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BIOLOGICAL EFFECTS OF MILLIMETER AND SUBMILLIMETER WAVES

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1 BIOLOGICAL EFFECTS OF MILLIMETER WAVES

1.1 Introduction

Millimeter (mm) waves comprise the region of electromagnetic spectrum between 30 and 300 GHz (wavelength (λ) = 10 - 1 mm in free space). However, in common practice, mm wave studies of biological effects cover the frequency range between 30 and 100 GHz. Submillimeter waves or terahertz radiation spectrum (1 THz=10¹² Hz) is typically defined as 100-10000 GHz or 0.1-10 THz, i.e., GHz and THz radiations are between the radiofrequencies and the far infrared radiations.

Humans are increasingly exposed to mm and THz waves from many different sources, such as wireless telecommunication devices [Kallfass et al., 2011], airport security scanners [Sheen et

al., 2001], automotive collision avoidance systems [Nicolson et al., 2008], nonlethal crowd control weaponry [Chen et al., 2012]. In addition, mm waves have been used as a therapeutic modality in medical devices [Rojavin and Ziskin, 1998]. Newly developed 5G mobile communication systems will significantly add to this exposure. The increasing utilization of mm wave and THz technologies creates considerable interest in the potential effects of mm waves and THz radiation on biological systems. Research of the mm wave and THz biological effects is especially important for establishment of accurate safety standards.

Pakhomov et al. [1998] presented a comprehensive review of biological effects of mm waves at different organization levels of organisms. This review covered about 50 papers published in peer reviewed Western sources and more than 300 papers published in the Former Soviet Union before the year 1997. Remarkable progress in bioelectromagnetics research has been achieved in the past two decades. In the present review, we provide the updated results of studies of biological effects of mm waves published since 1997 in the peer reviewed sources, except for a few essential citations of earlier publications. We review the studies of THz biological effects in Part II of this chapter. All of the publications reviewed are listed in the References located at the end of this Chapter.

It should be noted that some of the early results were confirmed in independent recent studies. However, most effects of low-intensity mm wave irradiation are still waiting for their replication. There have been systematic studies of mm wave effects on nervous and immune systems as well as on gene expression, cell proliferation, and artificial lipid membranes.

The present review is focused on experimental findings of mm wave effects on subcellular and cellular levels, effects on cellular proliferation and gene expression, effects on excitable tissues, involvement of nervous and immune systems in millimeter wave effects, physiologic

effects of high power mm waves, effects of mm waves on eyes, and skin heating. The review also includes the theoretical analysis of mechanisms of mm wave action on biological objects and typical artifacts encountered when conducting experiments with mm waves.

1.2 Basic Characteristics of mm Wave Radiation

1.2.1 Propagation of mm Waves in Free Space and Lossy Dielectric

The plane periodic mm waves traveling in free space in the z direction can be expressed as follows [Olver 1992]:

$$E = E_o e^{j(\omega t - \beta_o z)} \quad (1.1)$$

where E is the electric field (V/m), E_o is amplitude factor (V/m), ω is angular frequency, equal to $2\pi f$, f is frequency (Hz), β_o is the propagation constant (m^{-1}), $\beta_o = 2\pi/\lambda_o$, λ_o is the wavelength in free space (m), and $j = (-1)^{1/2}$.

In a lossy dielectric the equation (1) is transformed to the following form:

$$E = E_o e^{j\omega t - \gamma z} \quad (1.2)$$

where γ is the complex propagation factor expressed as

$$\gamma = \alpha + j\beta \quad (1.3)$$

The attenuation coefficient α (m^{-1}) is given by

$$\alpha = \omega \sqrt{\frac{\mu\epsilon'}{2} \left[\left(1 + \left(\frac{\epsilon''}{\epsilon'} \right)^2 \right)^{1/2} - 1 \right]} \quad (1.4)$$

The propagation coefficient β is given by

$$\beta = \omega \sqrt{\frac{\mu\epsilon'}{2} \left[\left(1 + \left(\frac{\epsilon''}{\epsilon'} \right)^2 \right)^{1/2} + 1 \right]} \quad (1.5)$$

The wavelength in a lossy dielectric is less than that in free space and can be found as

$$\lambda = \frac{2\pi}{\beta} \quad (1.6)$$

The attenuation coefficient α is also known as absorption coefficient of medium at the angular frequency ω . Its inverse is called the depth of penetration of mm waves, $\delta=1/\alpha$. δ equals the distance into a medium at which the amplitude of the E field drops to $1/e$ of the initial value.

The power penetration depth L is expressed as $L=\delta/2 = 1/2\alpha$.

The power density for a plane polarized field can be written as follows [Ramo et al. 1993; IEEE C95.1, 2005]:

$$PD = \frac{E^2}{\eta} = \eta \cdot H^2 \quad (1.7)$$

where η is the wave impedance (Ω):

$$\eta = \sqrt{\frac{\mu}{\epsilon'[1 - j(\epsilon''/\epsilon')]}} \quad (1.8)$$

$\eta = 377 \Omega$ in free space.

The amplitude reflection coefficient r of mm waves having parallel polarization, i.e., the E-field is parallel to the surface of incidence, is given by [suggested change in symbol. Save R for power]

$$r = \frac{\eta_2 - \eta_1}{\eta_2 + \eta_1} \quad (1.9)$$

where η_1 and η_2 are the wave impedances of medium 1 and 2, respectively. The transmission coefficient T is defined as

$$T = \frac{E_2}{E_1} = \frac{2\eta_2}{\eta_2 + \eta_1} \quad (1.10)$$

The energy absorbed by biological material is characterized by the specific absorption rate (SAR, W/kg) that is given by

$$SAR = \frac{\sigma \cdot E^2}{2\rho} \quad (1.11)$$

where σ is the conductivity (S/m), ρ is mass density (kg/m³), and E is the electric field strength (V/m).

In biological experiments, objects are exposed to mm waves in the far field or near field of antennas. The far field typically begins at a distance $l=2D/\lambda$, where D is the longest dimension of the radiating structure, and λ is the wavelength in air. Rectangular horn antennas frequently used in biological experiments have the longest dimension of 2 cm [Alekseev and Ziskin, 2003]. The far field distance at 42.25 GHz for this antenna is 11.3 cm. Actually, the far field is a term describing the plane wave exposure field. In far field exposure, the interaction or “coupling” between the antenna and object is absent. Irradiation at a distance less than l is termed near field exposure. The near field exposure especially in the reactive region of antenna is characterized by the strong interaction between the radiation source and object depending on the shape and size of the source and the geometrical and electrical properties of the object.

1.2.2 E-field Strength and P_D Measurements [suggested change in symbole]

The mm wave E-field distribution is measured with E-field probes. As the size of the commercially available E-field probes is relatively large (1-10 mm) their use in the near field of an antenna is limited. Moreover, in the reactive or radiating near field region where reactive fields or standing waves exist, the P_D distribution is too complicated. In this case, numerical calculation of the E-field and P_D distribution using the finite-difference time domain method (FDTD) [Zhadobov et al., 2017] or other numerical methods such as the Finite Element method (FEM), Finite Difference, and Monte Carlo are preferable [Wang and Wu 2007]. For measurement of the E-field strength at frequencies between 40-52 GHz Pakhomov et al. [1997]

successfully used a Narda miniature flat crystal detector with a custom made holder for the detector allowing precise movements in any direction over the irradiated area.

Mapping of the P_D distribution can be performed using a calibrated receiving antenna such as a horn antenna or open-ended waveguide connected to a power meter. More detailed mapping of the P_D distribution is achieved by using an open-ended waveguide due to small size of its aperture [Walters et al., 2000].

Most of the laboratory studies of bioeffects of mm waves have been performed in exposures where the biological structure is placed close to a small antenna. Thus, the object is exposed in the near field of the narrow irradiating mm wave beam. In this case, the SAR and P_D values can best be obtained by measuring the initial temperature rise rate on the object surface as described in paragraph 1.4.2.

1.2.3 Calibration of Measuring Devices

Calibration methods are addressed in IEEE C95.3 [2002]. Three basic methods of calibration of microwave survey devices are used: free- space standard field method, guided wave method, and transfer probe method. For mm wave devices, only a free-space standard method is suitable for accurate calibration in the frequency range above 1 GHz. Standard gain horn antennas are commonly used to establish accurate electromagnetic field intensities. The power density in the far field can be calculated from

$$P_D = \frac{P_r \cdot G}{4\pi \cdot r^2} \quad (1.12)$$

where P_D is the power density (W/m^2), G is the isotropic gain of the antenna, P_r is the radiated power (W), r is a distance from the horn antenna aperture to the on-axis field point (m). Gain is defined as $G = E_{\text{antenna}} \cdot D$, where E_{antenna} is efficiency and D is directivity of antenna. For common open-ended waveguide apertures with a two-to-one aspect ratio, i.e., $a/b=2$, the far-field gain is approximated by the following equation [Kanda and Orr, 1987]:

$$G = 21.6 \cdot f \cdot a \quad (1.13)$$

where f is frequency (GHz), a is the width (m) (larger dimension) of the waveguide aperture. For calculation of on-axis gain of the horn antenna, the equations given by Larsen [1978] can be used:

$$G(\text{dB}) = 10 \cdot \text{Log}(AB) + 10.08 - R_H(\text{dB}) - R_E(\text{dB}) \quad (1.14)$$

where A is the wavelength normalized width of the horn aperture, B is the wavelength normalized height of the horn aperture, R_H is the gain reduction factor due to the H-plane flair of the horn, and R_E is the gain reduction factor due to the E-plane flair of the horn. The gain reduction factors are given by

$$R_H(\text{dB}) = 0.01\alpha \cdot (1 + 10.19\alpha + 0.51\alpha^2 - 0.097\alpha^3) \quad (1.15)$$

$$R_E(\text{dB}) = 0.1\beta^2 \cdot (2.31 + 0.053\beta) \quad (1.16)$$

$$\alpha = A^2(1/L_H + 1/r) \quad (1.17)$$

$$\beta = B^2(1/L_E + 1/r) \quad (1.18)$$

where L_H and L_E are the wavelength normalized values of the slant lengths and r is the wavelength normalized distance from the aperture of the horn to the on-axis field point.

1.2.4 Generation of mm Waves

Millimeter wave generators use different sources of oscillators. Vacuum tube based sources include backward wave oscillators (BWO), orotrons (high-power BWO), magnetrons, gyrotrons, gyro-klystrons, and gyro- traveling wave tubes (gyro-TWT). Solid state sources include widely used Gunn diodes and impact ionization avalanche transit-time (IMPATT) diodes. Magnetrons, gyrotrons, gyro-klystrons, and gyro-TWT are used in high output power generators. Pulse magnetrons operate in the frequency range up to 220 GHz with peak power of 30 kW. Gyrotrons depending on cooling conditions can generate power at fixed frequencies up to 20 kW. Gyro-klystrons operate at fixed frequencies with output power up to 340 kW. Gyro-TWT can generate peak power up to 180 kW. The low output generators commonly used in biological experiments have oscillators such as BWO, Gunn diodes and IMPATT diodes. These sources of mm waves cover the frequency range of 30-178 GHz at maximum output power up to 400 mW. Generators based on BWO operate at frequencies from 36 to 178 GHz with output powers up to 80 mW. BWO are the most wide-banded sources with electronic control of frequency. Cavity stabilized Gunn oscillators generate from 40 to 140 GHz with maximum CW power at lower frequencies up to 200 mW, which drops at higher frequencies to 30 mW. CW IMPATT diodes are used for oscillators and amplifiers. They operate in the frequency range of 30-140 GHz with output power up to 400 mW. IMPATT diodes are used in noise generators. Some generators use frequency synthesizers. A frequency synthesizer is an electronic circuit for generating any of a range of frequencies from a single fixed oscillator. A frequency synthesizer uses the techniques of frequency multiplication, frequency division, direct digital synthesis, and frequency mixing (photomixing) to generate new frequencies which have the same stability and accuracy as the

master oscillator. Commercially available synthesizers cover the frequency ranges from 36 to 1250 GHz and 0.45 to 2.85 THz.

1.3 Permittivity of Biological Material

1.3.1 Electric and Magnetic Properties of Biological Material

The response of biological material (skin, tissue, cells) to mm wave exposure depends on its electric and magnetic properties at the exposure frequency. Magnetic properties of material are described by complex permeability, μ :

$$\mu = \mu' - j\mu'' \quad (1.19)$$

In biological non-ferromagnetic materials, magnetic response is very weak, and the permeability differs very little from the permeability of free space (μ_0), i.e., $\mu \approx \mu_0 = 4\pi \cdot 10^{-7}$ H/m.

The electric properties of material are characterized by the frequency dependent complex permittivity:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \quad (1.20)$$

where ε' is the relative dielectric constant and ε'' is the relative loss factor of the material. The total conductivity σ is related to ε'' by

$$\varepsilon'' = \frac{\sigma}{\omega \cdot \varepsilon_0} \quad (1.21)$$

The permittivity of free space is given by $\varepsilon_0 = 8.85 \cdot 10^{-12}$ F/m. The total conductivity σ includes a contribution from the frequency independent ionic conductivity and is associated with the

frequency dependent dielectric loss of material. The loss tangent is a measure of how “lossy” or energy absorbing a material is. It is defined as

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \quad (1.22)$$

Two relaxation functions are often used to fit dielectric data, the Debye and Cole-Cole equations. The Cole-Cole equation is given by:

$$\varepsilon^* = \varepsilon_{\infty} + \frac{\Delta\varepsilon}{(1 + j\omega\tau)^{1-\alpha}} + \frac{\sigma_i}{j\omega\varepsilon_o} \quad (1.23)$$

where ε_{∞} is the optical permittivity, $\Delta\varepsilon = \varepsilon_s - \varepsilon_{\infty}$ is the magnitude of the dispersion of material, ε_s is the permittivity at $\omega\tau \ll 1$, $\omega = 2\pi f$, ω is an angular frequency and f is the frequency (Hz), τ is the relaxation time (s), σ_i is the ionic conductivity (S/m), and α is the distribution parameter. At $\alpha=0$ equation 1.23 transforms to the well-known Debye equation.

According to Foster and Schwan [1986], the loss factor can be expressed as follows:

$$\varepsilon'' = \frac{\Delta\varepsilon \cdot \omega\tau}{1 + (\omega\tau)^2} + \frac{\sigma_i}{\omega\varepsilon_o} \quad (1.24)$$

The total conductivity is proportional to the loss factor ε'' (Eq. 1.21): $\sigma = \omega\varepsilon_o\varepsilon''$. The contribution of ionic conductivity decreases with increasing frequency. At mm wave frequencies, the total conductivity is defined mainly by the dielectric loss of material.

The dielectric spectrum of biological tissue is characterized by major relaxation regions such as α , β , and γ dispersions at low, medium, and high frequencies, respectively. Some minor

relaxation regions such as δ dispersion may also contribute to the dielectric spectrum of tissue [Schwan, 1977; Foster and Schwan, 1986]. The relaxation frequency of the γ dispersion in skin is about 22 GHz at 37°C [Gabriel et al. 1996], i.e., the γ dispersion is exhibited in the mm-wave frequency range.

The complex refractive index of a material is defined as

$$n^* = n' - j \cdot n'' = \sqrt{\epsilon^*} \quad (1.25)$$

1.3.2 Permittivity of Skin

In biological tissue (skin), water is a main absorber of mm waves. The photon energy of mm waves, $h\nu$, ranges from 0.12 to 1.24 meV, where h is Planck's constant, ν is frequency. The energy of electron removal from an atom or molecule is typically 12 eV. Unlike ionizing radiation, the photon energy of mm waves is not enough to disrupt molecular bonds. Therefore, mm waves are regarded as non-ionizing radiation. The energy of molecular vibration is in the range of 0.01-0.1 eV. The energy of thermal motion, kT , at room temperature is about 0.025 eV, where k is a Boltzmann constant, T is temperature. Hence, the photon energy of mm waves is even lower than the energy of thermal motion. Only the energy of orientation motion of the molecular dipoles (10^{-3} - 10^{-4} eV) is lower than the photon energy of mm waves. Thus, mm waves can interact with molecules that have rotational degree of freedom such as water molecules. This type of interaction raises the thermal energy of water molecules, i.e. produces heating of water.

With the exception of the stratum corneum layer, skin consists of about 70% of water. Some of the water molecules interact with soluble tissue components and form a hydration shell. This motionally restricted water is called “bound” water. The remaining water molecules are free in

motion and compose the “free” water of tissue. Dispersion of “bound” water and dispersion of tissue components such as proteins, lipids, etc. occur at lower microwave frequencies [Schwan, 1965; Grant, 1982; Smith and Foster, 1985; Mashimo et al., 1987]. The “free” water relaxation region is characterized by the γ dispersion that is well described by the Debye equation.

At higher frequencies (>100 GHz) Buchner et al. [1999], Ellison [2007] found an additional minor relaxation region in the dielectric spectrum of water. There is only a small difference between the permittivity dispersion curves calculated using the Debye equation with either one or two relaxation frequencies at mm waves. However, at THz frequencies the second relaxation region brings in a significant contribution to permittivity. The main absorber of mm waves in biological tissues including skin is “free” water. Permittivity of pure water at 298 K is shown in Fig. 1.1.

Fig. 1.1. Permittivity of pure water calculated using the double Debye equation (Eq. 2.2) with parameters from the paper by Pickwell et al. [2004a].

In most cases, skin permittivity is derived using the direct reflection measurements [Hwang et al., 2003; Alekseev and Ziskin, 2007], time domain reflectometry [Mashimo et al., 1987; Naito et al., 1997], and a quasi-optical method [Alabaster, 2003]. Direct mm wave measurements often require sophisticated equipment, such as mm wave vector network analyzers. The permittivity

data obtained for a homogeneous model of skin at 60 GHz is given in Table 1.1 [Zhadobov et al., 2011].

Table 1.1 Skin permittivity at 60 GHz obtained by different methods

Reference	ϵ^*	T, °C	Method	Sample type
Gabriel et al. [1966]	$7.98 - j10.90$	37	E	<i>In vivo</i>
Gandhi and Riazi [1986]	$8.89 - j13.15$	37	E	<i>In vitro</i>
Hwang et al. [2013]	$8.05 - j4.13$	24-26	M	<i>In vivo</i>
Alabaster [2003]	$9.90 - j9.0$	23	M	<i>In vitro</i>
Alabaster [2003]	$13.2 - j10.3$	37	E	<i>In vitro</i>
Alekseev and Ziskin [2007]	$8.12 - j11.4$	32.6	M	<i>In vivo</i>
Zhadobov et al. [2008]	$9.38 - j12.49$	30	T	-

E, extrapolation; M, direct measurement; and T, theoretical value.

Obtained data shows that in 10-100 GHz range the relative permittivity of skin decreases with increasing frequency while conductivity of skin increases with the increase of frequency.

1.4 Interaction of mm Waves With Skin

1.4.1 Skin Organization

The human skin consists of three individual layers: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer of skin, is about 0.1 mm thick but on the palms of the hands it can be 0.7 mm thick or more [Bloom and Fawcett, 1968; Odland, 1971; El Gammal et al., 1999; Welzel et al., 2004]. The majority of cells in the epidermis are keratinocytes. The surface layer of the epidermis is called the stratum corneum (SC). The SC is made up of flattened dead keratinocytes, which have lost their internal structure. The thickness of the SC in the forearm skin is about 0.012-0.018 mm [Kligman, 1964; Rajadhyaksha et al., 1999; Huzaira et al., 2001; Caspers et al., 2003; Sandby-Moller et al., 2003]. The rest of the epidermis (without the SC) consisting of live cells is called the viable epidermis. The dermis is organized into a papillary (outer) and a reticular (inner) region. The distinction between the two layers is based mostly on their differences in connective tissue. Literature data of the dermis thickness depend on location and on the methods used for thickness measurements, and vary from 1.0 to 2.0 mm [Meema et al., 1964; Black, 1969; Shuster et al., 1975; Dykes and Marks, 1977; Fornage and Deshayes, 1986; Branchet et al., 1990; Gniadecka and Quistorff, 1996]. The hypodermis is composed mainly of fat cells and connects the dermis to underlying tissues and muscle.

1.4.2 Depth of Penetration of mm Waves Into Skin

To evaluate the biological effect of mm waves it is important to know the power density (P_D) distribution within the skin, which can be defined based on the knowledge of the penetration depth of radiation and reflection of mm waves from the skin.

Two models were developed to describe the interaction of mm waves with skin: one-layer (or homogeneous) and multilayer skin models. Multilayer models consist of the SC, the viable epidermis and dermis, and the fat layer [Alekseev and Ziskin, 2007]. The power reflection coefficient for the homogeneous skin model in terms of refractive indices and at normal incidence of mm waves is expressed as

$$R = \left| \frac{n_1 - n_2}{n_1 + n_2} \right|^2 \quad (1.26)$$

where n_1 and n_2 are refractive indices of medium 1 and 2, respectively. If medium 1 is air, as commonly used in experiments, $n_1=1$. The power reflection coefficient decreases from 0.43 to 0.18 as the frequency increases from 30 to 100 GHz [Alekseev et al., 2008].

The amplitude penetration depth (m) is expressed as $\delta=c/\omega n''$, where c is the velocity of light (10^8 m/s), ω is an angular frequency (Hz), and n'' is the imaginary part of the refractive index of medium. The frequency dependence of penetration depth is presented in Fig. 1.2. As can be seen, the penetration depth decreases with increasing the frequency.

Fig. 1.2. Power penetration depth in the human skin. The penetration depth is calculated using permittivity data from Alekseev and Ziskin [2007].

As the wavelengths of mm waves are small in comparison with the size of human body, the geometrical optics concepts can be applied for calculations of interaction of mm waves with skin. That is, mm waves can be considered as rays. The skin can be presented as a flat semi-infinite surface exposed to plane mm waves. To characterize mm wave exposure intensity, the safety standards FCC [1996], ICNIRP [1998], and IEEE [2005] recommend using PD. The PD distribution in lossy homogeneous material is given by the Beer-Lambert's law

$$P_D(z) = P_{D_0} \cdot e^{-2z/\delta} \quad (1.27)$$

where δ is the amplitude penetration depth (m), and P_{D_0} is the incident power density (W/m²). More than 90% of mm wave energy is absorbed within the epidermis and dermis. The rest of mm wave energy is absorbed in the subcutaneous tissue.

In the reactive near field of a radiating antenna, it is not an easy task to derive accurate values of P_D . Therefore, an application of the specific absorption rate (SAR) commonly used at lower frequencies for characterization of absorbed energy is reasonable at mm wave frequencies also. Moreover, SAR is used for calculations of skin heating. SAR at a depth of z of the homogeneous skin model is given by [Gandhi and Riaz, 1986]

$$SAR(z) = \frac{2 \cdot (1 - R)}{\rho \cdot \delta} \cdot P_{D_0} \cdot e^{-2z/\delta} \quad (1.28)$$

where R is the power reflection coefficient, ρ is the mass density (kg/m³), other symbols are the same as in Eq. 1.27. The SAR increases rapidly with increasing frequency due to decreasing the

$$SAR = C \frac{dT}{dt} \Big|_{z=0}$$

power reflection coefficient and penetration depth. If P_{D0} is not known, SAR can be estimated by measuring the initial temperature rise rate in tissue:

$$(1.29)$$

where C is the specific heat capacity ($J/kg/^\circ C$). However, the thermal noise obscuring the initial phase of the heating kinetics reduces the accuracy in SAR determination. The most reliable way to determine SAR is by fitting of a thermal model to the experimental heating kinetics and then to calculate SAR from the model [Alekseev and Ziskin, 2003; Chahat et al., 2012; Zhadobov et al., 2017].

Millimeter waves penetrate deep enough into the skin to affect skin structures located in the epidermis and dermis. Main targets for the mm wave effect in the epidermis are the keratinocytes, melanocytes, and Langerhans cells as well as the Merkel cells located at the dermal-epidermal junction [Bloom and Fawcett 1968; Holbrook and Wolff, 1993; Mehregan et al., 1995]. In the papillary dermis, the mm wave power density is still high. The papillary dermis contains free nerve endings, the most widespread and important sensory receptors. Some free nerve endings extend into the epidermis. Another type of sensory receptor, the Meissner corpuscle, is also located in the papillary dermis. Monocytes and macrophages, as well as mast cells are commonly present in this region.

The microcirculatory beds in skin include arterioles, precapillary sphincters, arterial and venous capillaries, postcapillary venules, and collecting venules [Rhodin, 1967; 1968]. At the junction between the papillary and reticular dermis, terminal arterioles form a horizontal subpapillary plexus giving rise to the capillary loops extending into the papillary dermis. Venous capillaries drain into venules that lie above and below the arteriolar subpapillary plexus. Thus, a large amount of blood vessels and ultimately blood cells are exposed to mm waves at a relatively

high power density. Thus, most skin structures present in the epidermis and dermis could be primary targets for the mm wave action in human skin.

Most theoretical calculations of the power density and SAR distributions in skin were performed using equations 1.27 and 1.28 for a homogeneous skin model. The skin structures such as blood vessels and appendages (hair, sweat ducts, and sebaceous glands) with electrical properties different from the average electrical properties of skin tissue cause either selective absorption of mm wave energy or local distortion of the mm wave field in their vicinity [Alekseev and Ziskin, 2001; 2009a]. In the blood vessels located in the human and murine dermis and oriented parallel to the E-field the SAR could exceed the average SAR in the surrounding dermis by ~40% [Alekseev and Ziskin, 2009a]. In murine skin, SAR in blood vessels normally traversing the fat layer achieved its maximal value at the parallel orientation of the E-field to the vessel axis. At 42 GHz exposure, the maximal SAR in small blood vessels could be more than 30 times greater than that in the skin [Alekseev and Ziskin, 2011]. Analysis of mm wave absorption by blood vessels did not reveal strong frequency dependence of this effect. In evaluating the threshold intensities of biological effects in skin, it is important to take into account the heterogeneous structure of the exposed region. The greater the difference between the electrical properties of skin structures (blood vessels, hair, sweat pores, etc.) and skin, the greater is the distortion of the E-field distribution near these structures. Selective absorption of millimeter-wave energy by small cutaneous blood vessels could hardly result in excessive temperature elevation due to the effective heat dissipation by blood flow and heat conduction to the surrounding tissue. However, the increased E-field strength in the close vicinity of innervated skin appendages and blood vessels could result in a neural effect.

1.4.3 Heating of Skin

Absorption of mm wave energy of any intensity will produce a temperature elevation. If the intensity is sufficiently high, the temperature elevation in tissue will be significant. A modification in function or structure of a biological structure by a large temperature elevation is called a thermal bioeffect. However, thermoregulatory systems of mammals are capable of keeping integral body temperature at nearly the same level even when exposed to an intensity as high as 10 mW/cm^2 . Bioeffects occurring at low exposure intensities producing a temperature rise $\leq 0.1 \text{ }^\circ\text{C}$ are accepted as non-thermal [Betsky, 1993]. This occurs at intensities less than 10 mW/cm^2 . Any effect of electromagnetic energy absorption not associated with the production of heat or a measurable temperature rise is classified as non-thermal [IEEE C95-3, 2002].

As the skin heating is important for understanding the mechanisms of mm wave action and development of safety standards, human skin heating has been studied extensively and the results of these experimental studies are widely present in literature [Walters et al., 2000; 2004; Nelson et al., 2003; Alekseev and Ziskin, 2003; Alekseev et al., 2005]. In experiments, skin is exposed with antennas forming Gaussian beams on the skin surface of different size (between 4.8 to 40 mm in diameter) [Alekseev and Ziskin, 2003; Walters et al., 2000; 2004]. It is shown that the temperature elevation depends on the location of the exposed site on a body, the beam size, and blood flow rate [Walters et al., 2004; Alekseev et al., 2005]. Different thermal models are used for predictions of temperature distributions in skin exposed to mm waves [Nelson et al., 2000; 2003; Alekseev et al., 2005; Stewart et al., 2006; Kanezaki et al., 2008; 2010; Foster et al., 2010; 2016; Shafirstein and Moros, 2011; Zilberty et al., 2013; 2014; Morimoto et al., 2017]. Most of them are based on the Pennes bio-heat transfer equation [Pennes, 1948] that accounts for the

blood flow effect. One and two- dimensional bio-heat transfer equations (BHTE) applied to homogeneous skin have been frequently used, primarily due to their relative simplicity and accuracy in predictions of temperature fields in tissue regions without large (> 1 mm) blood vessels. Two-dimensional BHTE is typically applied for modeling radially-symmetric beam (Gaussian beam) exposure in the near- field of an antenna [Alekseev et al., 2005]. The one-dimensional BHTE is given by [Foster et al., 1978]

$$\rho C \cdot \frac{\partial T}{\partial t} = k \cdot \frac{\partial^2 T}{\partial z^2} - V_s \cdot T + SAR(z) \cdot \rho \quad (1.30)$$

where $T = T_t - T_b$, T_t is tissue temperature ($^{\circ}\text{C}$), T_b is arterial blood temperature ($^{\circ}\text{C}$), k is the coefficient of tissue heat conduction ($\text{W/m}\cdot^{\circ}\text{C}$), $V_s = f_b \cdot \rho_b \cdot C_b$, f_b is the specific blood flow rate (ml/s/ml), ρ_b (kg/m^3) and C_b ($\text{J/kg}\cdot^{\circ}\text{C}$) are the density and specific heat of blood, respectively, $SAR(z)$ is the SAR distribution in tissue, ρ is the density of tissue (kg/m^3), C is the specific heat of tissue ($\text{J/kg}\cdot^{\circ}\text{C}$), and z is distance from tissue surface in the direction of the beam axis (m). In the equation, a term accounting for metabolic heat production is neglected because its contribution to heat production in resting man is relatively low [Hardy, 1982; Nelson et al., 2000], and would not change much during mm-wave exposure.

The irradiating beam size plays a significant role in mm wave heating. The broader the beam, the higher is the surface temperature elevation and the deeper is the heat penetration into tissue [Alekseev et al., 2005].

The BHTE accurately predicts skin heating only at low blood perfusion rates. Blood perfusion produces a cooling effect and reduces the temperature elevation in skin. “Effective conductivity” models have been introduced by Crezee and Lagendijk [1990], Ducharme and

Tikuisis [1991], Crezee et al. [1994], Kolios et al. [1996]. To fit the experimental data the blood flow dependent effective thermal conductivity, k_{eff} , is applied to account for the thermal response in the areas of body (finger) with relatively high blood flow [Alekseev et al., 2005; Alekseev and Ziskin, 2009b].

Three or four layer thermal models are much closer to the real structure of skin and underlying tissue [Stolwijk and Hardy, 1977; Emery and Sekins, 1982; Xu and Werner, 1997; Kanezaki et al., 2008; 2010]. They include dermal, fat, and muscle blood flows. As the multilayer models reflect physiological conditions of different tissue layers they are more adequate for describing thermal events in the skin. In modeling skin heating with mm waves, Alekseev and Ziskin [2009b] used a 4 layer model consisting of the epidermis, dermis, fat, and muscle layers (Fig. 1.3). At skin blood flow rate typical for abdomen and the fat thickness of 2 mm the temperature elevation on skin surface at the incident P_D of 10 mW/cm^2 and frequency of 42 GHz was about 1.2°C . When applying a 1- layer model with the same blood flow rate the temperature elevation was about 2 times smaller. The fat layer plays a role of thermal insulator. With increasing the thickness of the fat layer, the temperature elevation in skin exposed to mm waves is also increased. The areas of skin with low blood flow and thick fat have the maximum temperature elevation. Heat diffuses deep into tissue reaching the fat and muscle layers. Kanezaki et al. [2010] obtained similar results with a model consisting of the skin, fat and muscle layers. The temperature elevation decreases when skin is exposed to a narrow Gaussian-type beam. Experimentally measured temperature elevation of skin surface exposed to 1.06 cm beam was only 0.5°C at the peak power density of 10 W/cm^2 [Alekseev et al., 2005].

Figure 1.3. Temperature increment distributions within tissue calculated for different thicknesses of the fat layer (2, 4, and 8 mm) in a four layer model. Blood flows in skin, fat and muscle layers were $1.4 \cdot 10^{-3}$, $0.43 \cdot 10^{-5}$ and $5.33 \cdot 10^{-4}$ ml/s/ml, respectively.

Thus, exposure of skin to 10 mW/cm^2 definitely can produce thermal effect on cells and structures located not only in the skin but also in the fat and muscle layers.

1.5 Millimeter Wave Effects on Eyes

The eyes, located on the surface of the body like skin, are directly exposed to mm wave irradiation. Exposure to high intensity mm waves can produce adverse effects in the eye. The eye is especially vulnerable to thermal damage because there is no blood flow to internal structures such as the lens. Within the anterior chamber of the eye, heat dissipates only via conduction through the sclera and convection from the surface of the cornea. Overheating of the eyes causes corneal burns and cataracts. Whether the exposure is accidental or occurs in military applications, it is important to determine the thresholds for damage in order to establish appropriate safety standards.

Kues et al. [1999] studied the ocular effects of 60 GHz mm waves at an intensity of 10 mW/cm^2 . Nonhuman primates and rabbits were exposed once for 8 h, and for 4 h per day during

5 consecutive days. The authors did not find any ocular changes at microscopic examination and diagnostic procedures that could be attributed to mm wave exposure. Thus, the low intensity mm wave exposure does not result in any detectable ocular damage.

However, mm wave exposures at high intensities produce adverse effects on eyes. Rosenthal et al. [1976] reported the results of the study of the mm wave effects on rabbit eyes exposed to 35 and 107 GHz at 50 mW/cm² for 15-80 min. These exposures induced both epithelial damage and stromal edema in the cornea. The immediate stromal damage produced by 107 GHz exposure recovered by the next day.

Chalfin et al. [2002] investigated the effects of pulsed 35 and 94 GHz exposure on the anterior segment of the nonhuman primate eye at the exposure intensities of 2 and 8 W/cm². The corneal temperature elevation at 94 GHz exposure was 35°C. The authors detected distinct lesions, involving only superficial layers of the cornea. The corneal damage caused by 35 GHz exposure at 2 W/cm² for 1.5-5 s was reversible within 24 h. The mean threshold fluence required to produce a corneal lesion was 7.5 J/cm² at 35 GHz and 5 J/cm² at 94 GHz.

The effect of high intensity 60 GHz exposure on the rabbit eye was studied by Kojima et al. [2009]. Exposure for 6 min at the intensity of 1,898 mW/cm² led to an elevation of the corneal surface temperature to 54.2°C accompanied with corneal edema and epithelial cell loss. Anterior uveitis also occurred resulting in acute miosis, an increase in flares, and iris vasodilatation. Heat generated by mm wave exposure could apparently penetrate below the surface of the eye inducing the observed thermal effects. The effects of mm waves were dependent on the intensity and duration of exposure.

Different numerical models were developed to describe the temperature distribution in rabbit, nonhuman primate, and human eyes following mm wave exposure [Scott, 1988; Foster et

al., 2003; Papaioannou and Samara, 2010; Karampatzakis and Theodoros, 2010; 2013]. For example, Foster et al. [2003] modeled the empirical temperature data obtained by Chalfin et al. [2002]. The results of calculations using one-dimensional heat conduction equation were in agreement with experimental data. The thresholds for damage to the cornea corresponded to temperature elevation by about 20°C at both irradiation frequencies 35 and 94 GHz. The authors concluded that the simple heat conduction equation could be used for predictions of thermal damage thresholds to the cornea.

The experimental results show that low intensity (10 mW/cm²) mm wave irradiation does not produce any harmful effect on eyes. However, the high intensity mm wave exposure results in adverse effects on eye. The ocular damage is induced by the thermal effect of mm waves and depends on the frequency, intensity and duration of exposure.

1.6 Millimeter Wave Effects on Subcellular and Cellular Levels

1.6.1 Effects on Membrane Level

6.1.1.1 *Artificial Lipid Membranes*

Cellular membrane is believed to be one of the main targets of mm wave action. This belief is substantiated by the theoretical works by Frölich [1968; 1970; 1972; 1978] suggesting that microwaves are capable of producing coherent longitudinal electrical oscillations in lipid membranes. Artificial lipid membranes are simple and convenient models for study of mm wave effects on ion transport, phase transition, hydration, membrane surface charge, lipid peroxidation and other processes.

Many studies were carried out on models of cellular membranes such as liposomes and bilayer lipid membranes [Alekseev and Ziskin, 1995; Logani and Ziskin, 1996; 1998; Ramundo-Orlando et al., 2009; Di Donato et al., 2012; Beneduci et al., 2012; 2013; 2014; Albini et al., 2014]. Di Donato et al. [2012] found the increase of permeability of cationic liposomes loaded with carbonic anhydrase and exposed to 53.37 GHz with an intensity as low as 0.1 mW/cm^2 . In a number of papers, the effects of mm waves on giant phospholipid vesicles were reported [Ramundo-Orlando et al., 2009; Albini et al., 2014]. Giant liposomes dispersed in 0.2 M sucrose solution were exposed from the top of a chamber to 53.37 GHz mm waves at 0.1 mW/cm^2 . Direct optical observations in real time allowed authors to discover induced diffusion of fluorescent dye di-8-ANEPPS into the bilayer membrane, elongation of vesicles, increased attraction between vesicles, and their vectorial movement. It was concluded that the observed effects cannot be attributed to local heating. However, the effect of convection, induced in the liquid medium by mm wave exposure, on the properties of lipid vesicles could not be excluded. Beneduci et al. [2013] reported the effect of mm waves on phosphatidylcholine vesicles using deuterium nuclear magnetic resonance ($^2\text{H-NMR}$) spectroscopy. Multilamellar vesicles were exposed to 53.57-78.33 GHz frequency range at $0.0035\text{-}0.010 \text{ mW/cm}^2$ with a Teflon antenna inserted into the NMR probe. Exposure at temperatures close to the phase transition of the membrane produced a transition of the membrane from the fluid to the gel phase. During phase transitions, the significant structural changes occur in the membrane, and the membrane becomes sensitive to external physical influences. The authors interpreted the results as a hydrogen-dependent reduction of the water ordering around the phosphatidylcholine headgroups [Beneduci et al., 2014].

Liposomes were used to study the effect of mm waves on lipid peroxidation in the presence and absence of melanin, a pigment produced in the epidermis by melanocytes [Logani and Ziskin, 1996]. Liposomes were exposed to 53.6, 61.2, and 78.2 GHz at incident power densities of 10, 1.0, and 500 mW/cm², respectively. The authors did not find an enhancement of the formation of lipid peroxides compared to unexposed samples. Direct exposure of melanin also did not exhibit an increased formation of superoxide or hydrogen peroxide.

Zhadobov et al. [2006] investigated the effect of millimeter waves on superficial pressure and microdomain distribution in phospholipid monolayers. Microdomains reflect functional specialization of the different membrane regions and play an important role in membrane interaction processes. Exposure at 60 GHz and power density of 0.9 mW/cm² for 5 h resulted in increase of lateral pressure. Low-power millimeter wave exposure did not induce significant transformations in the phospholipid domain organization in model membranes.

Direct measurements of the conductance and capacitance of lipid bilayer membranes (BLM) exposed to mm waves in the range of 54-76 GHz did not reveal any of the effects found in the experiments with giant liposomes which could affect the electrical properties of BLM [Alekseev and Ziskin, 1995]. A waveguide isolated from the solution by thin Teflon film was placed at specific distances d ($d \geq 0$ mm) from the BLM formed on a tip of plastic pipette. The pipette was located in the center of a chamber. Such an experimental setup excluded artifacts caused by the evaporation of water on the surface of solution when exposed from the top of a chamber, or influence of the bottom material and its size when exposed from the bottom of a chamber. Some membranes were modified by gramicidin A, amphotericin B, or tetraphenylboron anions, which increased the conductance of BLM. Gramicidin A and amphotericin B form ionic channels in a membrane, and model the ionic channels in excitable cells. Tetraphenylboron is a lipid soluble

ion. BLM was exposed to continuous wave (CW) and pulsed wave (PW) mm waves at repetition rates ranging from 1 to 100 pulses per second (pps) and PW at 1000 pps. In the 54-76 GHz range at 1 GHz steps, no resonance-like effects on capacitance and conductance of modified membranes were detected. All changes in membrane capacitance and conductance were independent on the modulation employed, and were equivalent to heating by approximately 1.1°C with an SAR of 2 kW/kg. These results clearly demonstrate that coherent oscillations in BLM are not excited in the frequency range of 54-76 GHz.

Thus, some results obtained in experiments with artificial lipid membranes are inconsistent with the thermal effect of mm waves. However, the mechanisms underlying these effects are not clear. It is obvious that more studies with better control of the exposure conditions are required to evaluate the validity of these results.

1.6.1.2 Cellular Membranes

The results of early studies of mm wave effects on ionic channels of cellular membranes are described in details in the review by Pakhomov et al. [1998]. Most of studies reported the positive effects of non-thermal low intensity mm waves on Ca^{++} activated K^{+} channels [Geletyuk et al., 1995; Fesenko et al., 1995], and chloride and calcium channels [Kataev et al., 1993]. Here we describe the results of two studies of mm wave action on K^{+} and Ca^{++} currents in snail neurons [Alekseev and Ziskin, 1999] and voltage-gated sodium and potassium channels expressed by *Xenopus laevis* oocytes [Shapiro et al., 2013].

Alekseev and Ziskin [1999] investigated the effects of mm waves 60.22-62.22 GHz and 75 GHz on A-type K^{+} currents, and the effects of 61.22 GHz on Ca^{++} currents of *Lymnaea* neurons. In a study of the frequency dependent effects of irradiation on the peak amplitude and

inactivation components of A-currents, the frequency was changed at 0.1 GHz intervals within the 60.22-62.22 GHz range. A neuron attached to a polythene pipette was exposed with a waveguide opening covered with thin Teflon film and placed into solution close to the neuron. The SAR was in the range of 0-2400 W/kg. This type of setup allowed authors to record ionic currents during exposure without artifacts. Millimeter wave exposure increased the peak amplitudes, activation and inactivation rates of both ionic currents. These changes were independent of frequency and resulted from the temperature rise produced by the irradiation. No additional effects of irradiation on A-type K^+ currents other than thermal were found when tested at the phase transition temperature. Millimeter waves had no effect on the steady-state activation and inactivation curves, suggesting that the membrane surface charge and binding of calcium ions to the membrane in the area of channel locations did not change. The absence of resonance-like frequency dependent effects of mm waves on BLM [Alekseev and Ziskin, 1995] and cellular membrane does not support Frölich's theory. However, it is not excluded that the resonance-like effects may occur in a different frequency range.

In the recent article by Shapiro et al. [2013], the results of study of 60 GHz exposure effects on the voltage-gated sodium and potassium channels in oocytes were presented. The irradiation power was delivered to oocytes through an open-ended waveguide from the bottom of a recording chamber. The power densities at the top and bottom of oocytes were 1-33 and 18-60 mW/cm², respectively. Oocyte sat on the bottom of a chamber on a 0.25 mm quartz coverslip. This setup excluded most of artifacts in recording ionic currents except those arising from convection currents resulting from the elevated temperature [Zhadobov et al., 2017]. The observed changes in the kinetics and activity levels of voltage-gated potassium and sodium channels were consistent with a thermal mechanism. The authors suggested that mm wave

exposure produced significant thermally mediated effects on excitable cells via basic thermodynamic mechanisms.

Thus, the more recent studies do not confirm the non-thermal effects of mm waves on electrical events in cell membranes.

1.6.2 Effects on Gene Expression

Gene expression is a process by which information from a gene is used to synthesize a functional gene product. These products are often proteins. Disturbances in gene expression if they are accompanied with DNA damage may result in serious medical conditions including cancer. That is why the studies of mm wave effects on gene expression and DNA damage, genotoxic effects, are very important and attract the efforts of the scientific community.

Vijayalaxmi et al. [2004] investigated the genotoxic potential of mm wave radiation on mice exposed to 42.2 GHz at the incident power density of 31.5 mW/cm². The radiation was applied locally to the nasal region of mice for 30 min/day for 3 consecutive days. The extent of genotoxicity was assessed from the incidence of micronuclei in polychromatic erythrocytes of peripheral blood and bone marrow cells collected 24 h after treatment. The results indicated that there was no evidence for the induction of genotoxicity in the peripheral blood and bone marrow cells of mice exposed to mm waves.

The influence of 60 GHz mm wave exposure at an intensity of 0.54 mW/cm² on a set of stress-sensitive gene expression of molecular chaperones, clusterin and Hsp70, in a human brain cell line was investigated in experiments with long exposure durations up to 33 h [Zhadobov et al., 2007]. The results demonstrated the absence of significant modifications in gene transcription, mRNA, and protein amount for the evaluated stress-sensitive genes. The authors

concluded that low-power 60 GHz radiation does not modify stress-sensitive gene expression of chaperon proteins.

The effects of mm waves on endoplasmic reticulum stress sensor gene expression were reported in a number of papers [Nicolaz et al., 2009a; 2009b; Le Quement et al., 2014]. Exposure at a frequency of 60 GHz (or 8 frequencies within the 59-61 GHz range) with different intensities up to 20 mW/cm² did not change the gene expression.

Soubere Mahamoud et al. [2016] analyzed modifications of the whole genome of a human keratinocyte model exposed to 60.4 GHz at the incident power density of 20 mW/cm² for 3 h. No keratinocyte transcriptome modifications were observed. The effects of mm waves on cell metabolism were tested by co-treating mm wave exposed cells with a glycolysis inhibitor, 2-deoxyglucose. Whole genome expression was evaluated along with the ATP content. In this case, the treatment did not alter the keratinocyte ATP content, but it slightly altered the transcriptome, which indicated the capacity of mm waves to interfere with the bioenergetic stress response.

The effect of mm waves on neuronal differentiation was studied using a neuron-like cell line (PC 12) [Haas et al., 2016a]. PC12 cell monolayers were exposed to 60.4 GHz for 24 h at the incident power density of 10 mW/cm². The PC 12 neurite outgrowth and cytoskeleton protein expression were assessed. No changes in protein expression were found. A slight increase in neurite outgrowth was related to the thermal effect of mm wave exposure. The same authors used a PC 12 subclone Neuroscreen-1 cell line to investigate the effects of mm waves on the expression of receptors of the Transient Receptor Potential cation channel subfamily TRPV1 and purinergic receptor P2X [Haas et al., 2016b]. Exposure to 60.4 GHz with the incident power

density of 10 mW/cm² revealed no effect on protein expression of Hsp70, TRPV1, TRPV2, and P2x3.

To test the genotoxic effect of mm waves at frequency of 60 GHz and intensity of 1.0 mW/cm² Koyama et al. [2016a] used human corneal and lens epithelial cells. Exposure for 24 h did not reveal any genotoxic effect of mm waves in human eye cells.

Changes in gene expression were discovered in rat skin following exposure to 35 GHz at 75 mW/cm² resulting in overheating of the skin up to 42-43°C [Millenbaugh et al., 2008].

Microscopic findings observed in the dermis of rats included aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen. Changes were detected in 56 genes at 6 h exposure and 58 genes at 24 h exposure. Genes associated with regulation of transcription, protein folding, oxidative stress, immune response, and tissue matrix turnover were affected at both times. The obtained results indicate that prolonged exposure to high intensity mm waves causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery.

In another paper, the effect of high intensity mm waves on biological activity of soluble factors in the plasma of exposed rats was reported [Sypniewska et al., 2010]. Rats were exposed to 35 GHz at 75 mW/cm² until core temperature reached 41.0°C. NR8383 rat macrophages were incubated with 10% plasma of exposed rats. Plasma from exposed rats increased the expression of 11 proteins and level of 3-nitrotyrosine, a marker of macrophage stimulation, in seven proteins. The altered proteins are associated with inflammation, oxidative stress, and energy metabolism.

Thus, no genotoxic effect was found in cells exposed to mm waves at intensities up to 75 mW/cm². Most studies show the absence of the effects of mm waves on gene expression at

exposure intensities $\leq 20 \text{ mW/cm}^2$. Changes in gene expression occur at much higher exposure intensities causing significant temperature elevations in skin.

1.6.3 Effects on Cellular Proliferation

The antitumor effects of millimeter waves are studied using animal models and in experiments on a cellular level *in vitro*.

The murine experimental model of B16 F10 melanoma was used to evaluate the ability of mm waves to influence tumor growth [Radzievsky et al., 2004a]. The nasal area of mice was exposed to 61.22 GHz with the average incident power density of 13.3 mW/cm^2 for 15 min a day during 5 consecutive days. The temperature elevation at the tip of the nose was 1.05°C . The mm wave treatment induced suppression of the subcutaneous tumor growth. Moreover, endogenous opioids were involved in this effect.

One of the major side effects of cyclophosphamide (CPA), a commonly used anti-cancer drug, is that it can enhance tumor metastasis due to suppression of natural killer (NK) cell activity. Logani et al. [2006] studied the ability of mm waves to inhibit tumor metastasis enhanced by CPA. Tumor metastasis was evaluated in mice that were injected through a tail vein with melanoma cells. Animals were exposed at the nasal area to 42.2 GHz with an incident power density of 36.5 mW/cm^2 and $\text{SAR}=730 \text{ W/kg}$ for a 30 min exposure that caused a maximum temperature rise of 1.5°C . CPA caused a marked enhancement of tumor colonies in lungs, which was significantly reduced when CPA-treated animals were irradiated with mm waves. Millimeter wave exposure increased NK cell activity suppressed by CPA, suggesting that a reduction in tumor metastasis by mm waves is mediated through activation of NK cells.

Antiproliferative effect of low intensity mm waves on a cellular level *in vitro* was described by a group of authors with Beneduci [Chidichimo et al., 2002; Beneduci et al., 2005a: 2005b; 2007; 2009]. Using a noise generator in the frequency range of 53.57-78.33 GHz or monochromatic frequencies in the range of 52-78 GHz with output powers of generator less than 1 mW, the authors were able to observe the effective inhibition of melanoma cell growth. Similar results were obtained under the same exposure conditions in a study of the antiproliferative effect of mm waves on human erythromyeloid leukemia cells in culture [Beneduci et al., 2007]. The power densities used in these experiments were too low to cause a thermal effect. The proposed explanation of the possible mechanisms of mm wave action underlying the antiproliferative effect is yet to be proved.

Li et al. [2012; 2014] reported on the different effects of mm waves on chondrocyte proliferation. Chondrocytes produce and maintain the cartilaginous matrix, which protects the ends of long bones at the joints. Chondrocytes were isolated from the rat knee. The second generation of chondrocytes was exposed to mm waves (7.0-10.0 mm) from the top of the Petri dish at an intensity of 4 mW/cm². Millimeter wave irradiation caused progression of the cell cycle of chondrocytes *in vitro* thus promoting chondrocyte proliferation. The actual intensity of mm waves at the cell layer located on the bottom of the Petri dish is much lower than the incident power density due to the strong absorption of mm waves by the liquid medium. However, a thermal mechanism of the mm wave action is not ruled out due to convective stirring of bulk culture medium above the heated medium on the surface.

E. coli bacterial cell viability and metabolic activity was used to evaluate the mm wave effect on cell proliferation [Cohen et al., 2010]. Cell suspension was exposed to 99 GHz at 0.2 mW/cm². One hour exposure produced no effect on *E. coli* viability and colony characteristics.

Exposure for 19 h caused a slight proliferation but did not influence the metabolic activity of cells. The authors concluded that the slight proliferation (five fold) following 19 h exposure was not biologically significant.

Thus, mm wave irradiation on cells cultures and in animals has an anticancer effect. However, the proliferative effect of mm waves on other cells is controversial in spite of the comparable exposure intensities. The indirect effect of mm waves in animals seems to be mediated through the nervous system and subsequently involving secretion of endogenous opioids.

1.6.4 Resonance Effects

Knowing the temperature responses of biological objects, the thermal effects of mm waves are well predictable. Therefore, investigation of mm wave specific effects (not related to heating) requires exposures with intensities that would be incapable of significant temperature elevation. Consequently, many if not most publications available in peer reviewed literature sources are devoted to the study of mm wave effects at low intensities $\leq 10 \text{ mW/cm}^2$.

Some early studies had discovered sharp resonance-like frequency dependent effects [Gründler et al., 1977, 1983, 1989, 1992; Belyaev et al., 1994, 1996; Gapeev et al., 1996]. Gründler and coauthors found that the growth rate of the yeast *Saccharomyces cerevisiae* was strongly dependent on exposure frequency in the very narrow range 41.8-42.0 GHz. The effect of about the same magnitude was observed in the wide range of exposure intensities from 5 pW/cm^2 to 10 mW/cm^2 . However, careful attempts to reproduce these results by other authors were not successful [Furia et al., 1986; Gos et al., 1997].

Belyaev et al. [1993a; 1993b; 1994; 1996] reported that mm wave exposure at intensities as low as 10^{-19} W/cm² produced the frequency dependent changes in the genome conformational state of *E.coli* cells with resonance peaks at 51.76 and 41.34 GHz. The dependence of resonance frequencies on the length of haploid genome allowed authors to suggest the involvement of the chromosomal DNA in the resonance interaction of mm waves with cells. These results were obtained using an anomalous viscosity time dependent technique. However, this method is not common for this type of study and its interpretation is questionable [Pakhomov et al., 1998].

Gapeyev et al. [1996] found the frequency-dependent inhibition of the production of oxygen active forms by neutrophils opsonized by zymozan. This effect was observed only in the near field of an antenna in the narrow frequency range 41.5-42.7 GHz at 2.5 mW/cm². Exposure in the far field of the antenna resulted in elimination of the frequency dependence of the effect. This result indicates that the frequency dependent effect may be caused by the “geometrical” resonances appearing in the near field of the antenna [Betsky, 1993; Khizhnyak and Ziskin, 1994].

It should be noted that the resonance frequencies mentioned in the above studies lay in the same frequency range 41-43 GHz. The interest to the possible resonance effects of mm waves has remained a constant subject of debates [Shneider and Pekker, 2013; 2014]. However, independent thorough replications of the above claimed resonance effects are necessary before the resonance mechanism can be accepted as a biological action of mm waves.

However, there has been a recent finding of an interesting resonant effect. Feldman et al. [2008] have proposed that sweat ducts in Human skin act as helical antennas in the millimeter and submillimeter wave range. Within the 95 to 110 GHz frequency range, there is an enhanced absorption due to a resonance with the sweat ducts twisted into a helical coil and filled with

sweat, a high water containing fluid. This enhanced absorption could lead to a highly localized temperature increase that would rapidly diffuse. Any bioeffect resulting from this effect would be from the temperature elevation if the incident intensities were sufficiently great.

1.6.5 Cellular Effects

Keratinocytes are the most numerous cells of the epidermis. The effect of mm waves on human keratinocytes was studied in experiments *in vitro* at exposures to 61.22 and 42.25 GHz with intensities 29 mW/cm² (SAR=770 W/kg) and 1.67 W/cm², respectively [Szabo et al., 2003]. The viability of keratinocytes, the productions of heat shock protein (Hsp70) and chemokines, including RANTES and IP-10, were analyzed. In addition, the intercellular gap junctional communication in keratinocyte monolayers was investigated. At low intensity exposure (29 mW/cm²) no significant changes in keratinocyte viability and in constitutive RANTES and inducible IP-10 production were found. No alterations in Hsp70 production and the gap junctional intercellular communication was observed. Exposure at intensity of 1.67 W/cm² induced cellular damage and heat stress reaction. The maximal temperature at the end of 30 min exposure reached 45.2°C. The authors stressed that exposure at low intensity (29 mW/cm²) provided no evidence of skin inflammation or keratinocytes damage.

The same authors studied the effect of mm wave exposure to 61.22 GHz with SAR of 770 W/kg on proliferation, adhesion, chemotaxis, and interleukin-1beta production in human keratinocytes [Szabo et al., 2001]. Exposure duration was 15-30 min. Exposure did not produce any effect on spontaneous proliferation, adhesion, chemotaxis except for a modest, but statistically significant increase in the intracellular level of IL-1beta. It seems that mm waves can cause activation of basal keratinocytes resulting in an elevated level of IL-1beta production.

The absence of the effect of mm wave exposures to 30.16 GHz with intensities of 1.0 and 3.5 mW/cm² on gap junction intercellular communication in human keratinocytes was confirmed by Chen et al. [2004].

The effect of 94 GHz exposure on skeletal muscle contraction was studied by Chatterjee et al. [2013]. Electric field magnitude in excess of 2.6 kV/m was able to cause a reproducible decrease in force production of skeletal muscle in the absence of measurable changes in temperature. The authors measured temperature only in the fluid forcibly circulated around the excised muscle. As calculated by Wu et al. [2015] the electric field in excess of 2.6 kV/m would result in an SAR_ρ level in muscle well in excess of 1000 kW/m³, which would definitely result in muscle heating.

A new mechanism of mm wave action on cellular level was discovered by Szabo et al. [2006]. Exposure of the human keratinocytes and murine melanoma cells to 42.25 GHz at the incident power density of 1.23 W/cm² induced reversible externalization of phosphatidylserine (PS) molecules without detectable membrane damage. The negatively charged PS molecules are normally localized within the internal leaflets of the phospholipid bilayer of plasma membranes in live cells. Their surface expression, that is, rotation of the negatively charged pole of the PS molecule from the inner surface of the cell membrane to the outer surface, is called externalization. The externally exposed portion of the molecule apparently possesses active sites upon which ligands and other molecules can attach and activate biological processes. Externalization is an early event of programmed cell death allowing recognition of apoptotic cells by phagocytes [Fadok et al., 1992; Martin et al., 1995]. Jurkat cells exposed to much lower intensity of 34.5 mW/cm² also showed reversible PS externalization. The authors could not exclude the effect of enhanced convection in liquid medium produced by local heating

of sample by 3.5°C as the possible mechanism of mm wave action. The involvement of non-thermal mechanisms in the effect cannot be ruled out either, but there is not enough data to develop a specific model.

Thus, low intensity mm wave exposure did not reveal changes in functioning of cellular systems. The effects induced by high power irradiation had most probably a thermal origin. The data on PS externalization show a new potential of mm wave action on cellular membranes and needs further verification in independent studies.

1.7 Millimeter Wave Effects on Excitable Tissues

A great number of early studies, mostly in the Former Soviet Union, were carried out exploring the effects of low intensity (≤ 10 mW/cm²) mm waves on conduction of the compound action potential (CAP) in isolated nerves. Several positive effects were reported in the literature [Pakhomov et al., 1998]. Pakhomov et al. [1997] investigated the effects of mm waves (CW, 40–52 GHz, 0.24–3.0 mW/cm²) on the CAP conduction in an isolated frog sciatic nerve preparation, and found none of the reported positive effects on CAP conduction. The low-rate electrical stimulation of the nerve mm wave exposure for 10–60 min at 0.24–1.5 mW/cm², either at various constant frequencies or with a stepwise frequency change (0.1 or 0.01 GHz/min), did not cause any detectable changes in CAP conduction or nerve refractoriness. The changes in CAP observed at higher intensities of 2–3 mW/cm² were both qualitatively and quantitatively similar to the effect of conventional heating causing a temperature rise of 0.3–0.4 °C. At high rate stimulation of the nerve, the gradual and reversible decrease of amplitude of the test CAPs

was retained when the nerve was exposed to 41.22 and 50.91 GHz. The effect was dependent on the frequency rather than on the intensity of the irradiation.

The effect of 94 GHz exposure on the assembly/disassembly of neuronal microtubules in *Xenopus* spinal cord neurons was studied by Samsonov and Popov [2013]. The microtubule array is regulated by a large number of signaling cascades, and may provide information about the biochemical status of neuronal cytoplasm. An exposure at 1.86 W/cm² increased the rate of microtubule assembly. This effect resulted entirely from the rapid temperature jump by 8°C induced by the mm wave exposure.

Modulation of calcium oscillations by mm wave irradiation in the mouse embryonic stem cell-derived neuronal cells was reported by Titushkin et al. [2009]. It is evident that the calcium spiking amplitude, frequency, and spatial distribution are important characteristics of cellular regulatory pathways. Fibroblast cell cultures were exposed to 94 GHz at 1.86 W/cm² to study the effect of millimeter wave exposure on actin filament organization [Titushkin et al., 2009]. Millimeter wave irradiation significantly increased the calcium spiking frequency. The N-type calcium channels, phospholipase C, and actin cytoskeleton appear to be involved in mediating increased calcium spiking. Reorganization of the actin microfilaments by a 94 GHz field seems to play a crucial role in modulating not only calcium activity but also cell biomechanics (decrease of cell elasticity). Millimeter wave exposure for 30 minutes caused some damage to the actin structure. Many but not all observed cellular responses to millimeter wave exposure were similar to thermally induced effects.

Alekseev et al. [1997] presented the results of a study of mm wave effects on the firing rate of intact pacemaker neurons in the isolated nerve ring of the snail *Lymnaea stagnalis*. The neurons were exposed to mm waves at a frequency of 75 GHz, and an SAR ranging from 600 to

4200 W/kg, causing temperature rises of 0.3 and 2.2°C, respectively. At an SAR = 4200 W/kg, exposure to mm waves produced biphasic changes in the firing rate, i.e., a transient decrease in the firing rate within the first 10 s of exposure by 69% below control, followed by a gradual increase with the time constant of 3.7 min to a new level exceeding the initial firing rate by 68%. The biphasic changes in the firing rate were reproduced by heating under the condition that the magnitude and the rate of temperature rise were equal to those produced by the irradiation. It was shown that the transient response resulted from the hyperpolarization of the neural membrane due to the thermal activation of the sodium pump. The slow increase in the firing rate was due to the depolarization of the membrane caused by its elevated permeability to sodium and potassium ions. The threshold stimulus for a transient response of a neuron found in warming experiments was a temperature rise rate of 0.0025°C/s. The firing rate changes occurred at temperature elevations as small as 0.3°C. The phase of transient decrease of the firing rate could be singled out by varying the exposure pulse duration [Alekseev et al., 2000]. Typically, the decrease in the firing rate occurs when the temperature is decreased. It is not clear what are the physiological consequences resulting from the transient response of neurons.

Shapiro et al. [2013] showed that 60 GHz mm wave stimulation significantly increased the action potential firing rate in oocytes coexpressing voltage-gated sodium and potassium channels. The time course of change in the spiking interval upon application of mm waves paralleled that of the temperature rise. The bulk temperature increases, similar to those achieved with mm wave exposure, also resulted in a similar acceleration in firing rate indicating a thermal nature of the mm wave effect. In addition, authors investigated the response of the sodium-potassium pump to mm wave exposure in oocytes. Upon application of a 100 s mm wave pulse (64 mW), producing a temperature elevation of about 6°C, the pump current increased by 53%

within the first 10 s of exposure. The changes in the kinetics and activity level of a sodium-potassium pump were consistent with a thermal mechanism.

Two studies were performed to explore the mm wave effects on electrical activity of organotypic cortical slices [Pikov et al., 2010] and individual neurons of leech ganglia [Romanenko et al., 2014]. Pikov et al. [2010] exposed the slices of cortical tissue located in the bottom of the exposure chamber from the top of the bath solution to 60.1 GHz at the highest incident power density of 90 mW/cm². Less than 1 μW/cm² reached the slice level located at 2.2 mm below the solution surface. One min exposure caused the reduction of the firing rate, narrowing of the width of the action potential, and decrease of the membrane resistance. These effects were accompanied by mm wave heating of the bath solution by 3°C. The authors compared these changes with the effects of conventional bath heating by 10°C and found that mm wave-induced effects cannot be fully attributed to heating and may involve specific mm wave interaction with tissue. It should be noted that in modeling mm wave heating it is necessary to reproduce not only the magnitude of the temperature rise, but also the temperature rise rate, which plays a significant role in neural responses.

In another study, Romanenko et al. [2014] investigated the effects of 1 min exposures to 60 GHz at the incident power densities of 1, 2, and 4 mW/cm² on the action potential of neurons of the leech ganglia. The firing rate of neurons was slightly suppressed during mm wave exposure while gradual bath heating caused a linear dose-dependent increase in the firing rate. Millimeter wave exposure produced narrowing of the action potentials, which was 5 times greater at 4 mW/cm² than at equivalent bath heating by 0.6°C. Qualitatively, the changes in the firing rate of leech neurons produced by mm wave exposure were similar to those of snail neurons [Alekseev et al., 1997], i.e., rapid mm wave heating induces a transient decrease of the firing rate followed

by a slower increase in the firing rate at longer exposure. The ganglion was exposed from the bottom of a chamber. The exposure beam passed through the polystyrene bottom of a Petri dish 1 mm thick and a paraffin layer 3.2 mm thick before reaching the ganglion. During exposure with the incident power density of 53-212 mW/cm², the bottom of the Petri dish was definitely heated. Due to the high thermal conductivity of paraffin (0.26 W/(m· °C)) the heat could pass from the polystyrene to the ganglion causing heating of the ganglion in addition to the direct effect of mm waves. Therefore, it would be worthwhile to reproduce the study with adequate temperature control.

Most studies showed the changes in the electrical activity and structure of the excitable cells induced by a thermal effect of mm waves. It was shown that the rate of heating plays a significant role in eliciting the electrical response of nerve cells. Some specific effects are apparently not due to a thermal action of mm waves, and require further investigation.

1.8 Involvement of Nervous System in Millimeter Wave Effects

Millimeter waves are widely used in Russia, China, and many Eastern European countries for the treatment of more than 30 diseases, including peptic ulcers, pain relief, cardiovascular diseases, and skin disorders [Rojavin and Ziskin, 1998]. Positive results were obtained at exposure to very low intensities (usually less than 1 mW/cm²). However, most findings made by scientists in these countries have not been replicated in the West. The systematic comprehensive investigation of hypoalgesic effects of mm waves on humans and animals was carried out in Temple University in the USA.

To evaluate the pain relief effect of mm waves in humans, double-blinded, randomized, cross-over, prospective experiments were conducted using cold pressor test as a model of tonic aching pain [Radzievsky et al., 1999]. Twelve volunteers participated in the study. They were exposed to 42.25 GHz at the incident power density of 25 mW/cm² for 30 min. Millimeter wave exposure produced a significant suppression of pain sensation with an average 37.7% gain in pain tolerance.

The hypoalgesic effect of mm waves on the experimentally induced cold pain in 20 healthy volunteers was studied in a double-blinded crossover investigation by another group of researchers [Partyla et al., 2017]. Volunteers were exposed to 42.25 GHz at the intensity of 17.2 mW/cm². The hypoalgesic effect of mm waves resulted in a 23-25% delay in the onset of cold sensation, pain threshold, and beginning of increasing pain compared with baseline condition. However, onsets in the placebo condition did not differ significantly from the exposure condition, only partially confirming the hypoalgesic effect of mm waves. Positive results were obtained in the treatment of chronic pain with mm wave therapy in patients with diffuse connective tissue diseases at the intensities of 2.5 mW/cm² [Usichenko and Hergert, 2003]. It is obvious that appropriate experimental investigations are required using large samples and improved methods of pain stimulation and data acquisition to better evaluate the hypoalgesic effects of mm waves in humans.

The first experiment involving the nervous system in animals revealed that mm wave exposure was able to extend the duration of anesthesia caused by ketamine and chloral hydrate in mice [Rojavin and Ziskin, 1997]. In these experiments, mice were exposed to 61.22 GHz at the incident power density of 15 mW/cm². Using the cold-water tail-flick test (experimental model of chronic non-neuropathic pain), it was found that the effect was pronounced and comparable to

that of 1 mg/kg of morphine, [Rojavin et al., 2000]. The authors showed that the hypoalgesic effect on mice was proportional to the incident power density. The effect reached the level of about 200% relative to the control, and disappeared after reducing the incident power density to 0.5 mW/cm². Radzievsky et al. [2000] compared the hypoalgesic effect of 61.22 GHz mm waves at 15 mW/cm² in different sites on the murine body with different innervation densities. They found that the maximal hypoalgesic effect was obtained where the innervation density was the greatest: the nose and paw areas.

The possible involvement of the sciatic nerve in the hypoalgesic effect of mm waves was studied in experiments with the transected nerve [Radzievsky et al., 2001]. The murine paw was exposed to 61.22 GHz with the average incident power density of 15 mW/cm². Dissection of the nerve resulted in a full loss of the hypoalgesic effect of mm waves. This result directly indicated the involvement of the peripheral nervous system in mm wave-induced hypoalgesia. The pretreatment of mice with naloxone, a nonselective antagonist of opioid receptors, also fully blocked the hypoalgesic effect of mm waves [Rojavin et al., 2000]. This result indicated that the antinociceptive effect of mm waves was mediated through endogenous opioids. Moreover, the involvement of δ and κ endogenous opioids in the mm wave-induced hypoalgesia was demonstrated using selective blockers of δ - and κ -opioid receptors and the direct ELISA measurement of endogenous opioids in central nervous system (CNS) tissue [Radzievsky et al., 2008]. Thus, a number of endogenous opioids seems to play an important role in the systemic effect of mm waves involving the CNS.

Using an experimental model of chronic non-neuropathic pain Radzievsky et al. [2008] studied the frequency dependence of the hypoalgesic effect of mm waves. The maximal effect was achieved with exposure of the nasal area of mice with 61.22 GHz mm waves. An equivalent

local heating of the same area with a laser did not produce a systemic hypoalgesia [Radzievsky et al., 2004b]. Based on these results, the authors concluded that the hypoalgesic effect had a non-thermal nature. However, the initial mechanisms underlying this effect remain unclear. It is suggested that mm waves could stimulate the skin sensory receptors.

Alekseev et al. [2010] studied the mm wave effect on the electrical activity of the sural nerve of mice *in vivo*. The sural nerve innervates the lateral side of the foot along the border between the hairy and glabrous skin. Electrical recordings were made in the nerve region located on the dorsal aspect of the lower leg. Millimeter wave exposure at 42.25 GHz was applied to the receptive field of the sural nerve in the hind paw. Exposure at the peak incident power density of a Gaussian beam $\geq 45 \text{ mW/cm}^2$ inhibited the spontaneous electrical activity of the nerve. The radiant heat exposure reproduced the inhibitory effect of mm wave exposure indicating the thermal mechanism of the mm wave action. It was shown that the cold sensitive fibers were responsible for the inhibitory effect of mm wave and radiant heat exposures. In addition, the nerve responded to the cessation of mm wave exposure with a transient increase of the firing rate. The threshold peak intensity for this effect was 160 mW/cm^2 . The radiant heat exposure did not reproduce this effect. The receptors and mechanisms involved in inducing the transient response to mm wave exposure are not clear. The authors suggested that mast cells were involved in this effect. It should be noted that in this study the authors selectively tested only one sciatic nerve branch. In order to determine if other skin sensory receptors are affected by mm waves, it is necessary to conduct experiments in which all the branches of the sciatic nerve are involved.

Most studies of the hypoalgesic effect of mm waves were performed at exposure to 61.22 GHz in the near field of antennas at the average incident power density of 13-15 mW/cm^2 . Mice

were exposed using a horn antenna. The exposure beam had a Gaussian-type distribution and was confined within the antenna aperture with a peak power density of 56 mW/cm^2 [Radzievsky et al., 2004b]. The threshold intensity for the inhibitory effect of mm waves (45 mW/cm^2) on cold receptors is within the peak intensities necessary for inducing the hypoalgesic effect. Hence, the cold receptors might be involved directly or indirectly in pain relief produced by mm waves.

Investigations of the reactions of the nervous systems of humans and animals to peripheral mm wave exposure were conducted in double-blind experiments by a number of authors [Lebedeva, 1993; 1997; Lebedeva and Kotrovskaya, 1996; Novikova et al., 2002; 2008a; 2008b; Shanin et al., 2005].

A group of scientists led by Korneva studied the potential of mm waves to stimulate the cutaneous nerve endings of rats using a c-fos immunoreactivity method [Novikova et al., 2002]. This method is based on the response of various hypothalamic structures to external stimulation by increased c-fos gene expression, an accepted marker of the activated neurons. Exposure of different parts of the rat body to 42.2 GHz at an output power of 20 mW resulted in activation of hypothalamic neurons. The electrical pain stimulation (EPS) and noxious mechanical stimulation (NMS) increased c-fos gene expression while combined exposure to EPS and mm waves or NMS and mm waves decreased the number of activated neurons [Novikova et al., 2002; 2008a]. Millimeter wave irradiation produced a modifying effect consisting of a reduction in the degree of activation of hypothalamic cells evoked by pain stimulations. Moreover, the painful electrical stimulation of the hind limbs of rats caused a reorganization of the central mechanisms that regulate splenic NK cell activity, resulting in a decrease of their cytotoxicity. Millimeter wave exposure protected NK cell activity from the impairment induced by EPS [Shanin et al., 2005]. The publications listed above do not provide an adequate assessment of the exposure intensity.

However, exposure in the near field of a horn antenna with an output power of 20 mW might be able for the mm waves to thermally activate neurons.

In a study of the mm wave effects on the human nervous system, the sensory reactions of humans were investigated using methods of sensory indications and electroencephalography [Lebedeva, 1993; Lebedeva and Kotrovskaya, 1996]. In these experiments, the hands of volunteers were exposed to 37.7, 42.25, and 53.57 GHz at the incident power densities of 5 and 10.5 mW/cm². Humans were able to recognize the mm wave stimulation. The predominant modalities of sensations were the pressure, pricking, and thermal reactions. The authors suggested that mm wave action as a stimulus is conveyed through nonspecific excitation pathways. It was speculated that mm-wave perception could involve some types of mechanoreceptors and nociceptors.

Thus, mm waves produce systemic effects on humans and animals. Based on the known biological effects of mm waves, the chain of events initiated by skin exposure to mm waves can be subdivided into four major steps: the initiation phase, the transmission of the signal to the CNS, modulation of the CNS function, and the systemic response [Radzievsky et al., 2008].

1.9 Millimeter Wave Effect on Immune System

It has been shown that mm wave irradiation enhances the immune response of organisms [Kabisov, 2000; Pletnev, 2000; Logani et al., 2011]. T cells have an impact on practically all aspects of immunity due to their ability to induce specific immune responses. T cells play an important role in the restoration process. Makar et al. [2003, 2006] studied the effects of mm waves on T cell recovery after suppression by cyclophosphamide (CPA), T cell activation,

proliferation, and effector functions in mice. CPA is an anticancer drug that prevents cell division by cross-linking DNA leading to the reduction of tumor growth [Goodman, 2000; Allison, 2000]. Mice were exposed at the nasal area to 42.2 or 61.22 GHz at the peak incident power density of 31.5 mW/cm² resulting in temperature elevation of 1.8°C during a 30 min exposure. These studies showed that mm waves can restore production of Interferon gamma (IFN- γ) and proliferation of T cells suppressed by CPA treatment. No significant changes were observed in the level of IL-10 and proliferation of B cells. These results suggest that mm waves accelerate the recovery process selectively through a T cell-mediated immune response.

The same group of authors investigated the effect of mm waves on the natural killer (NK) cell activity suppressed by CPA [Makar et al., 2005]. NK cells are known to kill a wide variety of tumor cells while sparing normal cells. In these experiments, the authors used the same exposure conditions as described above. Millimeter wave exposure of CPA treated mice resulted in restoration of CPA induced suppression of the cytolytic activity of NK cells. It was further demonstrated that mm wave exposure significantly augmented the tumor necrosis factor (TNF- α) production by NK cells suppressed by CPA administration.

Similar results were reported by Novoselova et al. [2002] who studied the effect of mm waves on the immune system of mice with experimental tumors. The whole-body millimeter wave irradiation (40 GHz, 0.5 μ W/cm²) caused a significant increase in the production of TNF- α , nitric oxide, and NK cell activity at the early stage of tumor development.

Millimeter wave irradiation was able to restore splenic NK cell activity suppressed by painful stimulation of the hind limbs in rats [Shanin et al., 2005]. As shown in experiments with neutrophils, the primed rather than intact neutrophils of mice were susceptible to exposure to 41.95 GHz at SAR=0.45 W/kg [Safronova et al., 2002].

The anti-inflammatory effects of mm waves were studied in mice exposed to 42.2-42.6, 43.0 and 61.22 GHz at intensities of 0.1-0.7 mW/cm² [Gapeyev et al., 2008, 2009]. Authors used a model of acute zymozan-induced paw edema. A single whole-body exposure of animals to continuous mm waves reduced the exudative edema of inflamed paws by 19%. The anti-inflammatory effects were dependent on both the exposure frequency and modulation frequency.

In the most studies, mm wave effects on immune system were obtained at the thermal intensities of exposure (>10 mW/cm²). It was suggested that this systemic effect is initiated by stimulation of free nerve endings in the skin [Radzievsky et al., 2008; Alekseev et al., 2010]. Then the signal is conveyed to the central nervous system where it modulates neural activity resulting in the release of endogenous opioids and development of various biological effects [Logani et al., 2011].

1.10 Physiologic Effects of High Power mm Waves

High intensity mm wave exposure causes overheating of skin and may induce structural protein damage, cell death, stress reactions, acute inflammatory responses, and circulatory dysfunctions.

Human sensory reception (warmth and pain sensations) of high power mm waves was reported in a number of papers. Blick et al. [1997] and Riu et al. [1997] studied the warmth-detection thresholds in human subjects. The pricking pain thresholds in volunteers were investigated by Walters et al. [2000]. The cutaneous thresholds for thermal pain was measured during 3 s exposures of each subject's back to 94 GHz at intensities up to 1.8 W/cm². The threshold for pricking pain was found to be 43.9°C. The power density required to evoke a

threshold sensation of pain was 1.25 W/cm^2 for a 3 s exposure. The results of modeling using a simple thermal model that accounted for heat conduction and penetration depth of mm waves were in good agreement with experimental measurements.

The physiologic effects of whole body exposure of animals to 35 and 94 GHz have been reported [Frei et al., 1995; Ryan et al., 1996; 1997; 2002; Millenbaugh et al., 2006; 2008; Jauchem et al., 2016]. The sustained overexposure of rats (~80 min) with intensities of 75 or 90 mW/cm^2 produced significant skin ($>46^\circ\text{C}$) and core heating and subsequent circulatory failure. During irradiation the heart rate increased, mean arterial pressure maintained until skin temperature reached 42°C , at which point mean arterial pressure declined until death. Death occurred at core temperature of 40°C and skin temperature of 48°C . Hypotension was accompanied by vasodilatation in the mesenteric vascular bed. The lethal effect became irreversible when the mean arterial pressure fell to 75 mm Hg even if exposure was ceased. Irradiation induced thermal injury to skin tissue that involved protein damage, oxidative stress, inflammation, immune cell response, and induction of repair processes involving extracellular matrix change. The common mediators involved in several forms of shock such as nitric oxide, platelet activating factor, and histamine did not play a role in mm wave-induced circulatory collapse. The results indicated that body core heating was the major determinant of induction of circulatory failure. The influence of mm wave heating of the skin and subcutaneous tissues became significant only when a certain threshold rate of heating of these tissues was exceeded. The obtained data suggested that mm waves induced the same thermoregulatory responses as environmental heating.

Xie et al. [2011] evaluated stress reactions in rats exposed to 35 GHz at the average incident power densities ranging from 0.5 to 7.5 W/cm^2 . Rats were exposed locally to the back area with

an exposure area of 1.8 or 3.6 cm². Stress reactions were quantitatively estimated from EEG analysis. The data showed that stress reactions are more intense during the first part of the irradiation than during the later part. The skin temperature elevation produced by millimeter wave exposure was the main factor responsible for the stress reactions and skin injuries.

The direct effect of mm wave exposure on cutaneous melanoma in mice was described in the paper by Szabo et al. [2004]. In this case, mm waves were used as the source of hyperthermia. In *in vitro* experiments, keratinocyte and melanoma cell monolayers were exposed from the bottom of a well to 42.25 GHz at an incident power density ranging from 0.78 to 1.48 W/cm² producing a maximum temperature rise up to 53°C. Melanoma cells revealed higher susceptibility to hyperthermia than keratinocytes. *In vivo* exposure of cutaneous melanoma at the power density of 1.25 W/cm² for 30 min resulted in a selective tumor destruction without tissue damage of normal skin. Since the penetration depth of mm waves varies with frequency, selective therapeutic heating of tumors at different depths in the body can be performed by choosing an appropriate frequency for the exposure and enforcing air convection near skin surface [Zhadobov et al., 2015].

1.11 Mechanisms of mm Wave Action

Many mm wave effects reported in the literature cannot be readily explained solely by the temperature elevation in irradiated objects. Up to now, the mechanisms underlying the non-thermal effects of mm waves are not clear. The resonance theory can be regarded as one of the well-developed hypothesis that is true could be specific for mm waves. As shown by Adair [2003] in his theoretical analysis, the excitation of resonance vibrational modes in molecules is

quite feasible due to coupling of the electromagnetic field to their dipoles. However, such mm wave excitable resonances are expected to be strongly damped by energy interchange with other vibrational modes of molecule and of molecules in the surrounding solution. This process cannot allow accumulating enough energy to change the properties of molecules.

Frölich [1968, 1970, 1972, 1978] has suggested another approach to explaining the resonance type frequency dependence of mm wave effects. His theoretical studies of phase correlations in condensed media show that many dipole moments in a lipid membrane can oscillate coherently. The resonance frequencies of these vibrations depend on the mechanical and structural properties of membranes. They are predicted to lie in the range of 50-150 GHz. It is assumed that vibrations in lipid membranes at resonance frequencies can produce changes in the physical and chemical properties of these membranes resulting in a biological effect. In such a system, the coupling of electromagnetic field greatly increases. However, the increased viscous damping suppresses the resonance effect [Adair, 2003].

For explaining the experimental results, some researchers suggested different non-thermal mechanisms of mm wave action. Beneduci et al. [2013; 2014] studied the effect of mm waves at temperatures close to the phase transition temperature of a membrane using a deuterium nuclear magnetic resonance spectroscopy. The authors interpreted the decrease of the heavy water quadrupole splitting produced by mm wave exposure by membrane dehydration due to coupling of the mm waves with the fast rotational dynamics of bound water molecules. However, it is not clear how mm waves can interact with bound water or charged phospholipid headgroups, which do not relax in the mm wave range [Klösigen et al., 1996].

Kotnik and Miklavčič [2000] evaluated theoretically the ability of cell membranes to absorb electromagnetic radiation in a wide frequency range. They found that local increase of power

dissipation in membranes occurred in the MHz and lower GHz regions. At frequencies above 20 GHz the power dissipation decreased. The overheating of membranes is excluded due to the effective heat dissipation from thin membranes to the surrounding medium. The authors suggested that high power dissipation could lead to structural changes in membrane proteins and the lipid matrix. However, the electric field induced in a membrane is too small in comparison with the internal static electric field of the membrane (about 10^5 V/cm) to produce a significant membrane effect.

Deformation of giant phospholipid vesicles reported by Ramundo-Orlando et al. [2009] could be produced by the effect of electrostrictive forces on liposomes arising during mm wave irradiation. Adair [2003] evaluated the magnitude of the electrostrictive forces acting on cells. The electrostrictive pressure is proportional to the permittivity difference between the medium and the cell and to the electric field squared. The calculated electrostrictive pressure at an intensity of 10 mW/cm^2 is about $2 \cdot 10^{-7} \text{ N/m}^2$, which is much less than the pressure from incident sunlight. Therefore, it is questionable that the electrostrictive pressure at low intensities of mm wave exposure could change the liposome shape.

The multilayer organization of skin might result in a resonance - type absorption of mm waves. As mentioned previously, Feldman et al. [2008] found an enhanced resonance absorption and reflection in the 95 to 110 GHz range, which was attributed to the coiled sweat ducts in skin acting as helical array antennas to mm waves. However, Alekseev et al. [2008] did not find any sharp resonance-type frequency dependence for either reflection from the human skin measured experimentally, or in the power density and SAR distributions within the skin layers calculated theoretically for frequencies up to 90 GHz. This can be explained by the small difference in permittivity of different human skin layers in this mm wave range.

The thermal effect of mm waves has two distinct features. Millimeter waves cause the temperature elevation in a relatively small volume of exposed matter. Due to the small volume, the rate of heating is high. The fast heating activates the sodium pump of neurons, which induces a fast trans-membrane current and hyperpolarizes the cell membrane inducing a transient decrease in the firing rate [Alekseev et al., 1997]. The hyperpolarizing voltage is proportional to the membrane resistance [Carpenter et al., 1968; 1973]. In neurons with higher resistance, the greater hyperpolarizing voltage and, hence, a greater transient response is expected. The depolarization of the membrane due to passive ion transport is a relatively slow process resulting in biphasic changes of the firing rate. The results obtained by Alekseev et al. [1997] allow one to draw an important conclusion: to reproduce the thermal effect of mm waves, especially in experiments involving the heating of nerve cells, it is not enough merely to increase the temperature to the maximum level reached, but it is also necessary to warm up an object with a rate of temperature rise equal to that caused by mm wave exposure. If a biological system is regulated by two different mechanisms having different thermal sensitivities and time constants of their thermal reactions, the fast heating that is typical for mm wave exposure may lead to a complex response of the system to exposure.

1.12 Indirect Effects of mm Waves

In experiments *in vitro*, different factors can mimic the mm wave effect on biological objects. When a liquid medium is exposed to a narrow mm wave beam, the energy deposition occurs in a very small volume. The rapid temperature rise in this small volume induces a

convection current, which occurs in nearly all *in vitro* mm wave experiments, and although undeservedly attracts little attention, is capable of producing biological effects.

A free-type convection appears in a liquid at the temperature gradient of $0.15 \cdot 10^{-3} \text{ }^{\circ}\text{C/m}$ [Landau and Lifshitz 1986]. When physiological solutions are irradiated by 40-70 GHz mm-waves, such a temperature gradient is reached within a few seconds after the start of irradiation at an incident power density as small as 10^{-9} W/cm^2 [Khizhnyak and Ziskin, 1996]. The background laminar convection can develop in liquid even without mm wave irradiation due to a non-uniform temperature distribution. For example, this convection may result from a lower temperature at the surface of a liquid due to its evaporation. Convection induced by mm waves in a liquid can affect the cellular functioning mimicking the direct mm wave effect on cells. Sharov et al. [1983] discovered the lipid peroxidation in liposomes produced by mm wave exposure at 46.15 GHz and an intensity of 0.5 mW/cm^2 . This effect was caused by an increased concentration of oxygen in the liquid due to convectional stirring of different liquid layers. At high intensities ($\text{SAR} \geq 4 \text{ kW/kg}$) exposure of a saline solution in a Petri dish can even induce temperature oscillations [Khizhnyak and Ziskin, 1996]. Hence, when interpreting the experimental results obtained *in vitro* studies it is important to take into account the effect of liquid convection. In experiments, stirring liquid during exposure may minimize this effect. In tissues, where the cells and other tissue components are tightly packed, the conditions for convection of fluid are limited.

Frequency dependent effects of mm waves can be result from the interaction of the irradiated beam of mm waves in the near field of an antenna with an object such as skin or an exposure chamber in *in vitro* experiments. It has been shown that exposure in the reactive zone of horn antennas produces non-uniform heating patterns in irradiated objects. These non-uniform

patterns are due to a “geometrical” resonance resulting from a secondary wave-mode interaction between an irradiated object and the corresponding critical cross-section of the horn antenna [Khizhnyak and Ziskin, 1994]. The location, quantity, number and size of the local field absorption maxima in irradiated objects are strongly depended on the specific antenna and the frequency of mm wave irradiation. These features of exposure in the near field of an antenna could provide an explanation for a number of frequency-dependent effects of mm wave irradiation.

In experiments *in vitro*, cells are usually exposed in plastic culture plates or dishes filled with a culture medium. When a plastic well filled with liquid is exposed from the bottom of a well, the power density and SAR distribution depend on the irradiation frequency [Alekseev et al., 2017]. In this case, a plastic bottom plays the role of a coupler between an antenna and the culture medium. Fig. 1.4 demonstrates the dependence of the power reflection coefficient on frequency, as obtained by Alekseev et al. [2017] and extended to THz frequencies. The position and number of maximums and minimums depend on the permittivity of the plastic and thickness of a well bottom. In studying the frequency dependent biological effects, the measurement of the incident power density is not enough to characterize the exposure intensity. It is necessary to define the power density reaching the cell layers. The frequency dependent exposure intensities can be calculated using analytical or numerical methods.

Fig. 1.4. Frequency dependence of reflection from the polystyrene bottom of a tissue culture well filled with water at 298 K. Permittivity of polystyrene, $\epsilon^* = 2.56 - j0.003$. Permittivity of water is calculated using the double Debye equation (Eq. 2.2) with parameters given by Pickwell et al. [2004a]. Dotted line shows the level of zero reflection. The thickness of the polystyrene well bottom is 1.5 mm.

In experiments with mm waves, the temperature measurements can be a challenging problem. The most common devices used for temperature measurements are thermocouples, thermistors, fiber-optic probes, and IR cameras. The IR camera is a useful technique for the measurements of surface temperature. Due to the shallow penetration of mm waves into biological solutions (e.g., 0.34 mm at 58 GHz), the absorbed power is locally concentrated close to the exposed sample surface, inducing a large temperature gradient. This makes it a very difficult task to measure the local temperature in thin cell monolayers, which is an unavoidable step for correct interpretation of experimental biological results. As shown recently, small thermocouples (25 μm in diameter) can be used for accurate temperature measurements [Pakhomov et al., 2000a; 2000b; Zhadobov et al., 2017]. On the other hand, fiber-optic probes and thermocouples of greater size (>0.1 mm) are not appropriate for measuring temperature in the thin absorbing layer of a solution because they disturb the temperature distribution in the thin heated area possessing a locally large temperature gradient.

1.13 Conclusion

Millimeter waves are entirely absorbed in the epidermis and dermis. Theoretical evaluations of skin heating show that mm wave exposure of skin to 10 mW/cm^2 could increase the skin surface temperature by 1.2°C . Due to effective heat transfer from the superficial regions of skin to deeper tissues, mm wave exposure also results in heating of fat and muscle layers. The fat layer plays the role of an insulator.

The experimental results show that low intensity ($\leq 10 \text{ mW/cm}^2$) mm wave irradiation does not produce any harmful effect on eyes. However, high intensity mm wave exposure results in adverse effects on the eye. The ocular damage is induced by the thermal effect of mm waves and depends on the frequency, intensity and duration of exposure.

Millimeter waves induce a thermal effect on artificial lipid membranes. However, some results obtained in experiments with artificial lipid membranes are inconsistent with a thermal mechanism. The mechanisms underlying these effects are not clear. Furthermore, recent studies do not confirm the non-thermal effects of mm waves on electrical events in cell membranes.

No genotoxic effects were found in cells exposed to mm waves at intensities up to 75 mW/cm^2 . Most studies show the absence of the effects of mm waves on gene expression at exposure intensities $\leq 20 \text{ mW/cm}^2$. Changes in gene expression occur at much higher exposure intensities causing significant temperature elevations in skin.

Millimeter wave irradiation can induce an anticancer effect. However, the data on proliferative effects in different cells is controversial.

Low intensity mm wave exposure did not reveal changes in functioning of cellular systems. The effects induced by high power irradiation have most probably been thermal in nature. The

data on PS externalization show a new potential of mm wave action on cellular membranes and need further verification in independent studies.

Most studies showed that the changes in electrical activity and structure of excitable cells induced by mm wave irradiation were thermal in origin. The rate of mm wave heating plays a significant role in eliciting the electrical response of nerve cells.

High power mm wave irradiation of animals produces severe thermal injury to skin tissue, and if the exposed area is sufficiently large, can eventually result in circulatory failure.

Millimeter waves produce systemic effect on humans and animals affecting the immune and nervous systems and involving the endogenous opioids. In most studies, a hypoalgesic effect and an effect on the immune system were obtained at thermal exposure intensities ($>10 \text{ mW/cm}^2$). It was suggested that the systemic effect was initiated by stimulation of free nerve endings in the skin. Then the peripheral signal is conveyed to the central nervous system where it modulates the neural activity resulting in the development of various biological effects.

The possible resonance effect of mm waves has remained a subject of debates. However, independent thorough replications of the claimed resonance effects are required, and more studies are necessary to be conducted to accept the resonance mechanism as a mode of biological action of mm waves.

2 BIOLOGICAL EFFECTS OF TERAHERTZ RADIATION

2.1 Introduction

Biological effects of THz radiation have not been studied as extensively as the effects of electromagnetic radiation in microwave and mm wave frequency ranges. This can be explained

by the absence of THz sources and detectors available for most researchers for a long time. However, the technology in the THz field has developed rapidly in recent years with its steadily increasing applications in different areas of human activity. Now THz devices are available in many research laboratories. Accordingly, the number of publications on the biological effects of THz radiation has been notably increased during the last 10-15 years [Hintzsche and Stopper, 2012]. Nevertheless, it is important that thorough scientific studies of biological effects of THz radiation in the frequency range above 300 GHz be continued.

With increasing use of THz radiation in security scanners, communication, medical applications, and scientific studies, the safety issue becomes very important. The safety limit for the general public for the frequency range between 2 and 100 GHz is 1 mW/cm^2 and increases to 10 mW/cm^2 at 300 GHz [IEEE Standard C95.1, 2005]. Similar limits are used by ICNIRP and the FDA. These safety standard limits are based on established adverse thermal effects. Safety limits of THz frequencies for the general public is 10 mW/cm^2 for whole body exposures and 100 mW/cm^2 for small beams.

The early publications on studies of biological effects at low THz frequencies first appeared in Russian literature over five decades ago. These studies were conducted using specialized backward-wave tube sources. Beginning in 2001, the European countries have been strongly involved in the investigation of biological effects of THz radiation through the large research program “THz-Bridge” [Gallerano, 2004]. Among others, the research program is focused on the potential genotoxic effects of THz radiation. To this day, a great number of studies of the effects of THz radiation on molecular and cellular levels, on microorganisms and animals, and mechanisms underlying these effects has been performed. Several comprehensive reviews have been published describing the current state of research on the subject [Fedorov et al., 2003;

Siegel, 2004; Wilmink and Grundt, 2011; Hintzsche and Stopper, 2012; Yu et al., 2012; Kirichuk and Ivanov, 2013; Zhao et al., 2014]. However, in spite of the great progress in the study of THz biological effects, it is often impossible to draw unequivocal conclusions due to the controversy of the published data.

Besides biological studies, THz radiation is successfully used in time-domain spectroscopy of water and biological molecules [Fischer et al., 2002; Møller et al., 2009]. THz time-domain spectroscopy allows measuring the size of the solvation shell around molecules [Arikawa et al., 2008]. The THz absorption spectra reflect the low-frequency internal motions or vibrations involving the weakest hydrogen bonds within molecules or between molecules [Globus et al., 2003]. The transmitted and reflected THz spectra of materials characterize the vibrational modes and provide information that is not available in other parts of the electromagnetic spectrum.

THz imaging has also been successfully used in medical applications [Woodward et al., 2002; 2003; Humphreys et al., 2004].

In this chapter, the interaction of THz radiation with water and skin, skin heating and the THz effects on biological objects at different organization as well as the mechanisms underlying these effects are reviewed.

2.2 THz Radiation Sources

The most common approach to generate THz frequencies is nonlinear reactive multiplication of lower frequencies from mm wave sources which include the solid-state electronic sources (Gunn diodes, IMPATT devices, tunneling transit time diodes (TUNNETT), and resonant tunneling diodes) and tube sources (BWO, klystrons, carcinotrons). The THz sources using the

frequency up-conversion approach are tunable and cover the entire range from 8 GHz to 1000 GHz. These devices provide an output power up to 100 mW at lower frequencies, which drops off between $1/f^2$ and $1/f^3$ with increasing frequency. They operate at room temperature, generate narrow line-width (10^{-6}) CW radiation, and are convenient to use in studies of THz biological effects.

The next most commonly used sources at terahertz frequencies are far-infrared (FIR), electrically- pumped solid state, and quantum cascade lasers. FIR lasers are usually based on grating tuned CO₂ pump lasers injected into a laser cavity with a vacuum envelope container filled with molecular gases (methanol). The high power CO₂ laser (20-100 W) excites the vibrational levels of gas molecules, which have transition frequencies in the THz spectrum. FIR lasers generate discrete frequencies at output powers up to 100 mW.

Another class of THz sources used in biological experiments is based on electron accelerator devices such as terahertz tubes (BWOs, orotrons, traveling wave tubes (TWTs), klystrons, and gyrotrons) and free electron lasers (FELs). It is well known, that charged particles can radiate electromagnetic (EM) waves only when accelerated. In BWOs an electron beam is accelerated in the opposite direction to the travelling EM wave. BWOs consist of a strong magnet system (typically 1 T) used to collimate the electronic beam and high-voltage source creating the strong electric field used for accelerating the electronic beam. Typical BWO sources are tunable in the range of 35 GHz to 1.42 THz at output powers up to 100 mW. Orotron based sources generate radiation in the frequency range of 120-370 GHz with output powers up to 50 mW. They have electronic and mechanical frequency tuning. Other terahertz tubes generate radiation at mW levels.

THz FELs consist of an electron accelerator and a wiggler magnetic array producing undulation of the electron beam. Laboratory FELs can generate tunable THz radiation in the wide range of THz frequencies at output powers up to several tens of W. Due to their large size and complexity, FELs operation requires a qualified staff.

Nowadays, most time-domain devices employ ultrafast lasers. Optical lasers are used for generating pulsed THz radiation by applying the frequency down-conversion technique. Two principal methods are used to generate THz radiation: difference frequency generation and optical rectification.

The principals of THz generation are described in detail elsewhere [Ferguson and Zhang, 2002; Siegel, 2002; Wolbarst and Hendee, 2006; Hosako et al., 2007; Lee and Wanke, 2007; Liu et al., 2007; Tonouchi, 2007; Williams, 2007; Lee 2009; Sirtory, 2009; Wilmink and Grundt, 2011].

The types of THz sources used for exposure of biological objects are usually given in articles on biological effects of THz radiation. It is shown that THz effects may depend on the type of THz source.

2.3 Absorption of THz Radiation by Water and Skin

Water is of the greatest importance in the absorption of THz energy. The electrical properties of body tissues in the THz frequencies are mostly determined by the concentration of water, which is approximately 70% by weight. Also, most biological studies are performed in aqueous media. Therefore, water is of utmost importance in the dosimetric evaluation of absorbed THz energy in objects.

The water molecule forms a tetrahedral structure of hydrogen bonds connected to its neighboring molecules. This facilitates forming the highly dynamic network of interconnected molecules. When a molecule is solvated in water, the water molecules rearrange themselves to accept the molecule. The photon energy of hydrogen bonds is within 0.043-0.22 eV. Thus, as opposed to mm waves, THz radiation can directly excite the vibrational modes of the weakest hydrogen bonds of water and solvated molecules.

If N relaxation processes occur simultaneously with M homogeneously broadened vibrational modes, then the general dielectric function can be expressed according to Møler et al. [2009] as

$$\varepsilon^*(\omega) = \sum_{j=1}^N \frac{\Delta\varepsilon_j}{1 - i\omega\tau_j} + \sum_{k=1}^M \frac{A_k}{\omega_k^2 - \omega^2 - i\omega\gamma_k} + \varepsilon_\infty \quad (2.1)$$

where $\Delta\varepsilon_j$ is the magnitude of the dispersion and τ_j is the relaxation time of the j^{th} relaxation process, ε_∞ is the optical permittivity, the coefficients A_k are the vibrational amplitudes, $\omega_k/2\pi$ are the resonance frequencies, and γ_k are damping rates of the k^{th} vibrational mode. The complex dielectric function of liquid water at room temperature can be decomposed into four components: a slow relaxation mode at $\tau_1=9.4$ ps, a fast relaxation mode at $\tau_2=0.25$ ps, an intermolecular stretching vibration mode at ~ 5 THz, and intermolecular vibration mode at ~ 15 THz [Kaatze, 1989; Yada et al., 2008]. The presentation of N relaxation processes as a sum in which each process is independent of all other processes is not obvious. In some studies, the double Debye theory was used to model the fast and slow relaxation processes of water [Rønne et al., 1997; Buchner et al., 1999; Pickwell et al., 2004a]. The best fit to the experimental data was obtained using the double Debye equation:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_2}{1 + i\omega\tau_1} + \frac{\varepsilon_2 - \varepsilon_\infty}{1 + i\omega\tau_2} \quad (2.2)$$

where ε_s is the static permittivity, ε_∞ is the optical permittivity, ε_2 is an intermediate frequency limit, τ_1 and τ_2 are the relaxation times of the slow and fast relaxation processes, respectively. The permittivity of pure water at 298 K calculated using the equation 2.2 and parameters given by Pickwell et al. [2004a] is presented in Fig. 1.1.

Water is a strong absorber at THz frequencies. In a homogeneous medium, the attenuation of the power density of a THz wave is given by

$$PD(f) = PD_0(f) \cdot e^{-2\alpha(f)z} \quad (2.3)$$

where f is frequency, $PD(f)$ is the transmitted intensity, $PD_0(f)$ is the incident intensity, and z is the sample thickness. The power absorption coefficient $a(f)=2\alpha(f)$ increases from about 40 up to 580 cm^{-1} following the frequency increase from 0.1 to 3.0 THz [Siegel, 2004]. The optical (power) penetration depth decreases from 140 to 40 μm following the frequency increase from 0.1 to 1.6 THz [Jepsen et al., 2007].

Several authors [Fitzgerald et al., 2003; Pickwell et al., 2004b; Sun et al., 2009; Huang et al., 2009; Wilmink et al., 2011b] studied the dielectric properties of skin and other biological tissues at THz frequencies. Because tissues are not 100% water, their absorption coefficients are lower than that of water. For example, the absorption coefficient $a(f)$ for skin, adipose tissue, and striated muscle at 1 THz is around 105, 105, and 130 cm^{-1} , respectively, while for water it is 225 cm^{-1} [Fitzgerald et al., 2003].

Fig. 2.1. Real index of refraction for water and skin calculated using double Debye equation (Eq. 2.2) with parameters from the papers by Pickwell et al. [2004a,b].

Pickwell et al. [2004b] studied the interaction of THz radiation with the forearm and palm skin *in vivo*. The reflection data was simulated using the FDTD model. As the power penetration depth for skin is about 60 μm at 1 THz [Wilmink et al., 2011b] the reflected waveform off the volar forearm is dominated by the reflection from the epidermis whose thickness is about 100 μm . The permittivity data of the epidermis was fitted to the double Debye equation (Eq. 2.2). Fig. 2.1 demonstrates the real index of refraction of the epidermis calculated using parameters of equation 2.2 given by Pickwell et al. [2004a,b]. The power penetration depth for human skin calculated using the same data given by Pickwell et al. [2004a,b] is shown in Fig. 2.2. It should be noted that the calculated values of the index of refraction and penetration depth for human skin and water and those reported in the literature [Jepsen et al., 2007; Nazarov et al., 2008; Wilmink et al., 2011b] are comparable.

Fig. 2.2. Frequency dependence of the power penetration depth for water and human skin calculated using the permittivity data from Pickwell et al. [2004a,b].

Thus, THz radiation is strongly absorbed by water and biological tissues. In the skin, THz radiation with a frequency >0.4 THz is almost entirely absorbed within the epidermis (~ 0.1 mm) affecting mostly the epidermal cells such as keratinocytes and melanocytes, Langerhans cells as well as free nerve endings. The epidermis does not contain blood vessels. Only a small part of THz radiation entering the skin can reach the blood vessels located in the dermis. Therefore, in evaluating the influence of THz radiation on blood components the in situ power density of THz radiation rather than the incident power density should be taken into account to define the threshold intensities of the effects.

2.4 THz Radiation Heating of Skin and Water

In experiments with THz radiation, the skin and biological samples are typically exposed to a Gaussian beam. In this case, the heat production due to THz radiation can be defined as follows [Alekseev and Ziskin, 2003]:

$$Q(z, r, t) = \frac{2 \cdot (1 - R)}{\delta} \cdot I_o \cdot e^{-\frac{2 \cdot z}{\delta}} \cdot S(r) \cdot u(t)$$

(2.3)

where $S(r)$ is the Gaussian distribution of the incident power density on the skin surface, I_0 is the peak power density (W/m^2), δ is the amplitude penetration depth of THz radiation into the human skin (m), R is the power reflection coefficient, and $u(t)$ is the unit step function. This equation used for modeling mm wave heating of skin is valid for THz radiation also. The only term in the bio-heat equations that depends on frequency is the heat production which in turn depends on the penetration depth. Therefore, the thermal models used for calculations of skin heating at mm wave exposure can be applied for the thermal modeling of THz heating of skin also, providing that the parameters of the Gaussian beam are defined.

Alekseev et al. [2012] studied the frequency dependence of mm wave heating of human skin experimentally. The authors showed that at the same power density entering the skin the temperature elevation due to mm wave exposure did not reveal any detectable frequency dependence. Modeling skin heating confirmed the experimental results, i.e., temperature elevation at exposure of skin to high frequency electromagnetic radiation above 30 GHz is not dependent on the irradiation frequency. The frequency dependence of heating was entirely due to the frequency dependence of reflection of mm waves from the skin. As the absorption of high frequency irradiation occurs in the thin surface layer of skin the absorbed energy is concentrated in a small tissue volume. The initial temperature gradient increases rapidly with increasing the frequency due to reducing the penetration depth. This results in a greater heat transfer from the thin heated tissue layer to surrounding tissue. Heat transfer strongly depends on thermal conductivities of tissue layers and blood flow. Therefore, in modeling skin heating at THz frequencies it is desirable to include in a thermal model all tissue layers in spite of the concentration of absorbed energy in the epidermis.

The heating of skin exposed to THz radiation was modeled in a number of studies [Kanezaki et al., 2008; 2009; 2010; Zilberty et al., 2013; 2014]. Kanezaki et al. [2008; 2009; 2010] used a one-dimensional three-layer model (skin, fat, and muscle) to calculate skin heating in the frequency range of 30-300 GHz. The authors found that at the incident power density of 50 mW/cm² the temperature elevations on the surface of skin exposed to 30, 60, 100, and 300 GHz were about 6, 7, 8, and 9.4°C, respectively. The model took into account the reflection of radiation from the skin. The increase of the temperature elevation was probably caused by increasing the exposure intensity due to the reduction of reflection from the skin with increasing frequency. Zilberty et al. [2014] modeled the transient heating of skin exposed to 0.1 and 1.0 THz. In this study, a three-layer model was exploited. It was shown that the skin temperature elevation was significantly higher during the first 1 s of exposure to 1 THz than to 0.1 THz. A parametric analysis showed that the properties of the skin played a major role in the computation of the maximum temperature rise.

Kristensen et al. [2010] theoretically evaluated heating of water exposed to THz radiation. As the biological samples in experiments *in vitro* are filled with culture medium the assessment of heating of culture medium or water exposed to THz radiation is very important from the point of view of understanding the mechanisms underlying THz effects and dosimetry. The THz source was focused to a spot with a diameter of 0.5 mm. The authors assumed that water was static. The steady-state temperature increase per mW of transmitted power was 1.8°C/mW. Obviously, this is a maximal temperature rise, which is achievable using the static water approach. In reality, heating of water induces convection currents that mix different regions of water possessing different temperatures and tends to smooth down local temperature non-

uniformities, thereby reducing the maximal temperature rise. Experimental verification of the model predictions is required before one can rely on the obtained results.

2.5 Effects of THz Radiation on Molecules and Membranes

The effects of THz radiation on a molecular level were evaluated by measuring enzyme activities, interaction of enzymes with substrate, spectroscopic changes and changes in fluorescence spectra of proteins.

The effect of THz radiation on albumin conformation was reported in several papers [Govorun et al., 1991; Cherkasova et al., 2009; Kirichuk and Tsymbal, 2013].

Measuring the circular dichroism (CD) spectra, Govorun et al. [1991] showed the changes in the albumin secondary structure following exposure to pulsed 3.3 THz radiation with pulse energies of 5 mJ. The amount of change was proportional to the number of pulses.

Cherkasova et al. [2009] evaluated the conformational changes of albumin by measuring the characteristic absorption spectra in the UV band and the CD spectra. The irradiation of albumin to 3.6 THz for 60 min with 10 mW (power density was not provided) resulted in statistically significant changes in both spectra resulting from the conformational rearrangements in protein. The observed changes depended on exposure duration and radiation power.

Kirichuk and Tsymbal [2013] measured the absorption and fluorescence spectra of a 2.5% water solution of human albumin. Exposure of samples to 0.129 and 0.150-0.151 THz (nitrogen oxide absorption band) for 30 min resulted in changes of the amplitude rather than the shape of the spectra. The authors attributed these changes to conformational changes of albumin. No power density was reported.

In addition, Govorun et al. [1991] investigated the effects of THz radiation with the parameters given above on the activity of alcohol dehydrogenase, peroxidase, and trypsin. The activity of peroxidase decreased slightly. The other proteins exhibited a nonlinear response.

The interaction of soluble or immobilized calf alkaline phosphatase with the substrate p-nitrophenylphosphate and the interaction between an antibody (mouse monoclonal anti-DNP) and its antigen (DNP) were studied in experiments with 0.1 THz irradiation at the power density of 0.008 mW/cm² [Homenko et al., 2009]. The samples with enzyme and the samples with enzyme and substrate were exposed for 15-90 min and 30-120 min, respectively. Irradiation of the enzyme without substrate, or during the enzymatic reaction with substrate, resulted in small but statistically significant reductions in enzyme activity. The immobilized enzyme was not affected. The previously formed substrate-antibody complexes reduced their stability following irradiation. The authors stated that 0.1 THz radiation could induce small but statistically significant alterations in enzyme reactions.

The dependence of the biological effect of THz radiation on the repetition rate of THz pulses was studied by Doria et al. [2004] and Ramundo-Orlando et al. [2007]. To investigate the effect of THz radiation on the permeability of artificial membranes, the liposomes were loaded with the enzyme carbonic anhydrase. Its substrate, p-nitrophenyl acetate, was added to the external solution. In the case of a permeability increase or membrane damage, the substrate could penetrate into the liposomes forming the reaction product p-nitrophenolate, which could be detected at the peak of absorbance ($\lambda = 400$ nm) with the UV spectrometer. In the two sets of exposures, the effect of 0.130 THz was investigated at different pulse repetition rates or pulses per second (pps) and power. The FEL generated macropulse duration was about 4 μ s. In the first set up, liposomes were exposed for 60 min with the power density of 0.16 mW/cm² at pps = 5 Hz

or with 0.23 mW/cm^2 at $\text{pps} = 7 \text{ Hz}$. In the second set up, liposomes were exposed for 2 min at 5.6 mW/cm^2 with $\text{pps}=5 \text{ Hz}$, at 7.8 mW/cm^2 with $\text{pps}=7 \text{ Hz}$, and at 11.1 mW/cm^2 with $\text{pps}=10 \text{ Hz}$. Only exposure at 7.8 mW/cm^2 with $\text{pps}=7 \text{ Hz}$ for 2 min resulted in a significant increase in enzyme activity. These experiments indicated that the repetition rate of pulsed THz radiation could play an important role in inducing a biological effect. However, the peak power of pulses was not given in the paper.

Ramundo-Orlando et al. [2007] investigated the effect of THz radiation on the same system used in the previous study. Liposomes were exposed to 0.130 THz for 2 min at room temperature (22°C) with different average power densities ranging from 5.2 to 17.1 mW/cm^2 and $\text{pps} = 5, 7$, and 10 Hz . Again, a significant effect was observed only at exposure of the liposomes to 7.7 mW/cm^2 with $\text{pps}=7 \text{ Hz}$. Continuous wave exposure to 0.130 THz at 6.2 mW/cm^2 did not induce any changes in permeability of liposomes. Thus, the present study confirmed the significance of the repetition rate of THz pulses in eliciting the membrane effect discovered by Doria et al. [2004]. The temperature elevations during all exposures did not exceed 0.05°C . For explaining the obtained results, the authors suggested resonance-like mechanisms.

In the studies referred above the molecular systems and liposomes were dissolved or dispersed in a liquid medium. Due to the low penetration depth of THz radiation, the absorption of radiation energy by objects was non-uniform. Therefore, it gives rise to skepticism regarding the direct effect of THz radiation on these systems.

2.6 Effects of THz Radiation on the Cellular Level

The effects of THz radiation on cellular systems have been extensively studied using a wide variety of mammalian (keratinocytes, fibroblasts, blood cells, lymphocytes, spleen cells, etc.) and microorganism (yeast, *E. coli*, etc.) cells.

Hadjiloucas et al. [2002] reported results of the study of 0.200-0.350 THz effects on *Saccharomyces cerevisiae* yeast growth. Yeast cells were exposed at room temperature to 5.8 mW/cm² for 30, 60, 90, 120, and 150 min. The maximal temperature rise after 2.5 h exposure was 2-3°C. The statistically significant enhanced growth rate was observed at 341 GHz. The authors suggested a non-thermal mechanism for explaining their results.

Extensive studies, performed on the effects of THz radiation on human primary keratinocytes were described in two papers [Clothier and Bourne, 2003; Bourne et al., 2008]. In the first study, the cells were exposed to pulsed 0.1-2.7 THz radiation with an average power of ~1 mW. The total pulse duration was 20-30 ps. The keratinocyte samples were exposed at room temperature (22°C) for 10, 20, and 30 min at up to 0.45 J/cm². Cellular activity and viability was examined using conventional assays. No inhibition or stimulation of cell activity induced by THz radiation was found. The differentiation of cells was also not affected.

In the second paper, the effects of pulsed 0.140-0.150 THz radiation at 0.1-0.25 mW/cm² on several human cell lines (human epithelial keratinocytes, corneal, and ND7/23 cells) were reported. The cells were exposed at 22°C for 24 h. The keratinocyte differentiation and viability, morphology, and expression of heat shock proteins and glutathione were tested using conventional assays. No exposed cells exhibited statistically significant changes.

In one paper, the effects of THz radiation on isolated neurons of mollusk *Lymnaea stagnalis* were reported [Ol'shevskaya et al., 2009]. Neurons were exposed to 0.7, 2.49, and 3.69 THz at the power densities 0.3-30 mW/cm². Irradiation caused significant effects on cellular growth,

adhesion (70-80%), membrane morphology, intracellular structures, and resting membrane potential. Though the temperature elevation during exposure was not measured, it could have been high enough to induce the observed effects.

Munzarova et al. [2013] studied the effects of pulsed 2.205-2.307 THz radiation on the morphology and aggregation of human erythrocytes. The erythrocytes were exposed to 8-10 W/cm² for 5-25 s. The power in the 5.6 MHz pulses was about 1 MW. It was found that exposure for 5 s did not cause visible changes in morphology or aggregation of cells. More prolonged exposure (>25 s) resulted in cell lysis. At exposure durations of 10-15 s the average number of erythrocytes in aggregates was reduced by 10-15%. The authors measured the intensity of an ultrasound wave in water exposed to THz radiation with the same parameters as used in the biological experiment. The ultrasound wave with a frequency equal to the pulse repetition rate of 5.6 MHz or its first harmonic, 11.2 MHz, was recorded. However, the intensity values of the ultrasound wave were not presented. The authors speculated that the induced ultrasound wave could result in the observed effects.

Demidova et al. [2013] investigated the effect of THz radiation on *E. coli/pKatG-gfp* biosensor cells, which are constructed on the basis of the stress-sensitive promoter of the catalase gene *katG*. Biosensors are the artificial genetic reporter systems that contain a sensitive element—the promoter of a sensor gene that is activated in response to external stress. The expression of green fluorescent protein (GFP) is an important indicator of stress in this system. The cell samples (volume 50 µl) were exposed to 1.5, 2.0, and 2.308 THz for 15 min at an average power density of 1.4 W/cm². Peak power in the pulses was ≤ 1.0 MW. During exposure the temperature of the cell samples was kept at 35 ± 2 °C using the thermal imager and by changing the average emission power. The majority of cells exposed to THz radiation exhibited intense fluorescence

signal of the GFP indicating that THz radiation induced the expression of GFP in *E. coli*/pKatG-gfp biosensor cells. No signal was detected in unexposed cells kept at 37°C. The authors did not present the quantitative values of the THz effects. It was suggested that the katG promoter was induced through the oxidative stress pathway.

In a study by Williams et al. [2013], three different cell types were used for THz radiation experiments: human corneal epithelial cells, human retinal pigment epithelial cells, and human embryonic stem cells. The samples were exposed in an incubator at 37°C to broad-band coherent synchrotron radiation with a cut off frequency up to approximately 0.5 THz, for time periods of up to 6 h and multiple 3 h periods. The average power density was in the range of 0.02-0.37 mW/cm². The peak power in the 2 ps pulses was 6 kW. The changes in cell morphology, attachment, proliferation, and differentiation were evaluated. Under all conditions, no statistically significant difference in any of these parameters between irradiated and control cell cultures was observed. Authors suggested that when the cells were grown under ideal conditions they could compensate for any stimulatory effect of THz radiation.

The effect of CW THz radiation on a rat glial cell line was investigated by Borovkova et al. [2016]. The samples were exposed to 0.12-0.18 THz at 3.2 mW/cm² at room temperature for 1, 2, 3, 4, and 5 min. THz irradiation induced a cytotoxic effect, which increased with the increase of exposure duration. The relative number of apoptotic cells increased 1.5 times following 1 min of exposure and this number was doubled after a 3 min exposure. The authors concluded that high intensity THz irradiation could cause an adverse effect on living organisms.

Thus, the controversial results obtained in the studies of the effects of THz radiation on cellular systems do not allow drawing justifiable conclusions concerning the existence of

cytotoxic effects. The experiments in many studies were performed under different conditions with uncertain dosimetry that complicate the evaluation of the obtained results.

2.7 Effects of THz Radiation on Cellular Genetic Apparatus

2.7.1 Effects on Gene Expression

Early works on the effects of THz radiation on the genetic apparatus of cells are described in detail in the comprehensive review by Wilmink and Grundt [2011]. Here we give only a short description of these publications mainly as reference sources. We will focus our efforts mostly on the description of the late publications on the subject related to the effects on gene expression without direct DNA damage.

Berns and Bewly [1987] and Berns et al. [1990; 1994] investigated the effect of 1.5 THz irradiation on DNA synthesis in different cell lines using a FEL source. Though the energy per 2 μ s pulse (2.6 mJ) was relatively high, the temporal average power density was 0.1 mW/cm². The authors found statistically significant increases in inhibition of DNA synthesis. In synchronized cells, the inhibition effect was greater (22%) than in asynchronized cells (17%). The temperature in exposed samples was not measured experimentally. Calculations estimated the temperature rise in samples to be 2.96°C. Nevertheless, the authors concluded that the effect had a non-thermal origin.

A group of authors [Bock et al., 2010; Alexandrov et al., 2011; 2013] performed a systematic study of THz irradiation effects on gene expression in mouse stem cells. In the first study, cells were exposed to broadband THz radiation (centered at 10 THz) at a 1 kHz repetition

rate with a peak power in each 35 fs pulse of 30 MW and an average power density of 1 mW/cm². Exposure durations were 2, 4, 6, and 9 h. The temperature of cells during exposure was monitored using an IR detector and thermo-sensors glued to the outside of the Petri dish lids. All exposures were performed at an ambient temperature of 26.3°C.

The gene chip survey of gene expression indicated that about 6% of the genes on the array increased more than two times, 5% decreased to the same amount and about 89% did not change. Using reverse transcription polymerase chain reaction (RT-PCR), four selected genes, including the transcription factor peroxisome proliferator-activated receptor gamma (PPARG) were confirmed to be present in the irradiated cells. The authors found out that following a 6 h exposure, lipid droplet-like inclusions appeared in the cellular cytoplasm. They suggested that THz exposure accelerates cell differentiation towards an adipose phenotype by activating PPARG. As the temperature elevation during exposure was low (about 0.3°C) the authors suggested a non-thermal mechanism for explaining the observed effects, namely the direct effect of THz radiation on the DNA conformation.

In the follow-up papers [Alexandrov et al., 2011; 2013], the effect of THz irradiation on the transcriptional activity of heat shock protein (Hsp90, Hsp105 and CRP) genes was reported. The mouse stem cells were exposed to the same broadband THz radiation (centered at ~10 THz) used in the previous study and to CW THz radiation at 2.52 THz and power density of 1.2 mW/cm². The temperature profiles in the Petri dish were calculated and experimentally measured using an IR camera. Temperature elevations at the bottom and at the top of the dish were about 1.5 °C and 1.0 °C, respectively. Exposure of the cells to the broad-band and CW THz radiation for up to 12 h did not activate the heat shock proteins. However, 9 and 12 h exposure to the broad-band THz radiation (rather than to CW) activated the expression of Adiponectin, GLUT4, and PPARG.

A group of researchers from the Air Force Research Laboratory conducted comprehensive studies of the effects of THz radiation on the cellular level at high exposure intensities [Wilmink et al., 2010a,b; 2011; Grundt et al., 2011]. These studies were performed using optically-pumped molecular gas lasers and an adequate dosimetry. Typically, samples were exposed under the controlled temperature conditions at 37°C. The computational dosimetry was performed using a FDTD program. Empirical dosimetry measurements were performed using an IR camera and thermocouples.

In the first two papers, Wilmink et al. [2010a; 2011a] reported the results of investigation of the effects of THz irradiation on human dermal fibroblasts to 2.52 THz at the power density of 84.8 mW/cm². The authors evaluated the cellular viability and the transcriptional activation of genes involved in protein and DNA damage pathways. Cellular viability was assessed using the MTT colorimetric assay. The transcriptional activation of heat shock proteins and DNA sensing genes was evaluated using the quantitative polymerase chain reaction (qPCR) technique. For all exposure durations tested (5, 10, 20, 40, and 80 min), more than 90% of cells remained viable. The exposed cells showed a minor increase in the expression of heat shock proteins. None of the DNA repair genes were up-regulated. The increases in the expression of heat shock proteins were comparable with those observed in hyperthermic controls.

In the second study, Wilmink et al. [2010b] determined the death thresholds and gene expression profiles in human dermal fibroblast and Jurkat cell lines exposed to 2.52 THz at the power density of 227 mW/cm². The MTT data showed that 60% of the Jurkat cells remained viable following 30 min exposure, and only 20% of them survived after the 40 min exposure. Similar results were obtained in the flow cytometry measurements. The data showed that cell death occurred via both apoptotic and necrotic pathways. The microarray data revealed that

dermal fibroblasts up-regulated several genes and activated many of the cellular stress response pathways when exposed to THz radiation. The primary genes induced by THz radiation were inflammatory cytokines.

The third study by the same group [Grundt et al., 2011] continued further examination of the cellular effects of THz radiation. Jurkat cells were exposed to 2.52 THz at the power density of 636 mW/cm² for 5, 10, 20, 30, 40, and 50 min. Viability of cells was assessed using the MTT assay. Gene expression was evaluated using an mRNA microarray. The authors found that cell death increased with exposure duration. The median lethal dose was calculated to be ~44 min. THz radiation induced the transcriptional activation of genes associated with cellular proliferation, differentiation, chaperone protein stabilization, and apoptosis. The magnitude of differential expression of genes was comparable with that induced by temperature controls. However, several genes were specifically activated only by THz exposure indicating that this effect was not thermal.

To evaluate spindle disturbances by THz radiation a human-hamster hybrid cell line was used in experiments *in vitro* [Hintzsche et al. 2011]. Monolayer cultures were exposed to 0.106 THz with power densities of 0.043, 0.43 and 4.3 mW/cm² for 0.5 h in an incubator at 36°C. Temperature elevation in cultures exposed to 4.3 mW/cm² was 1.4°C. THz irradiation at 0.43 and 4.3 mW/cm² produced statistically significant spindle disturbances in anaphase and telophase phases of cell division. The authors suggested that the THz radiation induced the effect on the spindle apparatus without directly affecting DNA.

Reported data on the effects of THz radiation on gene expression are controversial. Along with negative results, changes in gene expression were found to occur at low exposure intensities that excluded the thermal effect of irradiation. Alexandrov et al. [2011; 2013] suggested a

resonance mechanism of interaction of THz radiation with DNA. The changes in gene expression at high exposure intensities were attributed to a thermal effect of irradiation.

2.7.2 Potential Genotoxic Effects

Here we describe the results of studies of the potential genomic and DNA damage induced by THz radiation, mostly in human lymphocytes as well as in human skin cells, in human embryonic stem cells, in human corneal epithelial cells and in bacteria.

Scarfi et al. [2003] investigated the genotoxic effect of THz radiation in human peripheral blood lymphocytes. For exposure of blood samples, a FEL source was used generating a ‘train’ of pulses of about 50 ps duration at a frequency range of 0.120-0.140 THz. The peak power in 4 μ s pulses was about 1.5 kW at 0.120 THz. Samples were exposed for 20 min to 1 mW average power. To study micronucleus (MN) frequency and cell proliferation, the cytokinesis block technique was used. The results showed that the THz exposure did not change the cell cycle kinetics or the number of micronuclei, i.e. no direct chromosomal damage to lymphocytes occurred.

Doria et al. [2004] used human lymphocytes for studying genotoxic effects of THz radiation, which was evaluated using the micronucleus (MN) test and comet assay. Lymphocytes were exposed to pulsed 0.13 THz radiation for 20 min. Two experimental conditions were used. In the first set of experiments, an average power was 3.5 mW at a pulse repetition rate of 5 Hz. In the second set of experiments, the pulse repetition rate was 7 Hz at an average power of 5 mW. Both sets of experiments did not reveal chromosome damage (MN assay) or DNA damage (comet assay) in human lymphocytes. When samples were exposed using the second setup in the metal

cone a small increase in DNA damage was observed. The authors suggested that the cone played the role of a resonant cavity leading to a significant increase of THz power.

The genotoxic effects of THz radiation on human blood lymphocytes were investigated in another set of experiments using the same exposure system as described in the previous paper [Zeni et al., 2007]. The estimated temperature rise during a 20 min exposure of samples at room temperature (23°C) was 0.35°C. Samples were exposed to 0.12 THz at the SAR of 0.4 W/kg and 0.13 THz at the SAR of 0.24, 1.4, and 2 W/kg. Chromosomal damage and cell cycle kinetics were evaluated using the cytokinesis block MN technique. In addition, primary chromosomal damage was tested by applying the alkaline comet assay immediately after exposure. The authors concluded that under the described exposure conditions, DNA damage did not occur and the cell cycle kinetics of lymphocytes did not change.

Korenstein-Ilan et al. [2008] studied the effect of CW 0.1 THz radiation on chromosome number 1, 10, 11, and 17 in human blood lymphocytes using the fluorescence in situ hybridization (FISH) method. Exposure was conducted in an incubator at 37°C to 0.031 mW/cm² for up to 24 h. The temperature rise in samples during 24 h exposure did not exceed 0.3°C. Chromosomes 11 and 17 were most vulnerable (about 30% increase in aneuploidy after 2 and 24 h of exposure) to THz irradiation while chromosomes 1 and 10 were not affected. The asynchronous mode of replication of all centromeres was increased by 50% after 24 h exposure. The main conclusion of this work made by the authors is that the low intensity 0.1 THz irradiation induces genomic instability. As noted by Wilmink and Grundt [2011], the total incidence of aneuploidy of all chromosomes in cells following 24 h exposure and sham-exposure reported in paper was 10.6 and 9%, respectively. These indices are within the spontaneous levels

reported in literature [Vijayalaxmi and Prihoda, 2008]. Thus, the main conclusion of the paper, that low intensity 0.1 THz irradiation induces genomic instability, is questionable.

Hintzsche et al. [2012] studied the induction of genotoxic effect of THz radiation in two types of human skin cells (HaCaT and human dermal fibroblasts) and in the human-hamster hybrid cell line. The cells were exposed to 0.106 THz with power densities ranging from 0.04 to 2 mW/cm² for 2, 8, and 24 h. Genomic damage was evaluated using MN formation. DNA strand breaks and alkali-labile sites were quantified with the comet assay. The exposed cells showed DNA damage levels close to the sham-exposed cells at all exposure durations and power densities. No DNA strand breaks or alkali-labile sites were found.

Genomic damage in human skin cells induced by exposure to 0.380 and 2.520 THz was investigated using the comet assay [Hintzsche et al., 2013]. Cells *in vitro* were exposed to power densities ranging from 0.03 to 0.9 mW/cm² for 2 and 8 h. DNA damage was not detected at any frequency or exposure duration. In addition, the cell proliferation was quantified and found to be unaffected. According to the authors, they demonstrated for the first time that THz irradiation at higher frequencies (0.380 and 2.520 THz) did not induce genomic damage.

Titova et al. [2013a,b] studied the effects of pulsed THz irradiation on artificial full thickness human skin (EpiDermFT, MatTek) tissues. The top surface of tissue samples was exposed to 0.1-2.0 THz centered at 0.5 THz. THz pulses had a 1.7 ps duration, 1 kHz repetition rate, and peak pulse energy variable from 0.1 to 1 μ J with a power density of 57 mW/cm². THz exposure was conducted at room temperature (21°C). The maximal temperature rise was less than 0.7°C. In the first paper Titova et al. [2013a] used the presence of phosphorylated H2AX as the earliest cellular indicator of double strand breaks in DNA. Exposure to intense THz pulses for 10 min produced a significant induction of H2AX phosphorylation indicating that THz

irradiation may cause DNA damage in skin tissue. At the same time, THz irradiation increased the levels of tumor suppressors and proteins responsible for cell-cycle regulation. The authors suggested that DNA damage could be quickly repaired thereby minimizing the genotoxic effect of THz radiation.

In the second paper, Titova et al. [2013b] analyzed gene expression in the artificial skin tissues at the same exposure conditions. Exposure to intense THz pulses produced coordinated changes in gene expression of multiple genes involved in epidermal differentiation. The authors discussed the potential therapeutic applications of high intensity THz pulses.

Bogomazova et al. [2015] studied the DNA damage and transcriptome responses in human embryonic stem cells (hESC) exposed to narrow-band 2.3 THz irradiation. The hESC samples were exposed through a layer of polystyrene membrane covering the cell chamber. The average power density was 0.14 W/cm^2 at the peak power density of 4 kW/cm^2 . The temperature on the surface of the polystyrene membrane was monitored using an IR camera. The temperature on the surface of the polystyrene membrane was kept in the range of $36.5\text{-}37.5^\circ\text{C}$ by adjusting the shutter aperture. The warming of samples above the room temperature of 24°C was due to the THz exposure. The authors did not find any morphological changes in the exposed cells nor induction of H2AX phosphorylation, which indicated the absence of any genotoxic effect of THz radiation on hESC. The transcription of $\sim 1\%$ of genes was increased insignificantly following exposure.

The expression of heat shock proteins, morphological changes and genotoxic effects were studied following exposure of human corneal epithelial cells to 0.12 THz radiation at a spatially-averaged power density of 5 mW/cm^2 [Koyama et al., 2016b]. The chamber with cells was maintained under controlled conditions at a temperature of 37°C . The cells were exposed for 24

h. To evaluate genotoxicity, MN frequency was determined. The heat shock protein expression was estimated by Western blotting. Morphological changes were examined using a cell analyzer device. THz exposure produced no statistically significant increase in the MN frequency or morphological changes in cells. Expression of Hsp27, Hsp70, and Hsp90 α was unchanged in exposed cells as compared to control. The authors concluded that exposure to 0.12 THz under the experimental conditions produced none or little effect on the genetic apparatus and morphology of cells derived from the human eye.

The data on the induction of genomic damage and telomere length modulation in human primary fetal fibroblasts exposed to 0.1-0.15 THz radiation was reported by De Amicis et al. [2015]. The peak output power in 4 μ s pulses generated by a FEL was 1.5 kW. The average incident power density was 0.4 mW/cm² providing an SAR in the cell medium in the range of 15-20 W/kg. Samples were exposed for 20 min at an ambient temperature of 16°C. The cell culture temperature at the beginning of the irradiation (20°C) was higher than the ambient temperature. During exposure, it asymptotically reached the thermal equilibrium over the 20 min irradiation time. The maximal calculated temperature rise was 0.3 °C. DNA damage was evaluated using the Comet assay and phosphorylation of H2AX histone. No DNA damage and telomere length changes in exposed cells were observed. Apoptosis induction or changes in pro-survival signaling proteins were also not found. CREST analysis indicated that THz radiation could induce aneuploidy.

Sergeeva et al. [2016] studied the effect of 2.3 THz irradiation on bacteria *Salmonella* (S) typhimurium and *Escherichia coli* (E. coli) cells. The samples were exposed to 1.4 W/cm² for 5, 10, and 15 min. For the genotoxicity test, the authors measured SOS induction in E. coli PQ37. SOS response is a global response to DNA damage. No significant differences were found

between exposed and control cells indicating that THz radiation did not produce a genotoxic effect. Nevertheless, a small increase in total number of *S. typhimurium* after 15 min exposure and an increase in several enzyme activities in *E. coli* PQ37 were indications that THz radiation affected cell metabolism.

Most studies performed at irradiation intensities of 0.14-5 mW/cm² did not reveal a genotoxic effect of THz radiation in the frequency range of 0.106-2.52 THz. A genotoxic effect at higher intensity (57 mW/cm²) and broadband irradiation was reported by Titova et al. [2013a,b]. The data showing a genotoxic effect obtained by Korenstein-Ilan et al. [2008] requires further verification of its validity.

2.8 THz Radiation Effects on Skin

Dalzell et al. [2010] evaluated the tissue damage thresholds of THz radiation using computational and experimental approaches. Authors used two exposure setups: short exposure for 2 s to 0.1-1.0 THz with 2-14 W/cm², and long exposure for 60 min to 1.89 THz with 189.9 mW/cm². The short exposure was used in a study of the effect of THz radiation on wet chamois clothes. In this case, the tissue damage threshold (ED50) was determined to be equal to 7.16 W/cm². Computational modeling using an Arrhenius damage model predicted ED50 = 5 W/cm². The long exposure was used in a study of THz effect on freshly excised porcine skin and egg whites. No damage was observed in the porcine skin. Only several egg whites revealed visible signs of coagulation. In this case, the temperature had increased by 10-12°C. It is reasonable to suggest that the mechanism underlying the tissue damage was thermal as the temperature in chamois clothes increased up to 60°C by the end of the exposure.

Hwang et al. [2014] studied the inflammatory response in live mice following pulsed THz irradiation using a custom-built laser-scanning confocal microscopy system. The skin of the ear of anesthetized mice was exposed to pulsed THz radiation (2.7 THz, 4 μ s pulse width, 61.4 μ J per pulse, 3 Hz repetition rate) for 30 min with an average power density of 260 mW/cm². The beam was focused onto a small spot 300 μ m in diameter. The authors could not find any notable temperature change at the surface of the aural skin when the skin was examined with an IR camera. It might be explained by the small size of the irradiation beam. To monitor any potential inflammatory response, resident neutrophils in the area of exposure were repeatedly visualized before and after THz irradiation with a confocal microscope optimized for *in vivo* visualization. No changes in the number of resident neutrophils were observed in non-irradiated control skin areas. However, a massive recruitment of newly infiltrated neutrophils was observed in the exposed skin area 6 h following exposure, indicating the induction of an acute inflammatory response in the skin. The authors suggested that this effect was induced via a non-thermal mechanism.

The histological and molecular changes in the skin of 8-week-old male C57BL/6 mice exposed to an fs pulsed THz beam were studied by Jo et al. [2014]. The skin on the back of mice was exposed to 3 THz radiation with a pulse width of <200 fs and a repetition rate of 1 kHz for 1 h. The THz pulse energy was about 0.26 nJ/pulse. Accumulated energy in the exposed area (1 cm²) for 1 h was $\sim$ 1.15 J/cm². Skin biopsy samples were taken at 1 and 24 h after exposure. No histological changes were found in either 1 or 24 h post-exposure samples. The authors did not find any inflammatory cells or damaged skin cells such as sunburn cells. The collagen fibers in the dermis were also normal. A microarray analysis revealed that some genes in the exposed skin samples were either biologically activated or suppressed. For instance, the gene

transcription of substance P, which is involved in local inflammation, decreased while the transcription of calcitonin gene-related peptide, another neuropeptide that is associated with neurogenic inflammation in eczema, increased significantly. These results are at variance with the histological data. The authors did not provide any explanation for these effects. Any information about the temperature elevation in the exposed skin is also missing.

Kim et al. [2013] investigated wound healing in C57BL/6J and BALB/c nude mice exposed to fs pulsed THz radiation, as well as the genome-wide expression profile in the exposed mice skin. The frequency spectrum of irradiation ranged up to 2.5 THz. The pulse width was approximately 310 fs with an energy 0.26 nJ/pulse. The accumulated pulse energy for 1 h was up to 1.15 mJ/cm². Mice were exposed on their dorsal area with a beam 10 mm in diameter and an average power density of 1.15 mW/cm². Within the measurement error of the IR camera ($\pm 0.05^\circ\text{C}$), no temperature rise in the skin during exposure was detected. The authors did not observe any notable changes in expression of Hsp70 or in the histology of the exposed skin. Microarray analysis of exposed skin samples revealed 149 differentially expressed genes with a mean fold change of signal intensity ≥ 1.5 . Repeated fs pulsed THz irradiation of a punch wound in the skin significantly delayed the time for wound healing due to up regulation of transforming growth factor beta (TGF- β). The authors suggested that fs pulsed THz radiation induced a wound-like signal in skin, which inhibited wound healing *in vivo*.

Thus, exposure of skin to high intensity THz irradiation caused dermal damage and induction of an acute inflammatory response. At lower exposure intensities, there were no histological changes, inflammatory cells or damaged skin cells detected. However, some genes in exposed skin samples were either biologically activated or suppressed.

2.9 THz Radiation Effects on Animals

Due to the limited cross-sectional beam area available in early THz generators, small laboratory animals such as rats and mice were used in the initial studies of THz bioeffects. In these studies, the behavior effects of mice [Bondar et al., 2008] and responses of rats to immobilization stress [Kirichuk et al., 2009; Kirichuk and Tsymbal, 2009] following THz radiation were examined.

Bondar et al. [2008] exposed mice to 3.6 THz with the power density of 23.6 mW/cm^2 for 15 or 30 min. The behavior of mice was tested for anxiety. THz irradiation increased the anxiety level. The effect was maximum when the mice were in the area of the opening where radiation entered. Mice exposed for 30 min revealed the increased anxiety level on the next day. The observed effect might have resulted from the warmth sensation when the mice were in the area of the opening where the THz radiation intensity was maximum.

A study using albino rats evaluated the effect of 0.150 THz radiation on platelet aggregation and behavioral changes [Kirichuk et al., 2009]. Rats were exposed for 15, 30, or 60 min with the power density of 3 mW/cm^2 . Rats were subjected to immobilization stress and then exposed to THz radiation. Immobilization stress enhanced platelet aggregation. Exposure for 60 min produced an increased level of depression and an enhanced level of platelet aggregation even greater than that resulting from the immobilization stress. The mechanism of this effect is not clear.

In another study by Kirichuk and Tsymbal [2009] the effect of THz radiation on the level of lipoperoxidation (LPO) and antioxidant properties of the blood were examined in albino rats subjected to immobilization stress. Animals were exposed to 0.150 THz with the power density

of 0.7 mW/cm² for 15 or 30 min. Immobilization stress produced an elevation of stress markers for LPO and antioxidant levels. Irradiation for 30 min reduced the levels of stress markers to control levels. This result contradicted the data reported in [Kirichuk et al., 2009]. The authors speculated that the selective absorption of THz radiation by NO molecules was responsible for this effect. However, the mechanism underlying the observed effect, if verified, remains unclear.

The studies of the effects of THz radiation on animals are not numerous. More studies by different groups of researches are required to draw clear conclusions on the reactions of animals to THz radiation.

2.10 Mechanisms of Action of THz Radiation

Effects of THz radiation obtained following exposure to high intensities could be reproduced by equivalent temperature elevations indicating a thermal mechanism of THz action. However, in the most of *in vitro* studies the methods used for temperature measurement were not described in detail. Depending on the temperature measuring technique, the result may differ significantly [Zhadobov et al., 2017]. For example, with the same THz exposure intensity, the temperature in a cell monolayer may be much higher than that in bulk. Therefore, for the interpretation of the obtained results it is important to define accurately not only the exposure intensities but the temperature elevations also. This could be important for the correct identification of the mechanism of action of THz radiation.

Another problem in identification of mechanisms of THz radiation action is the exploitation of different exposure conditions in different studies. This creates a problem of adequate *in vitro* and *in vivo* dosimetry [Jastrow et al., 2010; Kleine-Ostmann et al., 2014]. Moreover, temperature

control of cell samples in experiments *in vitro* can be troublesome. The majority of experiments cited in this review were performed at an ambient temperature of 22-27°C. It should be noted that the growth of mammalian cells decreases following “cold stress” by lowering the temperature to 25-27°C [Wilmink and Grundt, 2011]. Moreover, mitochondria can undergo temperature-dependent phase transition at approximately 23°C [Raison et al., 1971]. To avoid these uncertainties, the experiments with cells should be conducted at physiological temperatures.

Effects of THz radiation at low intensities cannot be attributed to a thermal mechanism. They may be caused by a resonance interaction of THz radiation with biomolecules. Using THz time-domain spectroscopy Fisher et al. [2002] investigated absorption spectra of the four nucleobases and corresponding nucleosides forming DNA. They found numerous distinct resonances in the range of 0.5-4.0 THz. The origin of these resonances were interpreted as vibrations of hydrogen bonds between the molecules. Hence, THz radiation may be directly absorbed by DNA at resonance frequencies. Similar results were obtained by Globus et al. [2003].

Based on the data demonstrating DNA involvement in cellular effects of THz radiation Alexandrov et al. [2010] proposed a hypothesis of the existence of linear and nonlinear resonance interactions of THz radiation with DNA. For describing the local DNA pairing/unpairing (breathing) dynamics, the authors used the Peyrard-Bishop-Dauxois (PBD) model [Peyrard and Bishop, 1989; Dauxois et al, 1993] of double stranded DNA (dsDNA). The THz radiation was implemented in a model without specifying the precise mechanisms of physical coupling to dsDNA. The authors discovered a nonlinear discrete breather mode that resulted from a specific perturbation of the system. This mode can store energy for a long time.

The dsDNA molecule had two steady conformational states with different energies and amplitudes. Only in these two states, absorbed energy of an external source compensated for the losses due to friction. One of the states was vibrating at an applied frequency, f (2 THz), and the second was vibrating at $f/2$. A nonlinear mechanism required a spatial perturbation above a certain amplitude threshold. Thus, the model study showed that THz radiation could affect the dsDNA molecule producing large localized openings in the DNA double strand.

Swanson [2011] repeated calculations of the interaction of THz radiation with the dsDNA molecule with parameters including those used by Alexandrov et al. [2010] but varying them in a greater range. Swanson confirmed the previous observation of destabilizing dsDNA breather modes. It was found that parameter variation under reasonable physical conditions could eliminate the breather mode entirely or make it unrealistically strong. The author estimated the amplitude of the electric field necessary to generate breather modes to be 10^9 V/m. This value was much greater than the dielectric breakdown threshold. If these calculations are correct then the parameters used by Alexandrov et al. [2010] may not be relevant to real situations. To clarify this discrepancy further modeling of THz radiation with DNA is required.

The frequency dependent effect of THz radiation in experiments *in vitro* could also result from the complex interaction of the incident radiation with the exposure chamber filled with liquid medium. As shown in Fig. 1.4, reflection of the incident wave from the bottom of a chamber could drop to zero at some frequencies depositing the maximal radiation energy to a sample while at other frequencies reflection takes on a maximal value (30-15%) decreasing the deposited radiation energy. This result shows that the comparison of the magnitudes of frequency dependent changes induced by THz irradiation could be correctly evaluated only with adequate dosimetry.

As demonstrated by Munzarova et al. [2013] high intensity THz pulses generated an ultrasound wave in a liquid medium with a frequency equal to the repetition rate. Sound waves are generated in liquid or tissues by rapid thermal expansion caused by absorption of THz radiation. Under certain conditions, a sound wave may create a standing wave in the exposed system with a frequency and amplitude determined by the size of the system and its acoustic properties [Lin, 1977a; 1977b]. At the coincidence of both frequencies, resonance amplification of the amplitude of the sound wave could occur. It is known that ultrasound exposure at even moderate intensities (0.3 and 0.4 W/cm²) can produce DNA double-strand breaks [Furusawa et al., 2012]. However, the intensity of ultrasound wave generated by THz exposure was not evaluated, and it is not clear whether the intensity of the ultrasound wave was sufficient to produce a biological effect. The generation of an ultrasound wave might explain the bioeffects following some THz exposures.

2.11 Conclusion

Reflection and absorption of electromagnetic energy at THz frequencies are well characterized empirically and theoretically. Heating of skin and water by THz irradiation was evaluated only theoretically and need to be confirmed experimentally.

Changes in the molecular systems and liposomes exposed to THz radiation were found in many studies. However, due to the low penetration depth of THz radiation in a liquid medium, the absorption of radiation energy by objects is extremely non-uniform. Therefore, it gives rise to skepticism regarding the direct effect of THz radiation on these systems.

The controversial results obtained in the studies of the effects of THz radiation on cellular systems prevents the drawing of clear-cut conclusions about the existence of the non-thermal THz bioeffects. Moreover, the experiments in many studies were performed under very different conditions and without adequate dosimetry thereby complicating the evaluation of the obtained results.

Reported data on the effects of THz radiation on gene expression are controversial. Along with negative results, gene expression was found to occur at low exposure intensities that excluded the thermal effect of irradiation. To explain a non-thermal effect of THz radiation, Alexandrov et al. [2011; 2013] suggested the resonance mechanism of interaction of THz radiation with DNA. The changes in gene expression at high exposure intensities were attributed to the thermal effect of irradiation.

In spite of the relatively short time of THz studies, the genotoxic effect was investigated in a very comprehensive way. Most studies performed at irradiation intensities of 0.14-5 mW/cm² did not reveal any genotoxic effect of THz radiation in the frequency range of 0.106-2.52 THz. A Genotoxic effect at higher intensity (57 mW/cm²) and broadband irradiation was reported by Titova et al. [2013a,b]. The data showing the genotoxic effect obtained by Korenstein-Ilan et al. [2008] requires further verification.

At exposure of skin to high intensity THz irradiation, dermal damage and induction of an acute inflammatory response were observed. At lower exposure intensities, no histological changes, inflammatory cells, or damaged skin cells were found. However, some genes in exposed skin samples were either biologically activated or suppressed.

The studies of the effects of THz radiation on animals are not numerous. More studies by different groups of researches are required in order to draw clear conclusions on the reactions of animals to THz radiation. Studies regarding the THz radiation interaction with humans are also required.

Three main mechanisms of THz effects were suggested:

1. Thermal mechanism;
2. The nonlinear resonance interaction of THz radiation with DNA;
3. Generation of ultrasound waves in liquid samples by pulsed THz radiation.

The resonance and sound wave hypotheses need to be explored further.

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