms are spherically symmetrical, and thus have vanishing electrical multipole moments, molecules will exhibit some non-zero multipole moments. The set of possible multipole moments (charge, dipole moment, quadrupole moment, octupole moment, etc.) appear as molecular constants in the expression for the electrostatic potential, $V_{es}(R)$, in Equation [9]. In fact, $V_{es}(R)$ can be expressed as an inverse power series in the intermolecular distance R, with constants in this series containing combinations of multipole-multipole, and multipole-induced-multipole interactions between the two molecules. An important aspect of the electrostatic, as well as the induction, terms in V is that $V_{es}(R)$ and $V_{ind}(R)$ are functions of the angular orientation of one molecule with respect to another, allowing positive and negative values of V depending upon the orientation. The term $V_{ind}(R)$ in Equation [9] describes the induction of multipoles in a molecule via the perturbation of permanent multipoles of an adjacent molecule. Finally, in systems containing H atoms chemically bonded to atoms such as O, N, F, and permitting the proximity (within 1—2 Å) of non-bonded atoms (O, N, F), an additional, strong interaction results in the formation of the hydrogen bridge X—H...Y, which may be said to contribute to V a term $V_{hb}(R)$. This term is separated from the other terms in V because the nature of the hydrogen-bond interaction is not adequately expressed in the form of any of the other intermolecular forces, although it is frequently implied that the hydrogen bridge is essentially an electrostatic interaction. The hydrogen bridge (often, less descriptively, called the hydrogen bond) is an extremely important intermolecular interaction in biological systems, as well as in such liquids as H_2O. Because the energy of the hydrogen bridge is extremely sensitive to the X—H...Y angle, this interaction exhibits even stronger angular characteristics than the electrostatic and induction interactions.

In a typical molecular system relevant to a biological entity, all of the molecular interactions summarized in Equation [9] and briefly traced above will be operative. In fact, the secondary and tertiary structures of biologically important molecules, in particular biopolymers, are strongly determined by intermolecular forces. The three-dimensional architecture of biologically important molecules is at once flexible and programmable in that a multitude of configurations of the isolated molecule are attainable by internal rotation about C—C, C—N and C—O bonds. The potential energy for such intramolecular conformational alterations is of the same order of magnitude as the intermolecular interaction energies given by Equation [9]. As a consequence, there

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

exists a fairly large set of conformations of slightly different energies for a biopolymer, each component of which has a slightly different stability depending on its intermolecular environment. Equilibria among different conformers have been studied extensively, and the effect of thermodynamic variables, such as pressure and temperature, on such equilibria are reasonably well understood. The dynamics of conformational equilibria are less well characterized, and the time evolution of conformations in the open, steady-state systems that characterize real biological entities is not fully understood to date.

The dynamics of molecular systems of biological interest are affected by the thermodynamic variables of state which characterize their macroscopic environment and by any external potentials, such as EM fields. Given the viability of known biological systems in the presence of natural background of temperature, pressure, gravitational fields and non-ionizing radiation, it is of interest to inquire into the EM background in the biosphere, before considering the effect of man-made perturbations.

NATURAL BACKGROUND OF NON-IONIZING RADIATION IN THE BIOSPHERE

Apart from certain natural sources of ELF and VLF fields, to be touched upon later, the natural background of EM non-ionizing radiation in the terrestrial biosphere arises from (a) radiation from the sun and (b) reradiation from the earth. Although transmission through the atmosphere modifies the solar spectral irradiance R (1) at the surface of the earth, the envelope of radiation flux (i.e. the upper limit of R (1) as a function of the wavelength 1) is quite accurately described as a blackbody distribution, with T = 5700°K (2). Similarly, the terrestrial reradiation can be treated as arising from a blackbody at 288°K. Characteristic of the blackbody model is that the total radiant energy emitted over all wavelengths is cT^4, where c is the Stefan-Bolzmann constant, and that the wavelength associated with the maximum value of R (1) occurs at $1_{max} = 2898\ m/T$. The latter relation predicts maximum values for R (1) at 0.5084 μm and 10.06 μm for the solar and the terrestrial blackbody radiation, respectively. Measured solar spectral irradiance at 38 000 ft shows a maximum R (1_{max}) = 208.5 mW cm^{-2} μm^{-1} at 0.480 microns wavelength, consistent with a solar constant (total radiation emitted at all wavelengths) of 135.1 mW cm^{-2} or 1.938 cal cm^{-1} min^{-1}. Measurements at 1, 10, and 20 microns wavelength give values of 74.6 mW^{-2} 2.5×10^{-2} mW cm^{-2}, and 1.6×10^{-3} mW cm^{-2}, per unit micron wavelength interval, respectively. The percentages of the solar constant associated with shorter wavelength radiation at 1, 10, and 20 microns are 69.42, 99.935, and 99.9893 percent, respectively. It is evident from these considerations that the natural blackbody radiation background in the terrestrial biosphere at wavelengths beyond the far-infrared (beyond 100 microns) is below microwatts cm^{-2} in the long-wavelength limit. R(1) falls off essentially exponentially with wavelength, so that the natural background at microwave and RF frequencies is several orders of magnitude below the far-infrared flux.

Ionization processes in the atmosphere, produced mainly by cosmic radiation and natural terrestrial radioactivity, produce electric fields of the order of 100—200 V m^{-1}; under disturbed weather conditions, fields of 10^3 V m^{-1} are observable. Emissions from lightning further provide ELF fields in the region 3—300 Hz and VLF fields in the region 1—10 kHz; then, former may produce resonances in the earth-ionosphere cavity with typical resonance frequencies near 10 Hz for the first few modes. In the RF and microwave regions, the only natural background is the superposed blackbody distributions of solar and terrestrial radiation. For a bandwidth of 1 MHz, thermal or "Johnson" noise produces a power output of the order of 10^{-14} W in amplificatory

WHO – Biologic effects & health hazards of microwave radiation 1974

systems at 300°K. Power sources for scientific purposes thus have power outputs many orders of magnitude above the background. As a result, the EM field perturbation term in Equation [5] may be taken, to extremely good accuracy, to arise purely from the externally applied field, neglecting the natural background, in the microwave and RF regions.

MOLECULAR DYNAMICS IN THE PRESENCE OF EM FIELD PERTURBATION

In principle, the prescription of the time-dependent energy operator in Equation [5] permits complete formulation of the problem of the dynamics of a molecular system being perturbed simultaneously by intermolecular forces and an external EM field. It will be assumed that an adequate expression for $H_I(t)$ can be written in the form:

$$H_f(t) \cong E_0^{int} \cdot \mu \left[e^{ivt} + e^{-i\omega t}\right] G(t,\omega) \qquad [10]$$

Here, ω is the angular frequency of the EM field, and $G(t, \omega)$ is a function which describes the time-profile of the EM field. For CW radiation, $G = 1$; for AM or FM modulated fields, $G(t, \omega)$ takes on the appropriate form. More general fields, containing more than one frequency, can be written as a superposition of terms as in Equation [10]. E_o^{int} is the amplitude of the internal field, i.e. the effective field at the molecule, and μ is the dipole-moment operator. The problem of computing accurate values for the internal field in a general dielectric is a formidable one; however, for the present purpose it will suffice to consider it of the same order of magnitude as the macroscopic field in the dielectric. This argument does not include the attenuation of the external macroscopic field as it penetrates through a dielectric. The magnetic vector of the EM field has been neglected in Equation [10], since magnetic interactions, except for ferromagnetic and paramagnetic systems, are much weaker than electric-field interactions (25). It should be noted, however, that the magnetic vector of an EM field is capable of inducing eddy currents in a conducting dielectric which, in turn, produce macroscopic electric fields in the dielectric. This effect, of particular importance at frequencies below the microwave region, requires knowledge of the detailed radiation pattern of an EM source for both the electric and magnetic vector.

An accurate description of the dynamics of a molecular system under the perturbations summarized in Equation [5] requires, aside from the form of $H_I(t)$ in Equation [10], an accurate formulation of the time-dependent intermolecular potential energy $V(r, t)$. Although the description of a molecule in the gas phase undergoing collisions and interacting with an EM field can be treated, to varying degrees of approximation, by scattering theory, (19, 51) the solution of the problem for condensed states provides serious difficulties. In particular, some real biological entities are inhomogeneous systems with characteristics intermediate between those of a simple liquid and those of solides. Thus, it is expected that the ordinary quasi-continuum models for the dielectric properties of fluids are not completely adequate for the general problem of the perturbation of biodynamic systems by EM radiation. Nevertheless, some qualitative features of the simple theories are expected to provide a guide toward actual behavior of these systems. At ordinary temperatures in simple fluids, molecular interaction reduces the lifetimes of individual rotational eigenstates to such an extent that, owing to the indeterminacy relation $\Delta E \Delta t > \hbar$ (where Δt is the lifetime of an eigenstate and ΔE is the associated indeterminacy in the energy), the rotational degree of freedom becomes quasi-classical. (The translational degree of freedom is quasi-classical even in a dilute gas, since the spacings of the translational energy levels are within ΔE as

determined by the lifetime of translational states, even at very low pressures.) As a result, the interaction of the rotational and translational degree of freedom in a simple fluid with an EM field is characterized by a relaxation process. In some solids, the rotational degree of freedom, as well as presumably certain inversion modes, can remain quantized (36). Furthermore, in certain cases, at sufficiently low temperatures, quantized rotational motion may persist in the liquid state (3). For a simple fluid, the molecular constant which characterizes the dynamics of the interaction to first order is the rotational diffusion constant or the correlation time for rotational diffusion. Extensive theoretical and experimental work (7, 14, 17, 48) on simple fluids and simple solids has been carried out to determine rotational relaxation behavior. The orders of magnitude of rotational correlation times of many molecular and of representative intramolecular segments capable of internal rotation have been measured. Although the prediction *ab initio* of rotational correlation times is a task that still confronts dielectric theory, empirical and semi-empirical schemes are available to correlate experimental findings on simple fluids (49), polymer solutions and colloids.

In spite of the extensive treatment of dielectrics, a complete predictive theory for the perturbation of biodynamical systems by microwave and millimeter wave EM radiation cannot be said to exist. Conformational changes in biologically active molecules can, in principle, be induced by EM radiation. A crucial question is the relaxation toward the biologic steady-state of such induced changes, if any, within the time scale of biologically significant processes. In particular, the extent to which biologically active molecular systems (and their reactions and/or transport at biomolecular surfaces, membranes, etc.) are describable by a quasi-solid rather than quasi-homogeneous liquid, remains an incompletely solved research problem. The accuracy of the description of the medium or substrate in which a biologically active molecule is found *in vivo* may be crucial to an adequate model for the interaction with EM fields. The latter requires an accurate description of the intramolecular potential for conformational change as well as the intermolecular potential V(r, t) *in situ*. Finally, although the effect of (all but extremely large) external fields on the equilibrium constants of chemical and conformational equilibria is expected to be small, the actual *in vivo* biodynamical system is a (non-equilibrium) open, steady-state system with finite relaxation times. All these aspects render an accurate description of collisional and EM perturbations of real biological systems difficult but challenging.

Although the foregoing discussion has hardly paved the way toward computational elucidation of Equations [5-9], the formalism presented may aid in the conceptual appreciation of the treatment of actual systems. In fact, the crucial question as to whether collisional perturbations in a given system are associated with adiabatic or diabatic processes may be examined numerically with the aid of a criterion for adiabaticity (33):

$$A = \left| \int_0^{\delta} (\Delta E_{ab})^{-1} \left(\frac{dH}{dt}\right)_{ab} \left[\exp\left(\frac{i}{\hbar} \int_c^{\delta} \Delta E_{ab}\, dt'' \right) dt' \right] \right| \ll 1 \qquad [11]$$

Here, ΔE_{ab} is the energy difference between the levels of energy E_a and E_b being bridged by the EM photon, and $(dH/dt)_{ab}$ is the time derivative of the total Hamiltonian, Equation [5], appropriate to the states *a* and *b*, and δ is of the order of the duration of a collision. It is enlightening to perform an order-of-magnitude evaluation of the integral A. In the case of a molecule in the dilute gas, the average value of the intermolecular potential, V(t), in Equation [5]), is small, tending toward zero as the pressure decreases. If the relative velocity of the perturber and the absorber is 10^4 cm s^{-1}, the duration of a typical collision in the gas phase is 10^{-12} s; the intermolecular potential varies roughly between 10^{-13} erg and zero. If we consider $(dH/dt)_{ab}$ to be

WHO – Biologic effects & health hazards of microwave radiation 1974

constant during the collision (i.e. a triangular interaction potential), the value of A to first order is given by: $A = (1/h)(V_0/\omega_{ab}) = 10^{14}/\omega_{ab}$. It is apparent that transitions for which the adiabetic assumption holds cannot be less than 10^{14} cp s^{-1} in frequency. This frequency is in the infrared region; in the microwave region, the adiabetic approximation is clearly inapplicable. Experimental confirmation of this fact exists in studies of the effect of microwave power saturation on line widths in the spectra of gases. This result leads to the assumption of the very opposite of the adiabatic approximation: one can assert that gas-phase collisions are sudden, and strongly diabatic, in the time scale of rotational transitions (50). Molecules in a liquid experience an average intermolecular potential several orders of magnitude larger than that operative in the dilute gas; superimposed upon this average are excursions in H(t) due to individual collisions. In the solid, the rotational and translational degrees of freedom of individual molecules are in general transformed into lattice vibrations, although molecular rotation or group rotation may persist in certain cases. Whilst analysis via Equation [11] in the case of condensed states is rendered difficult because of the lack of detailed knowledge of (dH/dt), recent direct experimental measurements (27) of the lifetimes of vibrational states in simple fluids confirm the magnitude of 10^{-11} s, indicating that the regime of strongly adiabatic interactions lies considerably above 1 cm^{-1} (30 GHz). This order of magnitude for the lifetime of molecular states is consistent, via the uncertainty principle, with an indeterminacy of the energy of the order of 1 cm^{-1}, corroborating the fact that molecular transitions in the microwave region in condensed states are nonresonant, whereas those in the infrared are strongly resonant, and those in the far-infrared represent an intermediate case.

RF AND MICROWAVE ABSORPTION IN BIOPOLYMERS

The microwave absorption of biological systems is dominated by the intense rotational relaxation of H_2O, centered at 17.2 GHz, corresponding to a relaxation time of 9.24×10^{-12} s at 20°C, with an E_{max} near 35. The H_2O absorption, which fortuitously fits with great accuracy the form of the complex dielectric constant function predicted on the basis of a single relaxation time, provides efficient attenuation of a microwave EM field in the GHz region into a biological entity.

FIELD-STIMULATED PROTON SHIFT IN THE H-BRIDGES OF BIOPOLYMERS

Since the tertiary structure of many molecular systems of biological importance is closely connected with the existence of hydrogen bridges between pairs of atoms within the molecule, the effect of possible motion of the proton in such environments on the fidelity of molecular replication, in particular that of DNA and hence the genetic code, has been frequently discussed. Since the small mass of the proton imparts a finite tunneling rate ($<<$ lifetime of an individual) to its motion, the question of spontaneous proton tunneling in DNA has been given some attention (28, 29) and appeared to have been corroborated by an early quantum-mechanical study of the energy surface of the DNA base-pairs as a function of the position of the three protons linking the bases (42). The limited basis set of electronic wave functions employed in that study *inter alia* conspired to imply a double-minimum potential for the motion of a single proton across one of the three hydrogen bridges in the guanadine-cytosine base-pairs of DNA. A subsequent study (6), with state-of-the-art sophistication in electronic

computing, permitted considerable refinement of the basis set of electronic wave functions and numeral techniques in general. The cuts through the potential surface pertaining to the motion of a single proton across one of the hydrogen bridges in the DNA base pair were found, on the basis of this computation, to show a single minimum. Simultaneous motion of two protons, involving two hydrogen bridges, is associated with a double-minimum intramolecular potential energy function in the case of formic acid, HCOOH, (circular) dimer (6); even the refined computations in Ref. (6) were not deemed sufficiently accurate by their authors to include or exclude the possibility of such a double minimum for simultaneous motion of two protons in the DNA base-pair problem. Viewed conservatively, their conclusions render doubtful any field-induced single-proton transfers in DNA by RF and microwave EM radiation, although field-stimulated proton shifts have been invoked (38) in analyzing dielectric saturation in aliphatic alcohols (31).

REFERENCES

1. ABE, Y., KRIMM, S.: *Biopolymers*, 1972, **11**, 1817.
2. American Institute of Physics Handbook, 3rd Ed. McGraw-Hill, New York, 1972 (Sect. 6k).
3. BIRNBAUM, G.: *Mol. Phys.*, 1973, **25**, 241.
4. BLOCK, H., HAYES, E. F.: *Trans. Faraday Soc.*, 1970, **66**, 2512.
5. BURKE, J. J., HAMMEA, G. G., LEWIS, T. B.: *J. Chem. Phys.*, 1965, **42**, 3520.
6. CLEMENTI, E., J. MEHL, VON NIESSEN, W.: *J. Chem. Phys.*, 1971, **45**, 508.
7. COLE, R. H.: *Progr. in Dielectrics*, 1961, **3**, 47.
8. DAVIES, M., MAUREL, D., PRICE, A. H.: *Trans. Faraday Soc.*, 1972, **68**, 1041.
9. DAY, E. P., PHILO, J. S., FAIRBANK, W. M.: *Bull. Am. Phys. Soc.*, 1973, Ser. II **18**, 61.
10. FANCONI, B., PETICOLAS, W. L.: *Biopolymers*, 1971, **10**, 2223.
11. FANCONI, B.: *J. Chem. Phys.*, 1972, **57**, 2109.
12. FANCONI, B.: *Bull. Amer. Phys. Soc.*, 1973, Ser. II, **18**, 373.
13. GREGSON, M., JONES, G. P., DAVIES, M.: *Trans. Faraday Soc.*, 1971, **67**, 1630.
14. HILL, N. E., VAUGHAN, W. E., PRICE, A. H., DAVIES, M.: *Dielectric Properties and Molecular Behavior*, Van Nostrand Reinhold, London 1969.
15. HIRSCHFELDER, J. O., CURTISS, C. F., BIRD, R. B.: *Molecular theory of gases and Liquids*, John Wiley and Sons, New York 1954.
16. ILLINGER, K. H.: *J. Chem. Phys.*, 1961, **35**, 409.
17. ILLINGER, K. H.: *Progr. in Dielectrics*, 1962, **4**, 37.
18. ILLINGER, K. H., FREEMAN, D. H.: *J. Molec. Spectroscopy*, 1962, **9**, 191.
19. ILLINGER, K. H., TRINDLE, C. O.: *J. Chem. Phys.*, 1968, **48**, 4427.
20. ITOH, K., SIMANOUCHI, T.: *Biopolymers*, 1970, **9**, 383.
21. JONES, G. P., GREGSON, M., DAVIES, M.: *Chem. Phys. Letters*, 1969, **4**, 33.
22. JONES, G. P., GREGSON, M., KRUPKOWSKI, T.: *Chem. Phys. Letters*, 1972, **13**, 266.
23. KIELICH, S.: *Acta Phys. Polon.*, 1969, **A36**, 495.
24. KIELICH, S.: *Acta Phys. Polon.*, 1970, **A37**, 447.
25. KOLIN, A.: *Physics Today*, 1968, **21**, 39.
26. O'KONSKI, C. T., YOSHIOKA, K., ORTTUNG, W. H.: *Phys. Chem.*, 1959, **63**, 1558.
27. LEUBEREAU, A., VON DER LINDE, D.: *Phys. Rev. Letters*, 1972, **28**, 1162.
28. LÖWDIN, P. O.: *Rev. Mod. Phys.*, 1964, **35**, 724.
29. LÖWDIN, P. O.: *Biopolymers Symposium*, 1964, **1**, 161, 293; Electronic Aspects of Biochemistry, Ed. B. Pullman, Academic Press, New York, 1964.
30. LUMRY, R., LEGARE, R., MILLER, W. G.: *Biopolymers*, 1964, **2**, 484.
31. MAŁECKI, J.: *J. Chem. Phys.*, 1962, **36**, 2144.
32. MARCHIAL, E.: *Biopolymers*, 1971, **10**, 417.
33. MARGENAU, H., LEWIS, M.: *Rev. Mod. Phys.*, 1959, **31**, 569.
34. MICHAELSON, S. M.: Biological Effects of Microwave Exposure, In: *Ref. 42*, p. 35.
35. NAGAYAMA, K., WADA, A.: *Chem. Phys. Letters*, 1972, **16**, 50.
36. NARAYANAMURTI, V., POHL, R. O.: *Rev. Mod. Phys.*, 1970, **42**, 201.
37. PIEKARA, A.: *Proc. Roy. Soc.*, 1939, **A172**, 360.
38. PIEKARA, A.: *J. Chem. Phys.*, 1962, **36**, 2145.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

39. PIEKARA A., KIELICH, S.: *Acta Phys. Polon.*, 1958, **47**, 209.
40. PIMENTEL, G. C., McCLELLAN, A. L.: *Ann. Rev. Phys. Chem.*, 1971, **22**, 347.
41. POWERS, J. C., PETICOLAS, W. L.: *Adv. Chem. Series*, 1967, **65**, 217.
42. REIN, R., HARRIS, F. E.: *J. Chem. Phys.*, 1964, **41**, 3393.
43. SCHWAN, H. P.: Interaction of Microwave and RF Radiation with Biological Systems. In: *Biological Effects and Health Implications of Microwave Radiation*. Ed. S. F. Cleary, U.S. Dept. of Health, Education and Welfare. Report BRH/DBE 70-2 (FPB 193 858) Rockville, 1970, p. 13.
44. SCHWARZ, G.: *Molecular Relaxation Processes*, Chemical Society, London, 1966 (Spec. Pub. No. 20, p. 191).
45. SCHWARZ, G.: *J. Phys. Chem.*, 1967, **71**, 4021.
46. SCHWARZ, G.: *Rev. Mod. Phys.*, 1968, **40**, 206.
47. SCHWARZ, G., SEELIG, J.: *Biopolymers*, 1968, **6**, 1263; 1969, **8**, 429.
48. SMYTH, C. P.: *Dielectric Behavior and Structure* (McGraw-Hill, New York, 1955).
49. SMYTH, C. P.: *Ann. Rev. Phys. Chem.*, 1966, **17**, 433.
50. TRINDLE, C. O., ILLINGER, K. H.: *Chem. Phys.*, 1967, **46**, 3429.
51. TRINDLE, C. O., ILLINGER, K. H.: *J. Chem. Phys.*, 1968, **48**, 4415.
52. TOWNES, C. H., SCHAWLOW, A. L.: *Microwave Spectroscopy* McGraw-Hill, New York, 1955, p. 407.
53. WADA, A.: *J. Chem. Phys.*, 1958, **29**, 674; 1959, **30**, 328.
54. WADA, A., TAHAKA, T., KIHARA, M.: *Biopolymers*, 1972, **11**, 587.
55. WATSON, J. D.: *Molecular Biology of the Gene*, W. A. Benjamin, New York, 1965, Chap. 4.
56. WEBB, S. J., BOOTH, A. D.: *Science*, 1971, **174**, 72.

WHO – Biologic effects & health hazards of microwave radiation
1974

EFFECTS OF MICROWAVE IRRADIATION IN VITRO ON CELL MEMBRANE PERMEABILITY

S. Barański, S. Szmigielski and J. Moneta

Military Institute of Aviation Medicine, Warsaw, Poland

Biologic effects of irradiation of living organisms with very high frequency electromagnetic waves were demonstrated several years ago (for review, see ref. 3). Nevertheless, the mechanism of extrathermal biologic effects of microwaves in living organisms remains still unknown, although various hypotheses have been proposed. Paramagnetic resonance (9), ionic conductivity (10) and changed dielectric characteristics of certain compounds (7, 14) are suggested as the phenomena leading to the extrathermal effects of microwave irradiation. Only a few papers have been published on the effect of microwave irradiation on isolated cells *in vitro*, although a rather strong cytopathic effect of microwaves have been demonstrated (11, 16).

In view of the above it seemed interesting to evaluate the effect of microwave irradiation on cell and lysosomal membranes. The cell membrane, because of its morphologic structure (5, 12, 18) and function (13) should be highly susceptible to the action of any factor leading to changes in dielectric parameters of protein molecules. As increased permeability, the displacement of potassium ions from cells to the extracellular fluid and the disturbed function of the sodium-potassium pump, followed by higher permeability for larger molecules, the well-known signs of cell membrane injury (15) it seemed interesting to test these phenomena in cells undergoing microwave irradiation *in vitro*.

MATERIAL AND METHODS

Erythrocytes. Erythrocytes were isolated from heparinized (50 I.U./ml) rabbit venous blood by centrifugation and washing in saline three times. The cells were suspended in chemically clean saline, containing no potassium, to form a 10% suspension and were irradiated in thin-wall Plexiglas tubes with continuous electromagnetic waves of 10 cm length at 1, 5 and 10 mW/cm² power densities, in each case at the far field. The cell suspensions were irradiated during 15, 30, 60, 120 and 180 minutes, the temperature of the suspensions being checked during the whole period of irradiation. The control erythrocytes were stored at room temperature. In irradiated and control RBC suspensions the following estimations were performed:

1. Concentration of hemoglobin in the supernatant (after centrifugation at 3000 rpm during 15 min) with use of the Beckman colorimeter;
2. Osmotic resistance curve at NaCl concentrations of 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1%. The amount of hemoglobin liberated was measured with Beckman's colorimeter and compared to erythrocytes suspended in a 0.1% solution of NaCl.
3. Potassium concentration in the supernatant by the titration method of Pincussen (8).

Granulocytes. Rabbit granulocytes were isolated from the peritoneal cavity 8 h after an infusion of 0.5% glycogen in saline (6) and suspended in heparinized saline 50 I.U./ml). The suspensions were diluted with saline to obtain 10^6 cells/ml and the percentage of granulocytes was checked on slides stained according to the May-Grunwald-Giemsa method. Suspensions containing at least 90% of mature granulocytes were used for further studies. Granulocytes were irradiated in thin-wall Plexiglas tubes with continuous 10-cm waves at 1 or 5 mW/cm² during 15, 30 and 60 minutes, in each case at the far field. In control and irradiated suspensions of granulocytes the following estimations were performed:

1. Supravital staining with 0.1% nigrosin (6) and observation of the percentage of stained (dead) cells in a light microscope. 500 cells were counted in each case.

2. Activity of acid and alkaline phosphatases and lysozyme (muramidase) in supernatants after centrifugation at 5000 rpm during 15 min at 4°C (in plastic tubes). The phosphatases were determined according to the King-Armstrong method (for details see ref. 6), lysozyme by means of the turbidimetric technique of Litwack (4) based on lysis of lyophilized *Micrococcus lysodeikticus*. The activities of the above enzymes were compared to the activity found in supernatants of granulocytes (10^6/ml) frozen and thawed three times and centrifuged, as above. Activity in frozen/thawed suspensions was estimated as 100% and the results obtained in irradiated suspensions were expressed as a percentage of the above value.

RESULTS

Hemoglobin concentration, osmotic resistance and concentration of potassium are presented in Figures 1 and 2.

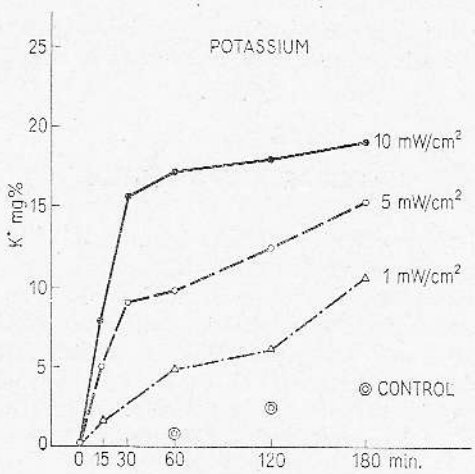

Fig. 1. Concentration of hemoglobin in the supernatant of suspensions of erythrocytes irradiated with microwaves.

Increased permeability of erythrocyte cell membranes to hemoglobin was observed only after irradiation at 1 mW/cm² during 60 min.

Osmotic resistance of erythrocytes irradiated with microwaves decreased during irradiation (Fig. 1), being time- and dose-dependent. During the first two hours of

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

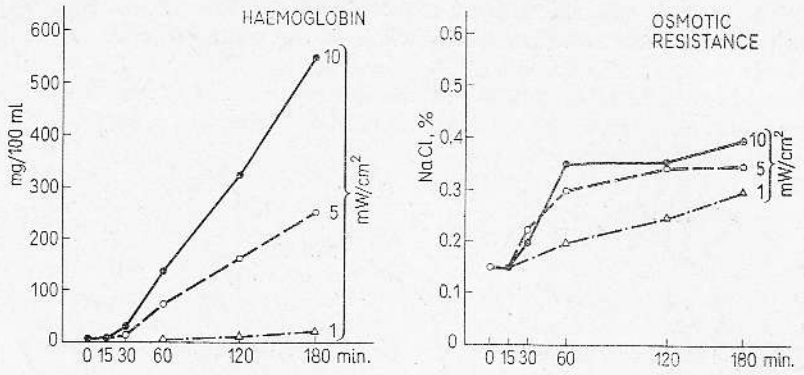

Fig. 2. Osmotic resistance of erythrocytes and concentration of potassium in supernatants of erythrocytes irradiated with microwaves.

irradiation at 1 mW/cm² no significant changes, as compared to control solutions, were observed. After 120 min of irradiation, total hemolysis occurred already at 0.3% NaCl, while at higher power densities osmotic resistance markedly decreased after 30 minutes (Fig. 1).

Changes in potassium metabolism were the earliest observed disturbances in erythrocytes irradiated with microwaves. After only 15 min of irradiation at 1 mW/cm² power density a significant increase in potassium concentration in the supernatant was noted (Fig. 2). The values were still higher after longer lasting irradiation or after irradiation at 5 or 10 mW/cm² power density.

A marked increase in the percentage of dead (stained with nigrosin) granulocytes was observed only in suspensions irradiated at 5 mW/cm² (Fig. 3). After a 60-min irradiation at this power density, about 80% of granulocytes showed diffuse staining

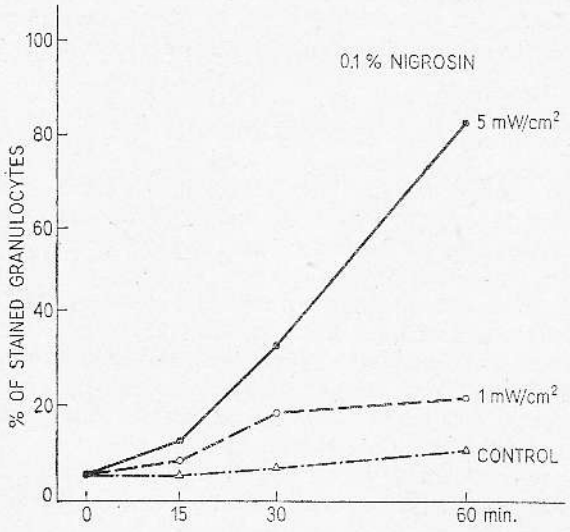

Fig. 3. Staining of granulocytes irradiated with microwaves with 0.1% nigrosin (percentage of stained cells per 500 cells).

WHO – Biologic effects & health hazards of microwave radiation 1974

with nigrosin, while in suspensions irradiated at 1 mW/cm² only about 20% of granulocytes were stained, the last figure being close to the value observed in control solutions, not irradiated with microwaves.

Activities of acid and alkaline phosphatases and lysozyme in supernatants of granulocytes irradiated with microwaves are summarized in Figure 4.

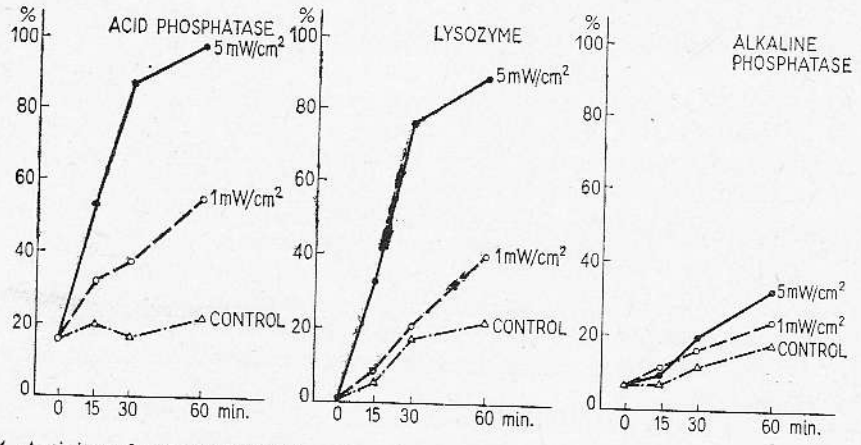

Fig. 4. Activity of acid and alkaline phosphatases and lysozyme in the supernatant of suspensions of granulocytes irradiated with microwaves.

After a 60-min irradiation with 10 cm waves at 5 mW/cm², about 85% of the whole activity of acid phosphatase and lysozyme were liberated from granulocytes and found in the supernatant, while only about 45% of the whole activiy of alkaline phosphatase were liberated from cells under this condition (Fig. 4). Liberation of higher amounts of acid phosphatase and lysozyme, as compared to alkaline phosphatase was also noted in suspensions irradiated at 1 mW/cm² (Fig. 4). In control suspensions not irradiated with microwaves only trace activities of all the three enzymes were found.

DISCUSSION

Irradiation of erythrocytes and granulocytes *in vitro* resulted in marked injury of cell membranes. After 15 minutes irradiation with 10 cm microwaves at 1 mW/cm², the power density that causes no thermal effect, increased potassium concentration in the supernatant was observed. This was followed by decreased osmotic resistance of the cells and increased cell membrane permeability for hemoglobin at higher power densities. Injury of the cell membrane function seems to be time- and dose-dependent. In granulocytes irradiated at 5 mW/cm² a rapid increase of the percentage of dead cells and liberation of lysosomal enzymes (acid phosphatase and lysozyme) from the cells was seen, while at 1 mW/cm² partial liberation of the hydrolases was the only phenomenon observed during 60 minutes of irradiation.

Lack in the literature of reports concerning the effect of microwave irradiation on the cell membrane function suggested that it would be best to begin by testing the most simple experimental system — isolated erythrocytes and granulocytes *in vitro*. The cell membrane of these cells does not differ in its function and morphology from that of other cells (2, 17). In granulocytes two distinct forms of intracellular granules, differing

in their morphology and function were found (1, 4): azurophilic granules containing lysosomal hydrolases, among them acid phosphatase and lysozyme, and specific granules containing alkaline phosphatase. Damage of the above granules results in liberation of their enzymatic "markers" into the cytoplasm or extracellular fluid, depending on the character and intensity of cell injury (1, 4). The amount of enzymes liberated from granulocytes proved to be a useful test for measuring the cytopathic effect and its specifity (4). The results obtained in granulocytes irradiated *in vitro* with microwaves suggest a damaging effect of higher power densities (5 mW/cm²) for lysosomes, while under these conditions the liberation of enzymes localized in specific granules (alkaline phosphatase) is much smaller.

On the other hand, the disturbed function of erythrocyte cell membranes was observed already after 15 minutes of irradiation at 1 mW/cm². The increased permeability for potassium ions, observed under these conditions, seems to be the effect of the disturbed function of the sodium-potassium pump. This system is very active in erythrocytes (2) and a high activity of the sodium-potassium activated adenosine-triphosphatas (Na-K-ATP-ase) was demonstrated in red blood cells (2). A longer time of irradiation of erythrocytes in microwave fields resulted in decreased osmotic resistance and increased permeability of cell membranes to hemoglobin. These phenomena are probably the consequence of earlier disturbances in the sodium-potassium pump and the signs of an irreversible injury of the cell membrane, leading to the death of cells. Under similar conditions and time of irradiation (30—60 min at 5 mW/cm²) about 80% of granulocytes showed staining with 0.1% nigrosin. Staining with nigrosin, widely used for determining cell vitality (6), depends on cell membrane permeability for large molecules and the positive results suggest an irreversible injury of the cell membrane.

In view of the results obtained it is our feeling that irradiation of cells *in vitro* with microwaves at non-thermal power densities causes injury of the cell membrane function and the efflux of potassium from cells seems to be the first sign of disturbances in the cell membrane function.

REFERENCES

1. ACKERMAN, C. A., *Lab. Invest.*, 1968, **19**, 290.
2. ALBERS, R. W.: *Ann. Rev. Biochem.*, 1967, **36/II**, 727.
3. BARAŃSKI, S., CZERSKI, P.: *Biological effects of microwaves.* Pol. Med. Publ., Warsaw, 1975 (in press).
4. CLINE, M. J.: *Physiol. Rev.*, 1965, **45**, 674.
5. COOK, G. M. W.: *Brit. Med. Bull.*, 1968, **24**, 118.
6. CUTTS, M.: *Cell separation.* Acad. Press, New York, London, 1970.
7. HAGGIS, G. H., BUCHANAN, T. J.: HASTED, J. B.: *Nature*, 1961, **167**, 607.
8. PINCUSSEN, A. C.: *Mikromethodik.* Leipzig, 1950.
9. PRESSMAN, A. S.: *Usp. Sowr. Biol.* 1963, **56**, 161.
10. PRESSMAN, A. S.: *Usp. Sowr. Biol.* 1963, **61**, 40.
11. PRESSMAN, A. S.: *Zarub. Radioelectron.* 1964, **3**, 63.
12. ROTHSTEIN, A.: *Ann. Rev. Physiol.*, 1968, **30**, 15.
13. SCHOFFENIELS, E.: *Cellular aspects of membrane permeability.* Pergamon Press, London, 1967.
14. SCHWAN, H. P.: *J. Phys. Med.*, 1953, **32**, 144.
15. SKOU, J. C.: *Physiol. Rev.*, 1965, **45**, 596.
16. STODOLNIK-BARANSKA, W.: *Nature*, 1967, **214**, 102.
17. TROSHIN, A. S.: *Problems of cell permeability.* Pergamon Press, London, 1966.
18. WHITTAKER, V. P.: *Brit. Med. Bull.*, 1968, **24**, 101.

ASSESSING MICROWAVES AS A HAZARD TO THE EYE — PROGRESS AND PROBLEMS*

R. L. Carpenter, E. S. Ferri and G. J. Hagan

Northeastern Radiological Health Laboratory, Bureau of Radiological Health, Food and Drug Administration, Department of Health, Education and Welfare, Winchester, Massachusetts, U.S.A.

In 1948, Daily et al. (9) and Richardson et al. (19) independently reported development of opacities in the lens following exposure of the eyes of rabbits or dogs to microwave radiation. Subsequent research has both confirmed their findings and extended our knowledge of the action of microwaves on the lens. Much remains to be learned regarding the relation of factors such as radiation frequency, power density, frequency of successive exposures and intraocular temperature to the degree of lens damage. More studies are needed on biochemical, metabolic, cellular and fine-structure changes associated with microwave-induced opacities. We particularly need well grounded information on how microwaves affect the human eye.

Many investigators have reported development of lens opacities after one exposure of the eye at various frequencies from 0.8 to 10 GHz (2, 3, 4, 5, 6, 7, 8, 15, 20, 21, 22, 25). When the eye was at any distance from the transmitting source, opacities characteristically developed in the posterior subcapsular cortex of the lens. However, when microwaves were transmitted directly by having the eye closely applied to an aperture in a diaphragm which terminated a waveguide (8) or to the corneal-fitted end of a dielectric waveguide transition terminating either a waveguide or a coaxial conductor (5) then the opacities which developed were in the anterior subcapsular cortex, as has been reported also for experimental electric cataracts (10, 11).

In most experiments involving microwave irradiation of the eye, the radiation source was a dipole antenna backed by a reflector and the eye was positioned only a few inches from it. The radiation was largely confined to the animal's head and the field was a near-zone one, with consequent difficulties in attempting to assess the level of microwave power acting on the eye. Additionally, in much of the earlier work, instrumentation suitable for reliable measurement was not available. As a result, investigators had to resort to describing the conditions under which irradiations were performed or, using instruments, providing values which, unfortunately, were not applicable to the conditions of the near-zone field. In view of these circumstances, it is futile to compare the results of experiments performed in many different laboratories, although the attempt has been made (18).

Even without reliable power measurements, it was still possible to determine, for any given output of a particular microwave source, the minimal single exposure period required to induce opacity formation in the lens. With power outputs expressed in some term of relative measurement common to all the experiments of a given series,

---

* Presented by Dr. R. L. Elder.

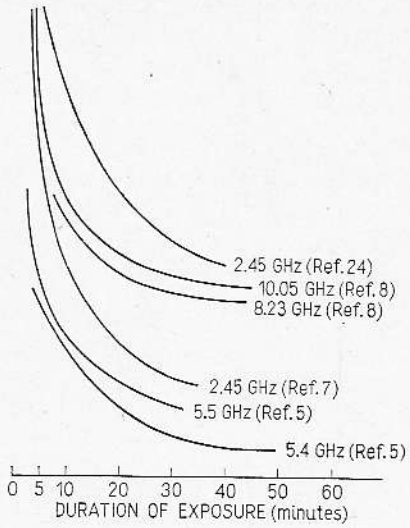

Fig. 1. Shapes of curves showing time-power thresholds for induction of lens opacities in the rabbit eye at various microwave frequencies. (Note: Ref. 24 should read Ref. 25).

these minimal single exposure periods could yield a curve showing time and power thresholds for the induction of lens opacities by single irradiations of the eye. Although data derived in different laboratories could not be expected to be identical with respect to time-power relations, the threshold curves reported by three different laboratories and covering several frequencies — 2.45, 5.4, 5.5, 8.2, and 10 GHz — were similar in shape. (Fig. 1).

The threshold curve for a given experimental series was also useful for identifying specific time and power combinations which constituted subthreshold exposures, incapable of producing changes in lens transparency. Consequently, it became possible to test the effect on the lens when a subthreshold exposure was repeated at regular intervals. If multiple exposures, separate in time and each incapable of causing opacity formation, were to result finally in a lens opacity, there would be demonstrated a cumulative, or additive, effect. A number of such experiments were performed and did, indeed, demonstrate that episodes of irradiation which apparently do not permanently harm the lens when experienced only once may cause lasting damage when they are repeated daily or even weekly (7, 8).

In our most recent experiments of this nature, eyes of non-anesthetized mature New Zealand white rabbits were irradiated two inches from a dipole antenna at 2.45 GHz, continuous wave, one hour daily for 20 consecutive days. Defining this arbitrarily as chronic irradiation, we sought to find the lowest power level which would most consistently cause development of lens opacities. The results of 59 such experiments are summarized in Table 1.

The power levels there referred to were measured by a Narda Model 8100 electromagnetic radiation survey meter (1) with its sensing probe placed in the exact position to be occupied by the cornea of the rabbit's right eye during its irradiation. Although the figures in the left-hand column represent the meter readings in mW/cm², they may not be accurate for the near-zone field conditions of the experiments, inasmuch as the probe does not respond to a radial component of the electric field. Nevertheless,

WHO – Biologic effects & health hazards of microwave radiation 1974

Table 1

Lens opacities resulting from consecutive daily exposures of the right eye to 2.45 GHz radiation

Power density mW/cm²	No. Animals	No. Exposures 1 hour each	Positive response	
			No. Animals	Percent
50	10	20	0	0
80	9	20—24	1	11
90	10	20	1	10
100	10	18—32	4	40
110	10	21—30	4	40
120	10	13—20	8	80

the various power measurements are relative and afford a basis for comparison among these experiments.

Lens opacities developed in only 4 out of 10 experiments performed at the 100 and 110 mW/cm² levels but with an increase in power to 120 mW/cm², opacities developed in 8 of 10 experiments. A one-hour exposure at this power level was truly subthreshold, for when tested in seven experiments, no lens opacities resulted (Table 2). Ten anesthetized rabbits were subjected at the same power to single exposures ranging from $1^3/_4$ to $5^1/_2$ hours and with only two exceptions, no opacities were observed in the irradiated eyes nearly a year later.

When the eye is irradiated, it is a matter of days before the first sign of opacity formation is seen as discrete small granules scattered along or near the posterior lens suture. These small opacities are the end result of a complex series of events initiated within the lens when it has absorbed microwave power. It is important to learn what happens in the lens during this latent period; fortunately, a start has been made.

Table 2

Lens response resulting from single continuous exposures of right eye to 2.45 GHz radiation

Power density mW/cm²	No. of cases	Exposure duration (hours)	Right eye		Left eye	Post-irradiation observation days
			Opacity	No change	No change	
120	7	1	0	7	7	30—324
120	1	1.75	0	1	1	20
120	2	2	0	2	2	11
120	1	2.5	0	1	1	10
120	2	3	0	2	2	343, 363
120	1	3.5	0	1	1	340
120	1	4	1*	0	1	348
120	1	4.5	1**	0	1	329
120	1	5.5	0	13—20	1	315

* Small central opacity after 137 days; no change thereafter.
** Small central opacity after 173 days; no change thereafter.

Merola and Kinoshita (17) and Kinoshita et al. (14) made biochemical studies of rabbit lenses removed at different intervals following cataractogenic exposure of the right eye. The first identifiable change in the irradiated lens, occurring after 6 hours and before 18 hours post-irradiation, was a decrease of approximately 23 percent in lens ascorbic acid. This change took place some days before the first signs of an opacity would have been seen. There was no change in the ascorbic acid level of the aqueous humor and when isolated non-irradiated lenses were heated to the same temperature and for the same period of time as the lenses of the irradiated eyes experienced, the ascorbic acid concentration remained unchanged.

Van Ummersen and Cogan (24) employed autoradiography to investigate the effect of microwaves on the lens epithelium and found that in lenses which had been exposed to cataractogenic doses, there occurred in the epithelium an inhibition of DNA synthesis and a decrease of mitotic activity. This inhibition was most marked in the period of one to five days following irradiation, after which recovery gradually took place but not until two weeks after irradiation had DNA synthesis and mitotic activity returned to normal.

It has already been mentioned that most of the reported experiments on microwave induction of lens opacities were performed with the eye in the near-zone field, where the radiation pattern is complex and measurement of power density is not a reliable procedure. This objection does not apply to the far-zone field, where a properly designed and calibrated instrument can measure power density in the field exactly where the animal's eye will be subjected to irradiation. However, other problems arise. One is that while the eye as the target organ is being irradiated, the animal is at the same time being subjected to whole body irradiation and its possibly lethal effect.

Another problem is that an object in a microwave field, unless it completely transmits or absorbs the energy incident upon it, will tend to perturb that field. This field perturbation, in turn, may profoundly alter the power density incident on the eye. To ascertain the extent to which a microwave field might be altered through perturbation by the body of a rabbit, we undertook some experiments, using a Narda Model 8100 Electromagnetic Radiation Survey Meter (1), which has a cylindrical sensing probe 23 mm in diameter. Radiation was 2.45 GHz frequency, continuous wave, from a 17.3 db. standard gain horn into an anechoic chamber 2 m high, 2 m wide and 4.15 m long.

With only the probe in the chamber, 150 cm away from the horn, power was adjusted for a reading of 60 mW/cm² on the survey meter. The probe was then placed in exactly the same position but after it had been passed through the head of a freshly sacrificed rabbit from which both eyes and all tissues between had been removed. The rabbit was then positioned in the chamber as if for irradiation, with the sensing part of the probe where the cornea of the right eye would have been. With no change in transmitted power, the meter reading was now only 35 mW/cm². The presence of the animal thus so perturbed the field that the power density at the position of the eye was reduced by approximately 40 percent. This measurement was made with the rabbit's ears in their normal erect position. When the ears were fastened down against its back, power density changed to only 21 mW/cm².

To conduct a more detailed study of the effect of field perturbation, we designed and constructed apparatus for scanning and continuously recording power density along any horizontal line across the width of our anechoic chamber. By recording a series of such scans separated 2.5 cm in the vertical plane and transferring the data to graph paper, we could construct a plot showing equal power contours for all or any part of the vertical plane. We first scanned a vertical plane one meter square and 150 cm distant from the radiating horn. The transmitted power was adjusted to

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

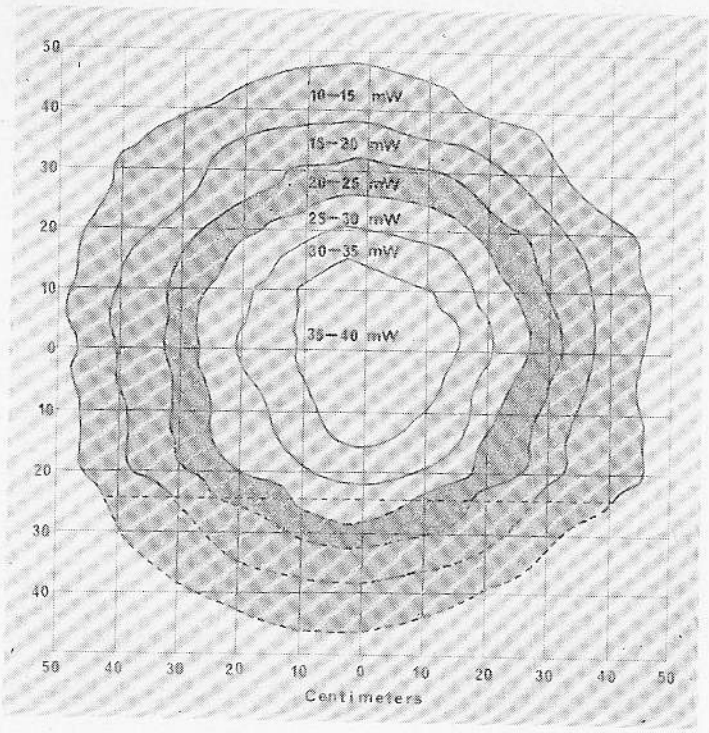

Fig. 2. Contours of equal power density for a vertical plane one meter square in a free field.

40 mW/cm² at the center of the plane, a point coincident with the projected geometrical axis of the horn. When power contours differing by 5 mW/cm² were plotted from these scans, the resultant plot (Fig. 2) represented the power distribution of a plane wave under the approximately free field conditions of our anechoic chamber. It resembled an archery target, with concentric circular zones decreasing in power density in a regular manner from 40 mW/cm² at the center to 10 mW/cm² at the periphery.

When the process was repeated at the same radiated power but with a rabbit placed just behind the vertical scanning plane, its right eye in line with the center of the field, the resulting plot was quite different (Fig. 3). In place of the symmetrical arrangement around a 40 mW/cm² central region was a haphazard pattern of irregular zones of differing power densities. In front of the eye, the power density was 50 mW/cm², a 25 percent increase. When the same animal was scanned in the same place and in the same position but with its long ears taped down instead of erect, the resulting plot was still irregular but the pattern was different (Fig. 4). Power density in front of the eye was now 60 mW/cm², representing an increase of 50 percent over the free field value. With the ears down, the pattern of field perturbation seemed conducive to reducing the hazard to the eye, as compared to the situation when the ears were erect. This agreed with the findings obtained when the survey meter probe was passed through the head to measure the power density at the plane of the cornea.

We also determined that cages, animal restrainers, or supports made of plastic, other than expanded polystyrene, can perturb the field and thereby alter the conditions of the experiment. We are forced to conclude that power density should be reported only as a measurement taken in the radiation field where the experiment is to be conducted

WHO – Biologic effects & health hazards of microwave radiation 1974

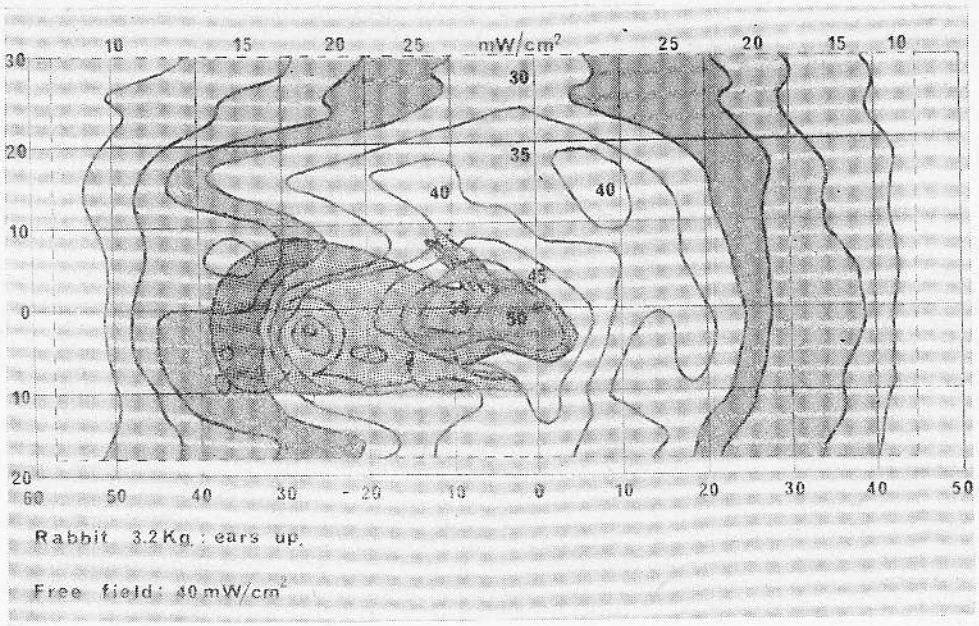

Fig. 3. Contours of equal power density made under the same conditions as was Figure 2 except for the presence of a rabbit just behind the measured plane. The animal's ears were in an erect position.

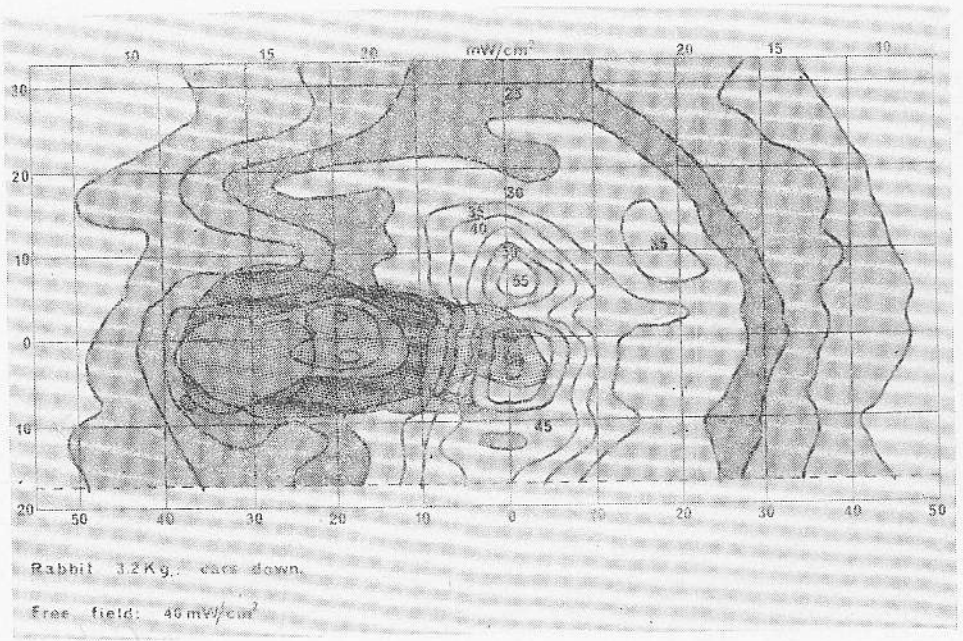

Fig. 4. Contours of equal power density made as in Figure 3 but with the rabbit's ears held flat against the body.

WHO – Biologic EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

and made in the absence of all perturbing factors, including the experimental animal. The size, shape and orientation of the animal are unpredictable factors.

Unfortunately, we cannot validly extrapolate to man the results of animal experiments. Even though a time-power threshold can be established for the induction of opacities in the rabbit eye, we cannot assume that the same conditions will produce a similar effect in another species. We have no reason to think that the human eye possesses a special immunity to the effects of microwaves but we have no information as to whether this radiation offers a greater or a lesser hazard to it than to the rabbit eye. We do not know how the human eye interacts with the microwave field or how its response to irradiation may be affected by field perturbations caused by the human head or the human form.

Our most insistent problems are in the field of dosimetry. Despite the many improvements in the reliability of electromagnetic survey meters for the evaluation of fields external to the body, we still must hope for an instrument sufficiently miniaturized so that it can be implanted in living tissues and there measure fields and absorbed power without modifying the field.

As of now, we do not know of a case in which microwave radiation has been the proven cause of a human cataract. The fact that a man develops posterior subcapsular cataracts and that at some previous time in his life he had been, or may have been, exposed to microwaves does not automatically establish a cause and effect relationship. Cases of presumed microwave cataract have been reported in the literature (2, 12, 13, 16, 23, 26, 27) but in no instance has convincing evidence been offered that microwaves were actually the causative agent. This is not to deny the possibility that they could have played that role but simply to point out that the supporting evidence which has been offered does not necessarily lead to that conclusion. In the assessment of microwave radiation as a hazard to the human eye, we must expect that any attempt to establish, *ex post facto*, a clear cause and effect relationship in suspected or alleged human cases will encounter difficulty. Nevertheless, we must insist that the scientific scrutiny accorded these cases be no less demanding than that applied to conclusions based on animal experimentation.

REFERENCES

1. ASLAN E.: *J. Microwave Power*, 1971, **6**, 169.
2. BAILLIE, H. D.: Thermal and Nonthermal Cataractogenesis by Microwaves. In: *Biological Effects and Health Implications of Microwave Radiation*. Ed.: S. F. Cleary, US Dept. of Health, Education and Welfare. Report BRH/DBE 70-2 (PB 193 858). Rockville, 1970, p. 59.
3. BELOVA, S. E.: The Effects of Microwave Irradiation on the Eye. In: *The Effects of Radar on the Human Body* (Results of Russian Studies on the Subject. Ed.: J. J. Turner). AD 278172. 1962, p. 43.
4. BELOVA, S. F., GORDON, Z. V.: *Bull. of Exp. Biol. and Med.*, 1956, **41**, 327.
5. BIRENBAUM, L., KAPLAN, I. T., METLEY, W., ROSENTHAL, S. W., SCHMIDT, H., ZARET, M. M.: *J. Microwave Power*, 1969, **4**, 232.
6. CARPENTER, R. L.: *Proc. Second Tri-Service Conf. on Biol. Effects of Microwave Energy*, Rome Air. Dev. Ctr., Air. Res. and Dev. Command, Rome, N.Y. ASTIA Doc. No. AD 131—477, 1968, p. 146.
7. CARPENTER, R. L., BIDDLE, D. K., VAN UMMERSEN, C. A.: *Biological Effects of Microwave Radiation with Particular Reference to the Eye*. Proc. 3rd Int. Conf. on Med. Electronics London, 1960, p. 401.
8. CARPENTER, R. L., VAN UMMERSEN, C. A.: *J. Microwave Power*, 1968, **3**, 3.
9. DAILY, L., WAKIM, K. G., HERRICK, J. F., PARKHILL, E. M.: *Am. J. Physiol.* 1948, **155**, 432.
10. FRAUNFELDER, F. T., HANNA, C.: *A.M.A. Arch. Ophthalmol.*, 1972, **87**, 179.

WHO – Biologic effects & health hazards of microwave radiation 1974

11. HANNA, C., FRAUNFELDER, F. T.: *A. M. A. Arch. Ophthalmol.* 1972, **87**, 18.
12. HIRSCH, F. G., PARKER, J. T.: *A. M. A. Arch. Ind. Hyg. Occup. Med.*, 1952, **6**, 512.
13. HIRSCH, F. G.: *Microwave Cataracts — A Case Report Reevaluation.* Lovelace Foundation for Medical Education and Research. Albuquerque., New Mexico, 1970.
14. KINOSHITA, J. H., MEROLA, L. O., DIKMAK, E., CARPENTER, R. L.: *Doc. Ophthalmol.* 1966, **20**, 91.
15. KRAMAR, P., EMERY A. F., GUY A. W., LIN J. C.: *Proc. IEEE-G-MIT Int.* Microwave Symposium, Boulder, Colorado, 1973, p. 265.
16. KURZ, G. H., EINAUGLER, R. B.: *Am. J. Ophthalmol.*, 1968, **66**, 866.
17. MEROLA, L. O., KINOSHITA, J. H.: *Changes in the Ascorbic Acid Content in Lenses of Rabbit Eyes Exposed to Microwave Radiation.* Proc. Fourth Ann. Tri-Service Conf. on Biol. Effects of Microwave Radiation. Plenum Press. New York, 1961, p. 285.
18. MILROY, W. C., MICHAELSON, S. M.: *Aerospace Med.*, 1972, **43**, 67.
19. RICHARDSON, A. W., DUANE, T. D., HINES, H. M.: *Arch. Phys. Med.*, 1948, **29**, 765.
20. RICHARDSON, A. W., DUANE, T. D., HINES, H. M.: *A. M. A. Arch. Ophthalmol.* 1951, **45**, 382.
21. SALISBURY, W. W., CLARK, J. W., HINES, M. M.: *Electronics*, 1949, **22**, 66.
22. SETH, H. S., MICHAELSON, S. M.: *J. Occup. Med.* 1965, **7**, 439.
23. SHIMKOVICH, I. S., SHILYAEV, V. G.: *Vestn. Oftal.* 1959, **72**, 12.
24. VAN UMMERSEN, C. A., COGAN, F. C.: Effects of Microwave Radiation on Lens Epithelial Cells. In: *Biological Effects and Health Implications of Microwave Radiation.* Ed.: S. F. Cleary, US Dept. of Health, Education and Welfare. Report BRH/DBE 70—2 (PB 193 858) Rockville. 1970, p. 122.
25. WILLIAMS, D. B., MONAHAN, J. P., NICHOLSON, W. J., ALDRICH, J. J.: *Biologic Effects of Microwave Radiation:* Time and Power Thresholds for the Production of Lens Opacities by 12.3 Cm. Microwaves. USAF School of Aviation Medicine, Randolph Air Force Base, Texas. Report No. 55—94. 1955.
26. ZARET, M. M.: *Ind. Hyg. Rev.* 1962, **5**, 11.
27. ZARET, M. M.: *Clinical Observations of Workers Exposed to Microwaves,* Zaret Foundation, Scarsdale, N. Y.

WHO – Biologic effects & health hazards of microwave radiation 1974

EXPERIMENTAL MICROWAVE OCULAR EFFECTS

B. Appleton

Ophthalmology Service, Walter Reed Army Medical Center, Washington D.C., U.S.A.

Albino rabbits were exposed to microwave radiation of the eyes under varying experimental conditions: acute single and chronic multiple exposures: two different frequencies (1700 MHz and 3000 MHz); anesthetized and unanesthetized animals; exposure of the head or eyes only and exposures involving more of the body; and

Tabela 1

One-year follow-up of single microwave exposures

Rabbits	Power density	Duration of exposure	Acute effects	Chronic effects
3	100 mW/cm²	15 minutes	0	0
3	100 mW/cm²	30 minutes	0	0
3	200 mW/cm²	15 minutes	0	0
3	200 mW/cm²	30 minutes	0	0
3	300 mW/cm²	15 minutes	+	0
3	300 mW/cm²	30 minutes	lethal	
3	400 mW/cm²	15 minutes	+	0
3	500 mW/cm²	15 minutes	(3)	0
3	Controls		0	0

Frequency: 3000 MHz CW
Anesthesia: none
New Zealand albino rabbits (51b)
Exposure to left eye placed one foot beyond focus

Table 2

One-year follow-up of five consecutive daily exposures

Rabbits	Power density	Duration of exposure	Acute effects	Chronic effects
3	100 mW/cm²	30 minutes	0	0
3	100 mW/cm²	60 minutes	0	0
2	200 mW/cm²	20 minutes	0	0

Frequency: 3000 MHz CW
Anesthesia: none
Left eye at one foot past focus of dish antenna
Albino rabbits (51b)

WHO - Biologic effects & health hazards of microwave radiation 1974

pulsed as well as continuous waves. In addition, power density of levels varying from 25 mW/cm² to 500 mW/cm² were used.

A summary of the results of these experiments is contained in Tables 1 through 4.

Table 3

Two-month follow-up of multiple microwave exposures

Rabbits	15 min daily exposure	Power density	Acute effects	Chronic effects
5	30	50 mW/cm²	0	0
5	30	100 mW/cm²	0	0
5	30	200 mW/cm²	0	0
5	30	300 mW/cm²	+	PSCI
5	30	400 mW/cm²	+	CAT.
5	14	500 mW/cm²	+	CAT.

3000 MHz CW
Anesthesia: ketamine HCl
Left eye at focus of elliptical dish antenna
New Zealand albino rabbits

Table 4

Two-month follow-up of multiple microwave exposures

Rabbits	15 min daily exposure	Power density	Acute effects	Chronic effects
5	30	50 mW/cm²	0	0
5	30	100 mW/cm²	0	0
5	30	200 mW/cm²	0	0
5	30	300 mW/cm²	+	PSCI
5	30	400 mW/cm²	+	CAT.

Frequency: 3000 MHz pulsed
Anesthesia: ketamine HCl
Left eye at focus of elliptical dish antenna
New Zealand albino rabbits

In addition, an experiment in which several rabbits were exposed simultaneously to 25 mW/cm² to the whole front of the body resulted in no deaths and no ocular damage, but the same experiment performed at 50 mW/cm² resulted in death of all the rabbits so exposed.

DISCUSSION

In would be virtually impossible within the resources of any of our present microwave research facilities to explore experimentally all the combinations and permutations of these variables: 1) number of exposures, 2) duration of exposures, 3) wavelength, 4) power level, 5) duty cycle, 6) head only versus whole body exposures, and species of experimental animal. Nevertheless, from the data at hand, some tentative conclusions can be drawn.

CONCLUSIONS

It appears that in the rabbit the lethal power level is somewhere between 25 mW/cm² and 50 mW/cm² if the front or one side of the animal is irradiated. But if the body is protected by shielding, or if the radiation is directed at the head only, the threshold for ocular damage appears to be around 300 mW/cm²; and the results of these experiments appear to offer little support for the hypothesis that repeated subthreshold exposures cause significant cumulation of damage. It also appears from these experiments that exposure to power levels approximately one order of magnitude below cataractogenic levels is sensed as an extremely noxious stimulus, probably thermal, causing the animal to struggle violently, to remove itself from the field.

The few initial observations gleaned from similar experiments using dogs (just recently initiated) appear to be roughly in quantitative agreement with those from the rabbit experiments, indicating an encouraging similarity with respect to microwave ocular effect between the two experimental models of the human case, despite their species-related differences from one another.

THE EFFECTS OF MICROWAVES ON HUMAN LYMPHOCYTE CULTURES

W. Stodolnik-Barańska

Institute of Biostructure, Medical Academy in Warsaw, Warsaw, Poland

Numerous reports indicate that microwave exposure may induce changes in the peripheral blood picture and the hematopoietic system both in man (2, 5, 9, 11) and in experimental animals (2, 3, 4, 9, 11). It should be stressed that long-term, low-dose exposure may induce peripheral lymphocytosis, stimulation of lymphopoiesis and anomalies of nuclear structure and mitotic abnormalities in lyphocytes and erythroblasts in guinea-pigs and rabbits (2, 3, 4, 6). In view of these data it seemed interesting to investigate the effects of exposure to microwave radiation on human lymphocytes cultured *in vitro*, with or without addition of phytohemagglutinin (PHA).

MATERIAL

Peripheral blood lymphocyte suspensions, purified in plastic containers (8), were incubated in TC 199 with 2% inactivated calf serum. The experiments were carried out in two series.

In the first series lymphocyte cultures without PHA were irradiated in two groups:

1. 4 h daily at 7 mW/cm² during 3 or 5 days.
2. 15 min daily at 20 mW/cm² during 3 or 5 days.

In the second series the effect of microwave exposure on PHA-transformed lymphocytes was examined, the experiments being carried out in the following groups:

1. Lymphocyte cultures following 66 h incubation were irradiated at 20 mW/cm² for 5, 10, 15, 20 minutes and 2 × 20 minutes with a 30 min interval.
2. Lymphocyte cultures following 64 h of incubation were irradiated 3 or 4 h at 7 mW/cm².
3. Lymphocyte cultures were exposed for 4 h daily during 3 days at 7 mW/cm².
4. Lymphocyte cultures were exposed to microwave irradiation for 10 min at various incubation periods: immediately after preparation of the culture (0 time) as well as following 59, 70 and 71.5 h of incubation.

METHODS

Lymphocyte cultures were exposed in a plastic thermostatic chamber (constant temperature of 37°C) at 7 mW/cm² or 20 mW/cm² to 2950 MHz pulsed microwaves (1200 Hz, 1 μs) using a horn antenna. Behind the exposure chamber an anechoic screen was placed, to avoid reflections and interference. The power density of the beam and the temperature of the incubation medium were controlled during each experiment.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

The temperature of the medium remained constant during 4 h at 7 mW/cm², at 20 mW/cm² it increased after 15 minutes by 0.5°C. Prolonged irradiation at this power density caused after 20 minutes an increase by 1°C.

In all experiments of the second series 0.05 mg of colcemide per 1 ml medium was added following 66 h of incubation, and the cultures were harvested after 6 h from the moment of colcemide addition (hypotonia, acetic alcohol fixation, Giemsa stain of air-dried preparations).

RESULTS

Microwave exposure at 20 mW/cm² induced changes in the mitotic index which depended on the exposure time (Tab. 1). 5 min exposure did not influence the proportion of dividing cells, slight differences compared with controls were observed after

Table 1

Percent of blastoid forms, lymphocytes and mitotic index in dependence from exposure time at 20 mW/cm²

Exposure time min	% Blastoid forms	% Lympho-cytes	Mitotic index % (M. I.)	Expected range for M. I. at 95% confidence level
0	78.0	10.0	12.0	10—15
5	79.0	8.3	13.7	9—16
10	78.2	4.6	17.2	13—17
15	75.8	6.3	17.9	13—17
20	73.5	3.0	23.5	18—22
40 (2×20)	74.0	1.0	25.0	23—27

10 and 15 minute exposures, significant differences were seen following 20 and 40 min exposures. Similar results were obtained following 3 or 4 h exposure at 7 mW/cm² at periods later than the 64th hour of incubation. The mitotic index was approximately two times higher in irradiated samples than in control ones. This effect became noticeable in cultures irradiated during the 59th hour of incubation and was pronounced in cultures irradiated after the 64th hour of incubation (Tab. 2). Irradiation after 70 or 71.5 hrs of incubation influenced the miotic index only in a very slight degree. Exposure of lymphocytes at 20 mW/cm² for various periods of time induced changes in numbers and structure of chromosomes which depended on the duration of exposure (Tab. 3). Only slight differences from control samples were noted after a 5 min exposure, more pronounced changes were seen after longer exposures. Stickiness of chromosome arms (Fig. 1) and aneuploidy were observed. Hyperploidal cells up to 85 chromosomes were encountered. Figure 2 shows a hyperploidal (about 3 N) cell. Dicentrics (Fig. 3) and chromatid breaks (Fig. 4) were also seen. It should be pointed out that changes in chromosomal morphology suggesting changes in spiralization were the most unusal finding (Fig. 5) Similar pictures were observed in chromosomes of

Table 2

Percent of blastoid forms, lymphocytes and mitotic index following 10 min exposure at 20 mW/cm² during various incubation periods

Hours of incubation	% Blastoid forms	% Lympho-cytes	Mitotic index % (M. I.)	Expected range for M. I. at 95% confidence level
0	73.0	15.0	12.0	10—14
59	80.4	4.0	15.6	12—18
64	78.0	5.0	17.0	14—19
70	79.8	10.0	10.2	8—13
71.5	80.0	9.0	11.0	9—14
Control	81.1	9.4	9.5	8—13

Table 3

Dependence of chromosomal aberrations on exposure time, 20 mW/cm²

Exposure time in min	Normal mitoses	Stickiness	Chromosomal aberrations			
			Dicentrics	Hypoploidy	Hyperploidy	Breaks
5	85.0	5.0	6.0	2.0	1.0	1.0
10	57.0	17.0	9.0	4.0	8.0	7.0
15	45.0	34.0	9.0	3.0	4.0	5.0
20	34.0	20.0	10.0	3.0	5.0	28.0
Control	92.0	2.0	4.0	0	0	2.0

irradiated rat kangaroo cells by Yao and Jiles (13). These authors call such changes "electromagnetic degeneration of chromosomes". Phagocytic forms containing nuclear fragments or phagocytized chromosomes were encountered. Moreover fragmentation of nuclei and nuclear vacuolization were seen. Certain pictures recalled cells in amitosis. Nuclear bridges were also not infrequent. The highest number of chromosomal aberrations was seen after exposure during the 70th hour of incubation. In this instance the number mitoses with chromosomal aberrations reached 50%.

The experiments demonstrated that microwave irradiation of PHA-stimulated human lymphocyte cultures at power densities of 7 mW/cm² and 20 mW/cm² causes an increase in the mitotic index as compared with control samples. A dependence between the value of the mitotic index and duration of exposure seems to exist. It should be stressed that irradiation of lymphocytes without PHA addition induces the appearance of blastoid forms and macrophage-like cells (Fig. 6).