Chapter 2
Experimental evidence

2.1 Introduction

The possible biological effects of non-ionizing radiation have been recently highlighted by the “mobile phone” debate and related public concerns (Hyland 2000) although the first reports of biological effects of millimetre wave radiation exposure actually date back to 1968 when Webb and Dodds (Webb & Dodds 1968) observed an inhibition of bacterial growth at 136 GHz. The thermal effects of mm-wave radiation are well documented and are not a subject of contention. However, the published evidence for non-thermal effects of millimetre wave absorption in biological systems is contentious. A largely contradictory body of evidence exists with a number of well-performed experiments not supporting the hypothesis that mm-wave induced effects are non-thermal in origin (Gos, Eicher, Kohli, & Heyer 1997b) (Furia, Hill, & Gandhi 1986b) (Blackman et al. 1975a). Some of the evidence indicates the possibility of non-thermal, low-dose effects with a non-linear-dose response. This evidence is critical to arguments on Health and Safety standards, which are currently based solely on dose-dependent thermal effects. It is also critical to progressing research into the possibilities of manipulating biological and biochemical systems via mm-wave radiation for medical and other purposes.

Several review articles have been published on the biological effects of mm-wave radiation. Most notable is the comprehensive review by Pakhomov in 1998 (Pakhomov et al. 1998) and one specifically concerned with mm-wave therapy (Rojavin & Ziskin 1998). The Russian language journal “*Millimetre wave radiation in biology and medicine*” for example, Kovalev et al and is published quarterly where the emphasis is on direct medical application.

The literature can be conveniently divided into a number of areas. Studies on the physical effects of mm-wave radiation e.g. convection, enhanced diffusion etc have shown a potential to impact on biological processes directly and via the cellular milieu. An understanding of these effects is important in determining the thermal or nonthermal nature of any induced effect with confidence.

The biological effects on living organisms can be categorised into those relating to single-celled organisms such as the prokaryotes, lower eukaryotes, tissue cultures, and animals. Publications relating to induced effects in higher organisms and animals actually represent the bulk of the literature. Annual publications from Russia, Ukraine and former Eastern Bloc countries number in the hundreds. These are mainly physician-orientated studies that relate the findings in the treatment of a range of conditions. Unfortunately, in many cases, established protocols for this type of experimentation such as the use of double-blind exposure have not been followed, and so some reports have to be considered largely anecdotal. This literature review is primarily concerned with effects relating to the prokaryotes, lower eukaryotes and cells cultured from the tissues of higher organisms.

2.2 Physical effects

More recently, a number of studies have contrasted the progress of conventionally heated reactions with “microwave assisted” ones. The authors claim that microwave radiation can accelerate organic reactions, increasing product yield and efficiency. Almost all studies describe effects that are operative at 2.45 GHz, the frequency commonly used in domestic and industrial applications. The possibility exists that such effects may also be important at higher frequencies. The findings are unanticipated as the coupling of microwaves with a dipolar molecule leads to dielectric heating (see Chapter 1) so, in this theory, the effects of microwave absorption on reaction kinetics should not differ significantly from those of equivalent energy input by conventional heating.

2.2.1 Effects on cell-free enzyme systems

Parker *et al* (Parker et al 1996) irradiated a hydrated lipase enzyme suspended in organic media at 2.45 GHz, which resulted in a 2 - 3 fold increase in reaction rate over classical heating. By controlling the enzyme-bound water content (thermodynamic water activity a_w) the observed increase was found to be dependent on the hydration state of the enzyme. The enzyme cutinase consistently exhibited a 2 – 3 fold increase in activity with a_w in the range 0.58 – 0.69 but with a_w at 0.97 the microwave irradiation decreased the reaction rate compared to conventional heating. The reaction rate of the enzyme cellulase was the subject of an investigation by Bose *et al* (Personal communication). An enhancement of enzymatic hydrolysis was observed, similar in magnitude to that found by Parker (Parker et al. 1996). Bose (Bose et al. 2000b) has also reported microwave-enhanced reactions with oligopeptides and the enhanced enantioselectivity of beta-lactams (Bose et al. 2000a). Hydration is an important factor in the catalytic potential of enzymes (Kurzynski 1998). Parker (Parker 1996) observed an increased rate of exchange between water molecules and protein following mm-wave exposure.

2.2.2 Enhanced diffusion effects

In 1988 Gibson *et al* (Gibson, Matthews, & Samuel 1988) reported accelerated diffusion of ethylene oxide with 2.45 GHz microwaves. An Arrhenius plot of change in diffusion coefficient for both conventional and microwave-assisted processes supported the hypothesis that hydrogen bonds were being actively disrupted.

2.2.3 Convection effects

The potential of mm-wave radiation to induce convection currents in fluids has been studied by Khizhnyak et al (Khizhnyak & Ziskin 1996). Convection currents can form stable vortexes, causing temperature oscillations that result in both a local decrease and increase in temperature.

$$-\frac{dT}{dz} \geq \frac{g\beta T}{C_{p'}} \quad (1.0)$$

Free type convection can occur if the above criterion (1.0) is satisfied (Khizhnyak & Ziskin 1996). Where *dT/dz* is the temperature gradient, T is the temperature $\beta = (\delta V/\delta T)_p/V$ and C_p is the molar heat capacity at constant pressure. In lossy dielectrics such as water, mm-wave radiation is rapidly absorbed (Gandhi 1983) and this results in high specific absorption rates. The rate of temperature rise within a fraction of a millimetre of the liquid surface can be extremely high although, owing to the large surface area available for cooling, absolute temperature gradients remain small. Convection phenomena have been reported at an incident power density level of 1×10^{-6} W cm^{-2}.

An additional factor may relate to horn antennas that under certain circumstances produce markedly non-uniform field patterns, particularly in the near field. In continuous-wave exposure regimes, this may cause complex thermal gradients near these non-uniformities (Khizhnyak & Ziskin 1996). Moreover, the field pattern can change in a frequency-specific manner and form the basis of a frequency-dependent artefact.

2.2.4 Exposure in the near and far field

Gapeyev (Gapeyev et al. 1996) compared heating patterns in a water phantom. A horn antenna was positioned to irradiate the phantom in the near field and far field. Irradiating the phantom in the near field resulted in a complex heating pattern, which was non-uniform. Moreover, changes in frequency result in significant changes in the irradiation pattern and so the potential for the induction of “quasi” resonant biological effects exists.

2.2.5 Fröhlich-Davydov effect in acetanilide

Acetanilide was selected for experimental investigation on the basis that its structure and bonding resembles that found in certain biopolymers (Mesquita, Vasconcellos, & Luzzi 1999). Investigators have shown an anomalous line (Careri, Buotempo, & Carta 1984) attributed to a Davydov soliton. A theoretical model shows good agreement with the experimental evidence (Vasconcellos, Mesquita & Luzzi 1998).

2.2.6 Fröhlich effect in aerosols

Miller (Miller & Gebbie 1996) investigated the effects of monochromatic mm-wave radiation and a continuum light source on the **phonon** spectrum of aerosols. According to Miller, aerosols meet certain isolation conditions and so a Fröhlich-type effect may be induced if pumped by an external source. Both continuum and monochromatic sources were reported to modify the aerosol phonon-mode spectrum (Miller & Gebbie 1996).

2.3 Effects of mm-wave radiation on Biological systems

2.3.1 Prokaryotes

Growth rate change was the first biological effect observed in the late 1960s when Webb and Dodds (Webb & Dodds D 1968) described an inhibition of growth in the bacterium *Escherichia coli* (*E. coli*) at 138 GHz. In 1969, the same group reported that growth rate could be either be enhanced by (up to 50%) or suppressed (50%) by exposure to different frequencies (Webb & Booth 1969). The switch from growth inhibition to enhancement occurred over a 2 - 3 GHz frequency range and represented the first report of a “resonance” or window effect. Blackman *et al* (Blackman et al. 1975b) failed to replicate the results of Webb’s 1969 study.

During the next decade groups in the former Soviet Union, France, Canada and Germany reported on mm-wave interactions with microorganisms, describing effects that were frequency dependent and occurred with relatively low power exposures. A review of Soviet work published in 1973 (Multiple authors 1973) related biological effects in fungi, yeasts, gram-positive and gram-negative bacteria. Changes in protein metabolism and a reduced spore-forming capability were observed. Some technical details of the exposure set-up and conditions and specific strains used were not reported. Berteaud *et al* observed a marked decrease in growth rate of *E. coli* at 70.5 and 73.0 GHz (Berteaud et al. 1975).

More recently, Tambiev (Tambiev & Kirikova 2000) describes mm-wave induced effects in photosynthesizing microorganisms such as the cyanobacterium *Spirulina platensis*. From an evolutionary perspective, *Spirulina platensis* is distant, in evolutionary terms, to *Escherichia coli,* the bacterium most commonly employed in experimental work. A significant increase in growth rate was observed compared with the control when the following exposure regime was employed: - 42 GHz / 5 mW cm^{-2} / 15-360 min (Tambiev & Kirikova 2000). No growth rate enhancing effects were observed in the absence of oxygen or in the presence of sodium selenite. Change in the membrane permeability to some ions was also noted.

2.3.2 DNA tropic effects

A major class of experimental findings relates to interactions with genetic elements of the cell such as the bacterial genome itself, extra-chromosomal elements such as **plasmids** and bacterial viruses (**Phages**). Belyaev (Belyaev et al 2000b) reported that mm-wave radiation induces conformational change in the fine structure of both eukaryotic and prokaryotic cell chromatin and change in the binding of chromatin–associated proteins. These effects were reported to be dependent on a range of physical, physiological and genetic factors. A technique (anomalous viscosity time dependence, AVTD) measured change in the radial migration of DNA-protein complexes in a hydrodynamic field. Radial migration of DNA macromolecules in the hydrodynamic field is said to be dependent on their size, shape and elasticity (Belyaev et al. 1999). Positive controls in the form of DNA intercalating agents such as ethidium bromide were employed.

Particularly notable are the extremely low power densities (10^{-19} W cm^{-2}) required to induce the DNA conformational change at resonance frequency (Belyaev et al. 1996). This is significantly lower than that achieved in any previous study. It may also overcome some of the objections to the concept of mm-wave therapy. It was also reported that the resonant frequency could be offset by changing **haploid** genome length. Differential effects were also reported with linear, circular (left and right handed) polarization of the incident mm-wave radiation. Another interesting feature is the physiological state of the organisms used in the studies. The induced changes in genome conformation were reported to be reproducible in **stationary phase** cultures of *E. coli* but not in exponentially growing (**exponential phase**) cultures. Osepchuk (Osepchuk & Petersen 1997) suggested that the actual power density could be much higher because higher order harmonics may have been propagated through flap attenuators used to control power density in the exposure system.

The effects of mm-wave exposure on **lysogenic** strains of bacteria have been reported by a number of investigators. In 1979 Webb (Webb S 1979) reported a frequency-specific induction of the λ-prophage in lysogenic *E. coli K12*λ^+ cells. A 10,000 fold increase in induction was reported to occur over a 200 MHz bandwidth centred at 71.4 GHz. Prophage induction has been independently replicated with slight variations in resonance frequency and power dependencies in 1980 and 1989 (Lukashevsky & Belyaev 1989) (Bannikov & Rozhkov 1980).

A number of investigators report mm-wave induced effects in plasmids. These are extra-chromosomal elements found in the cell cytoplasm. Smolyanskaya observed a 300% increase in colicin synthesis in *E. coli* at resonant frequencies (Smolyanskaya & Vilenskaya 1974) and also the modulation of penicillinase expression in *Staphylococcus aureus* (Smolyanskaya et al. 1981). Shub (Luneva & Shub 1982; Shub et al. 1989) reported effects on R-plasmids in *E. coli*, important in the transmission of antibiotic resistance.

2.3.3 Spectroscopic studies

Raman spectroscopy is a way of finding the vibrational modes of a cell or molecule. Some inherent problems with using metabolically active cells include photochemical decomposition and the need for high input powers. One solution is to employ a re-circulating system (Webb et al 1976) In the re-circulating system it was suggested that the observed effects were related to the clumping of cells (Furia et al 1986,1984).

2.3.4 Miscellaneous effects

Rojavin (Rojavin & Ziskin 1995) investigated the effects of mm-wave exposure on the survival of UVC exposed cells. Stationary phase cultures ($1x10^9$ CFU/ml) of *E. coli* K12 were exposed to a LD_{99} dose of UVC radiation. In different experiments, the cultures were irradiated with mm-wave radiation pre and post UVC exposure. The source had a 2 GHz bandwidth centred at 61 GHz and was of the type used in mm-wave therapy. Post UVC exposure with mm-waves resulted in a small but reproducible effect on survival. The authors suggest that mm-waves act either directly or indirectly on DNA dark repair mechanisms. Bulgakova (Bulgakova et al. 1996) investigated the combined effects of mm-wave radiation and antibiotics on growth of *Staphylococcus aureus*. Five antibiotics from the fourteen tested exhibited enhanced antibacterial activity the effect being particularly marked in the gramicidin group.

2.4 Lower eukaryotes

2.4.1 Growth and division rate effects

For the purposes of this section, growth is defined as a mass increase of an individual cell or bud and division as an increase in cell body number (Gos et al. 1997a). The effects of mm-wave radiation on *Saccharomyces cerevisiae* are perhaps the most controversial in the literature. Briefly, Grundler *et al* (Grundler & Keilmann 1978) irradiated yeast cultures with mm-waves at frequencies between 41.65 and 41.80 GHz. A frequency-dependent enhancement (+115%) or inhibition (-71 %) of growth rate was observed at particular frequencies. Furthermore, the width of resonant frequencies was incident power dependent (Grundler & Keilmann 1978). The findings were reproduced using different exposure systems and with reduced field intensities (Grundler W 1983). However, Furia failed to find statistically-significant effects in a carefully controlled replication attempt (Furia, Hill, & Gandhi 1986a). In addition, Grundler failed to replicate earlier studies when a klystron was used which is less noisy than the backward wave oscillator employed in the original study (personnel communication, O. Gandhi). Although the exposures were described as low intensity with a re-circulating system to dissipate heat, in some cases source power up to 40 mW was used. Furia also identified a number of experimental inadequacies (Furia, Hill, & Gandhi 1986a).

In later series of experiments (Grundler 1992; Grundler & Keilmann 1989), frequency-specific changes in division rate were reported in the 41.69 and 41.70 GHz frequency range. Again, an attempt was made to replicate the finding, which was unsuccessful. Since some of the counting procedures were subjective, Gos *et al* suggest that bias may have been introduced into the experiment. In both cases where independent replication of the effect was attempted, electromagnetic parameters were carefully controlled, but significant differences existed in the biological parameters.

Golant (Golant, Kuznetsov, & Bozhanova 1994), investigated growth rate and synchronicity effects in bud formation of the yeast *Saccharomyces carlsbergensis* after exposure at 46 GHz for 50 min. Short exposure was found to produce change of the budding period. A cell-cycle synchronization effect occurred after longer-duration exposure. Dardanoni (Dardanoni et al. 1985) observed a suppression of growth with 72 GHz radiation modulated at 1 KHz, an effect that was frequency-specific.

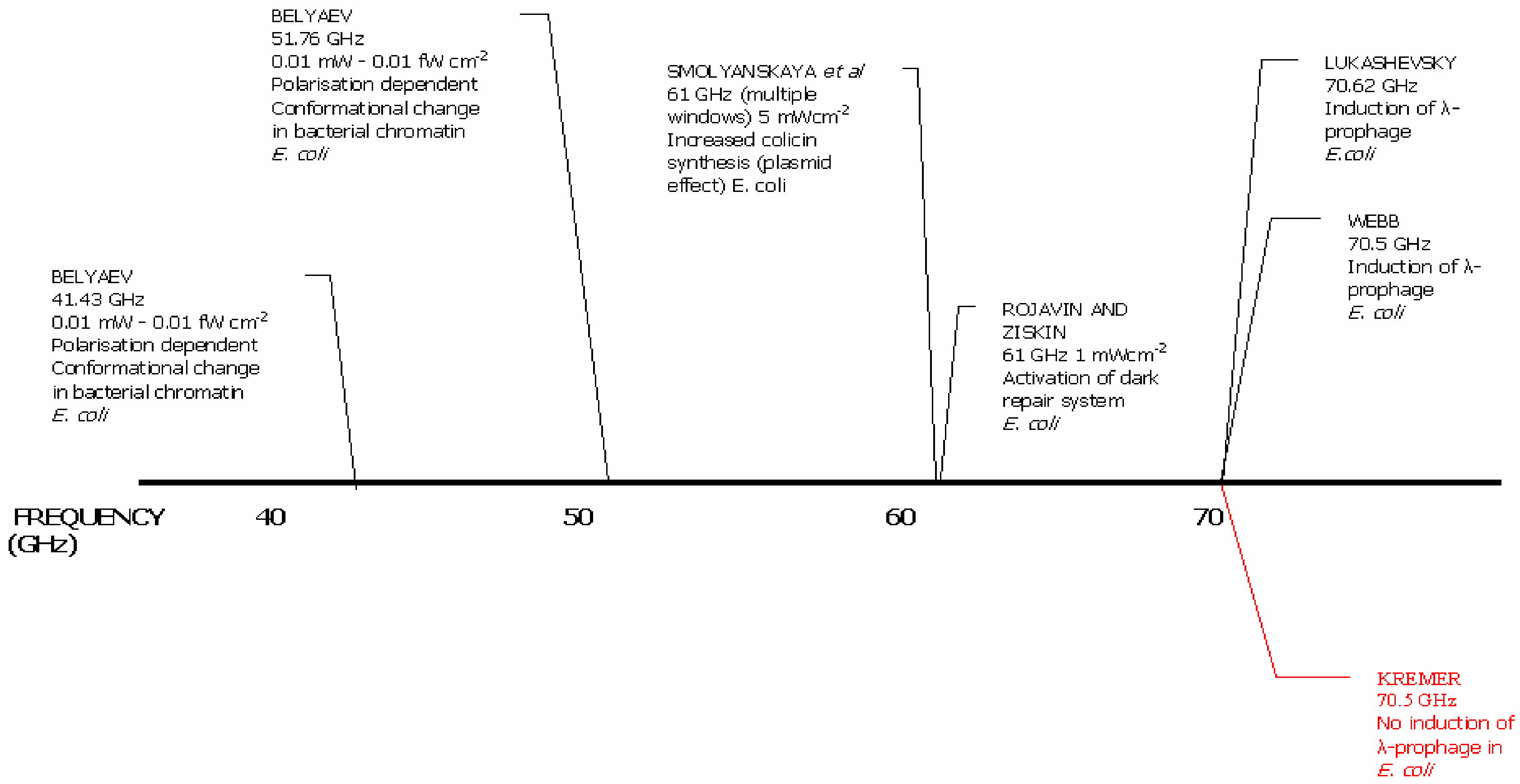

Fig. 3 epigenetic effects in cell-culture

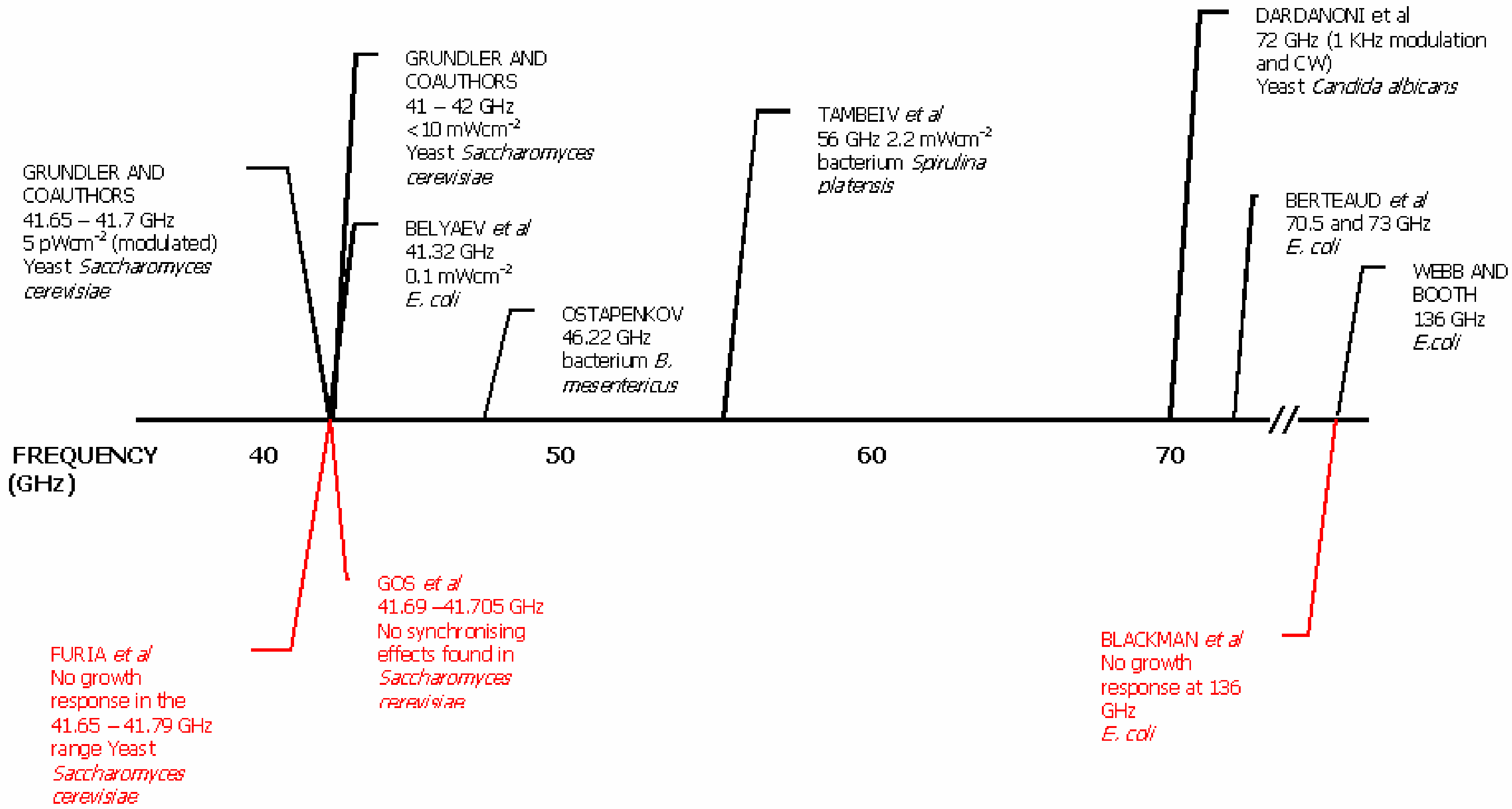

Fig. 4 Growth rate effects in bacteria and lower eukaryotes

2.5 Mammalian cells

Szabo *et al* investigated the effects of exposure on human keratinocytes *in vitro*. Exposure at 61.22 GHz with a SAR of 770 W kg^{-1} for 15-30 min resulted in elevated levels of IL- 1 beta production. The effects of mm-wave exposure on phospholipid metabolism and the neutrophil respiratory burst were reported. Using different antagonists, the authors emphasize the importance of cytochrome P- 450 and phospholipase A2 in the mm-wave induced effects. A Chemiluminescence-based technique was used to investigate the production of reactive oxygen species in murine peritoneal neutrophils in response to mm-wave exposure (50 μW cm^{-2} for 20 min). Significant enhancement and suppression were observed to occur in a frequency-specific manner.

2.6 Human and animal studies

Animal and human studies can be divided into two types: physiological studies and those performed in connection with mm-wave therapy. Because mm-wave radiation is rapidly absorbed in the superficial tissues, (see chapter 1), significant attention has been given to the biological effects of mm-wave radiation on the skin and peripheral receptors. Wound healing / tissue regeneration and peripheral receptor stimulation represent some of the more readily understood effects. However, a diverse range of conditions has been reported as responsive to mm-wave therapy.

Korpan and Saradeth (Korpan & Saradeth 1995) (see also Korpan and Saradeth 1994 (Korpan, Resch, & Kokoschinegg 1994)) investigated the effects of mm-wave radiation (37 GHz CW, 1 mW cm^{-2}) on septic wound healing. In the study, 141 patients with septic wounds (predominantly caused by pyogenic *Staphylococcus aureus*) were either exposed or sham exposed for 30 min per day for 7 days using a well-defined regime. Assignment to sham or mm-wave exposure groups was double blind. Findings included a significant enhancement of wound clearance, epithelization and granulation compared with sham exposure. Kolosova *et al* (Kolosova et al. 1998) reported enhanced nerve regeneration using the following regime: - 53.57 GHz; 4 mW cm^{-2} 10 min every 3 days for two weeks. Improvements were noted in nerve conduction velocity and included a long-term positive effect on regeneration was noted.

In a series of papers, Radzievsky *et al* investigated the analgesic properties of mm-wave radiation (Radzievsky et al. 1999), antipruritic effects (Rojavin & Ziskin 1997) and hypoalgesia (Radzievsky et al. 2000). Mice were exposed using a horn antenna (61.22 GHz; 15 mW cm^{-2}; 15 min). Pre-treatment of mice with the opioid antagonist naloxone (1 mg kg^{-1}) blocked the effect of mm-waves suggesting the *in vivo* release of endogenous opioids or enhanced activity of opioid signalling pathways.

The adjunctive use of mm-wave therapy in cardiovascular diseases and oncology is described in several review articles (Lebedeva 2000) (Pletnev 2000) . In a preliminary study Bellossi *et al* (Bellossi et al. 2000), investigated the effect of chronic exposure to 60 GHz radiation at 0.5 mW cm^{-2} (the same frequency as used in a indoor high-rate communication system) on healthy Swiss mice and mice grafted either with either L1210 cells or Lewis tumour cells. Compared with a sham exposed control Swiss mice showed increased activity, mice grafted with L1210 cells showed increased survival but enhanced growth of the Lewis tumour graft was reported. Potekhina *et al.* induced cardiac arrhythmias in anaesthetized rats at certain frequencies between 55 and 78 GHz, (> 10 mW cm^{-2} 20 min) (Potekhina et al. 1992). Extended exposure (150 min) at 51, 61 and 73 GHz resulted in the sudden death of 25 % of the animals under study as a consequence of the induced arrhythmia. The treatment of peptic and duodenal ulcers is also described in the literature by a number of authors.

2.6.1 Passive radiometry

The intracellular and extra-cellular make up of the cell is essentially aqueous. Some studies have suggested that mm-wave radiation may be implicated in the ordering or “structuring” of water associated with biological systems. Sinitsyn (Sinitsyn et al. 2000) described apparatus that concurrently irradiated the biological system and measured emission using microwave radiometry at 1 GHz. Microwave radiometers are used to make non-invasive temperature measurements deep within human tissue (Jacobsen & Stauffer 2000). Sinitsyn reports that at certain resonant frequencies in the mm-wave spectral range the radiometer detected a decrease in emission. The authors hypothesize that this was due to the structuring of “biological” water. It should be noted that an important feature of the Fröhlich model is that energy is channelled into excited mode (s). Because of the laws of conservation of energy, energy channelled into the excitation of low frequency modes will result in the depletion of others.

2.7 Discussion

The literature comprises a diverse list of biological effects. The majority of papers relate to positive findings where the mm-wave radiation induced some kind of effect. Negative findings are seldom published except where they provide support for public health policy and so a positive-effects bias exists in the literature.

The term non-thermal is used in the literature in connection with a wide range of power densities. In many cases, “low-intensity” might be a more appropriate term because the non-thermal nature of the effect has not been convincingly established. It is suggested here that thermocouple-based temperature measurements are unreliable. The term “non-thermal” should be reserved for exposures power densities that are below 0.1 mW cm^{-2}, or where there is strong biological evidence for the non-thermal nature of the effect.

Belyaev (Belyaev et al) describes extremely low power density effects (10^{-19} W cm^{-2}) on genome conformation. Speculatively, these may indicate that mm-wave radiation could be deployed from orbit to modify genome conformation state. However, these power densities are not measurable and are of comparable intensity to background black body noise levels. Belyaev et al introduces the concept of selection rules e.g. helicity to account for the low-intensity interaction.

The literature review contained reports of “quasi-resonant” phenomena. Khizhnyak (Khizhnyak & Ziskin 1996) reported the existence of convection effects at incident power densities normally considered well within the non-thermal domain. Furthermore, the convection effect could be detuned by shifting frequency a few MHz, which closely resembles the detuning of biological effects often reported in the literature. Another potentially important source of artefact relates to near and far-zone exposure. Using a horn antenna, Gapeyev (Gapeyev et al 1996) made thermographic measurements of a phantom in the near and far zone. Near-zone exposure produced complex interference patterns with potential to induce biological effects in a “quasi-resonant” manner. Far-zone exposure greatly minimized the potential for quasi-resonance. The above findings represent the basis for caution in the interpretation of frequency effects that emphasise the significant challenge associated with exposure cell design.

The biological effects of mm-wave radiation on lower eukaryotes such as yeast are perhaps the most contentious. Synchrony and growth enhancement and inhibition effects have been reported which would implicate cell-cycle regulators such as the *cdc 2* and *wee 1* genes (Gos, Eicher, Kohli, & Heyer 1997a). Mutations in these genes can result in premature cell division.

Lloyd (Lloyd et al 2001) has recently shown that with the fission yeast *Schizosaccharomyces pombe*, an ultradian clock modulates the passage of cells from G_2 into mitosis at the *cdc 2* control network. The ultradian clock is required for the timekeeping of convergent processes necessary for successful completion of the cell division cycle. Frequently, it is difficult to determine if mm-wave induced effects are thermal or non thermal with confidence. The ultradian clock is a temperature-compensated type of oscillator that can “gate" the cell cycle to give incremental increases in doubling times. A mathematical model has been created whereby mitotic oscillator and ultradian clock interact and which can explain observed dispersion and quantization of cell division times.

Golant *et al* irradiated *S. cerevisiae* at 46 GHz, (0.03 mW cm^{-2}) for 50 min. In the control culture, Golant observed a periodic fluctuation (60 min) in growth rate and bud formation. In the exposed culture, fluctuations were much stronger and had a longer period of 80 min. The existence of oscillatory cellular dynamics in exposed and control cultures must be addressed in the experimental design. Because the ultradian clock has a genetic basis and the period is different for each cell type, it represents a novel therapeutic target.

More recently, mm-wave exposure has been reported as therapeutic modality has gained acceptance in Russia and former Eastern Bloc countries. A significant volume of literature relating the efficacy of mm-wave therapy in a diverse range of conditions has been published (Rojavin & Ziskin 1998). In some cases, local irradiation of wounds or skin was performed but more often, application was to areas not immediately connected with the disease process being treated. The authors conclude that mm-wave radiation has a systemic effect. This could be mediated by an immune response, which has been confirmed in several clinical and cell-culture studies. Rojavin (Rojavin & Ziskin 1998) provides some independent support (from outside the countries of the former Soviet Union) for these findings.

Summary

The mm-wave bioeffects literature relates to a diverse gamut of organisms, ranging in complexity from bacteria to humans. In many cases, induced effects are said to be “frequency-specific” or “resonant” which is inconsistent with a thermal effect. However,

they are controversial because of associated difficulty with independent replication. In addition, a number of confounding experimental factors have been identified which could account for the frequency-specificity in some circumstances.