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”Non-thermal“ effects and microdosimetry – a relational system

During discussions with members of environmental/action groups, again and again the accusation is made that in setting limit values concerning high-frequency fields of mobile radio only ‘thermal’ effects are taken into account; ‘non-thermal’ effects occurring at lower field strengths are alleged to be neglected. Apart from the fact that such assertions are untenable, first we need to know what the terms ‘thermal’ and ‘non-thermal’ are all about.

At a quick glance, there seems to be no doubt as to what is meant by both terms: ‘thermal’ refers to those responses of the biological system that are caused and accompanied by a measurable temperature rise, whereas ‘non-thermal’ responses occur without showing a measurable heating of the system. But the expression ‘caused and accompanied by’ alone is ambiguous. Is heating the cause of an effect or its result? Electroration, i.e. the rotation of cells in a rotating HF field, or dielectrophoresis, i.e. cell movements within a field gradient, for example are not thermally caused, though accompanied by a considerable heating of the medium. But can we therefore speak of a ‘thermal’ response?

Several experts point to the fact that temperature measurement in general requires a thermal balance between the system to be measured and the measurement head, whether it is made by the mercury

sphere of a thermometer or via the sensitive area of a thermistor or any other sensor (Laurence et al. 2000, Moros and Pickard 1999). In each of these cases, stationary temperature of a huge mass or huge volume – in relation to cell or even biological receptor measurements – is determined, though at different time constants. The German law ‘26. BImSCHV’ even decrees the determination of an average body part SAR value using 10 g tissue and based upon a time interval of 6 minutes. But what is it that happens within these 6 minutes and inside this volume of approximately 10 cm³ tissue? Several authors emphasise the need to redefine pulsed fields limit values (Raslear et al. 1993, Jauchem 1998, Laurence et al. 2000).

In this context, there is a growing number of experts calling for ‘microdosimetry’ (Foster 1997, Foster et al. 1999, Jauchem 1998, Schwan 1999). It is long since known that so-called ‘hot spots’, i.e. spots of increased energy absorption, occur in the exposed organism caused by its dielectric heterogeneity. When at these spots a temperature effect is triggered in its biological impact ultimately concerning the whole organism, but without leading to a heating worth mentioning, this could be considered as a ‘non-thermal’ (or ‘athermal’) effect, since it is not connected with a heating of the whole system. Schwan and Piesol (1954) call such responses ‘specific thermal effects’.

LIMIT VALUES

How small can such areas of local heating be? At present, this question is the centre of serious controversies. As early as during the 1930s local heating was discussed as a possible interaction mechanism of high-frequency fields. Calculations of Krassny-Ergen (1935) later on were added to by Schaefer and Schwan (1943). Though it proved impossible to analytically solve the partial differential equations, the conclusion was that even under the conditions of extremely different dielectric properties as those of oil and water the radius of droplets has to lie within mm-dimensions for showing significant stationary temperature differences. This viewpoint – supported by further calculations – today is shared by Schwan (1999) and Foster (1997).

According to the estimate, the time constant at which a sphere emits heat to its surroundings is proportional to the value r^2 (r – radius). The characteristic cooling time is inverse proportional to this value (fig. 1). Though this might be an idealised case, assuming more or less realistic parameters the temperature of a heated cell aligns with the temperature of the surrounding milieu in less than a second, that of a mitochondria in less than a millisecond and that of a protein in less than a microsecond. Liu and Cleary (1995) calculated a duration of picoseconds for tem-

perature alignment of a structured water layer in the vicinity of a membrane. Consequently, we must ask what biologically relevant temperature gradients may exist at these dimensions?

This question can not be answered just by means of calculations using phenomenological parameters. To gain insight into the processes actually occurring within the system during electromagnetic fields absorption, first we must take a look at the range of quantum energies of this radiation type (fig. 2). As is known, the range of ionising radiation begins with the ionising energy of water requiring a quantum energy of at least 12,56 eV. This value lies within the frequency range of ultraviolet light. If a given quantum energy exceeds this value, electrons are knocked out of the atomic bond resulting in a break of covalent bindings - in other words, in ionisation, i.e. a chemically induced molecular alteration. In the range of visible light, or rather from infrared to ultraviolet light, radiation quantum energy can only temporarily elevate electrons to a new energy level. Molecules are excited and subsequently transmit the obtained energy via fluorescence or via a process not involving radiation. The excitation processes occur within an exceptionally narrow frequency range. The biological system uses just this frequency range for an extremely

effective transformation of irradiated photon energy supported by the photosynthesis apparatus of the plant into chemical binding energy, all this without destroying system molecules by ionisation. During radiation of lower frequencies, i.e. in the infrared range and even more so in the HF range, such processes are not longer possible. Here, the energy of single quanta only is sufficient to excite thermal movements of molecules or molecular parts.

On one side, all this leads to the conclusion that practically each primary effect of high-frequency fields is thermal. Further, it has become clear that field intensity, more precisely the absorbed energy, resulting in any biological effect must lie above the level of thermal noise. This does not suffice to calculate the threshold value of field impact, as we do not know about the actual relation between the irradiated energy and that of thermal noise at the biologically relevant interaction site. However, we can be sure that such a threshold value principally exists. Thus, ‘non-ionising’ radiation is clearly different from ‘ionising’ radiation where each ‘hit’ causes molecular damage. In discussions on the effects of continuous HF radiation, this crucial distinction unfortunately is too often overlooked. However, it is not this aspect that is of utmost importance for the

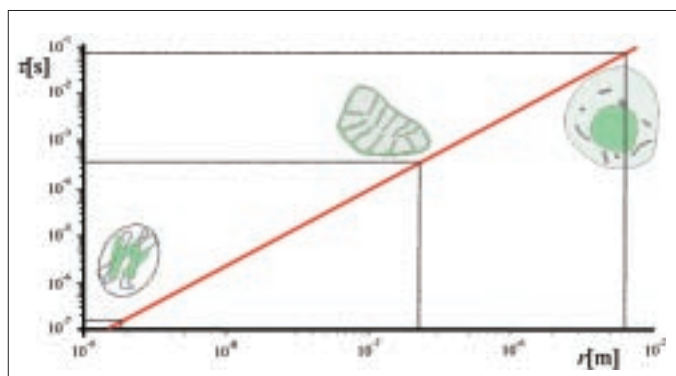


Fig. 1: Characteristic cooling time (τ) of a spherical body with radius r

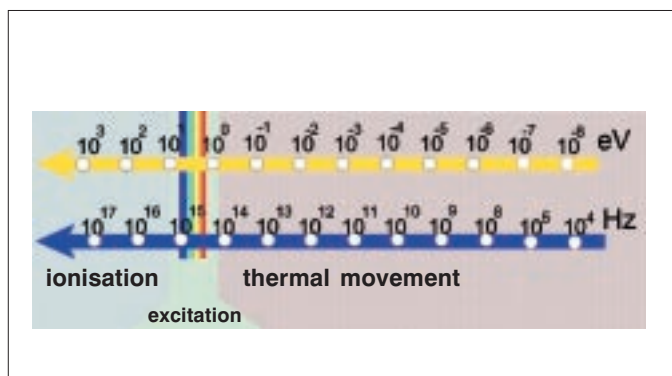


Fig. 2: Areas of different quantum energies in the electromagnetic radiation spectrum

Table 1: Consequences of high-frequency field absorption in biological systems

Possible effects of measurable heating of the whole system

- immediate biophysical consequences of the heating
 - increase of biochemical response velocity (Arrhenius equation)
 - alterations of response balances (LeChatelier principle)
 - changes of phase properties (f.e. of lipid membranes)
- physiological responses after activation of thermoreceptors
 - blood circulation changes in the tissue
 - other physiological responses aimed to prevent heating damage (f.e. production of heat shock proteins)

Possible effects of local energy absorption without measurable heating of the whole system

- local heating at the anatomical level ('hot spots')
- thermoelastic effects ('microwave hearing')
- differences of energy absorption at the molecular and supramolecular level
 - development of stationary microscopic temperature gradients involving thermodiffusion
 - conformation changes of proteins and adjacent water and lipid layers
- processes of molecular energy transfer

topic in question but the aspect above mentioned, i.e. the information that principally each high-frequency effect has to be 'thermal', regardless of whether the system heats measurably or not.

Table 1 tries to systematise possible effects of high-frequency electromagnetic fields. In the following, we will neglect classical 'thermal' effects, i.e. responses caused by direct heating of the system, and return to microdosimetry based upon the conclusions above mentioned. Are stationary temperature differences in the microscopic range really necessary for causing responses of the biological system? Or may we assume that molecular absorption of thermal energy quanta and subsequent energy transfer at the molecular and supramolecular level are sufficient to trigger such responses? Are these possibly those responses we call 'non-thermal'?

A short excursion may serve as a reminder of old but repeatedly discussed cal-

culations suggesting that even in vivo a stationary temperature gradient can exist across the cell membrane, fed by exothermal responses of asymmetrically localised enzymes, for example transport ATPase (Spanner 1954, 1964). A temperature difference of 0.01° across the cell membrane would cause a gradient of $0.01^\circ/10\text{ nm} = 106\text{ degrees/m}$. This would trigger for example processes of thermodiffusion, possibly producing an inner cell pressure of 130 kPa. However, such assessments remain hypothetical as long as a method for experimental verification is lacking.

But let's put aside this perhaps too bold hypothesis. Instead, we will try to understand the molecular processes occurring within the cell during high-frequency field absorption. Here, we deal with the classical frequency range of gamma radiation dispersion (Glaser 1996, 2000, Schwan 1957) where the alternating field induces dipole movements of the water, of small

molecules and compartments of macromolecules. Meanwhile, this area range has been thoroughly investigated by studies on electrorotation and dielectrophoresis, i.e. methods that contrary to impedance measurements at whole tissues or cell suspensions enable us to draw conclusions about the dielectric behavior of individual cells and cell components. Newer developments have demonstrated the usefulness of this method as well concerning cells in solutions of physiological ion strength and up to a frequency range of more than 1 GHz (Gimsa et al. 1996, Fuhr et al. 1996, Hölzel 1997). In addition, measurements and calculations enable us to draw reasonable conclusions about dielectricity constants of lipids and proteins (Cevc 1990, Laberge 1998).

In this context, it is important to bear in mind that measurement quantities from phenomenological physics such as temperature, dielectricity constant, conductiv-

ity etc., at the microscopic and molecular level too have to be seen as 'effective' quantities. This – for technicians maybe highly unusual – perspective is daily practice for molecular biophysicists and also extends to measurement quantities like spatial dimensions, viscosity, concentration, pressure etc. This must not only be put down to the small size of the system, i.e. to the small number of elements being part of the phenomena, but above all to the fact that cells at the microscopic range can not be seen as homogeneous system with isotropic properties. Because of the molecular organisation system involving electrostatic fields up to 10^7Vm^{-1} in the vicinity of electrical charges the motility of particles is limited. In this way, conductivity f.e. may become a vectorial parameter.

Next, we will take a look at one of the most important supramolecular structures of the cell - the cell membrane. In fig. 3 we see an approximately 6 nm wide bilayer-

er of phospholipids (blue-brown) in which proteins (green) are embedded. Protein functions – as ion channels, receptors, enzymes etc. – to a great part are determined by its lipid environment, particularly by the lipid ring immediately surrounding the protein (darker color). Phospholipids carry a polar, partially charged head group, each directed towards the aqueous phases of the outer/inner milieu of the cell, as well as polarised fatty acid chains creating a hydrophobic inner milieu. The phase state of this layer, comparable to a liquid crystal state, essentially determining the functions of the embedded proteins can be modified by temperature and many other factors. The charges of lipid head groups, but above all the outwards directed charged appendices of proteins produce electric bilayers of high field strength (for details see Glaser 1996, 1996a, 2000).

The membrane capacitatively fences off the inner cell with approximately 10mF/m^2 against the outer milieu. The average

static dielectricity constant of the membrane - dependent of protein contents - lies approximately around a value of 9. The hydrophobic internal layer (in fig. 3 colored brown) has a dielectricity constant of 2 - 5, polar head groups providing a crossing to the dielectricity constant of the outer and inner aqueous milieu of approximately 50 - 70 (Cevc 1990). The dielectricity constants of the stored proteins are hugely different and vary too functionally. These so-called 'microscopically defined' dielectricity constants accounting for local dipole elements and charges of the macromolecule (Laberge 1998) can internally reach a value of 2 - 4, in polar regions approximately a value of 40.

These considerations show that differences between dielectricity constants at the submicroscopic level are much more extreme than at the anatomical level of in comparison different tissues. However, the mentioned values only refer to the static dielectricity constants being modified in

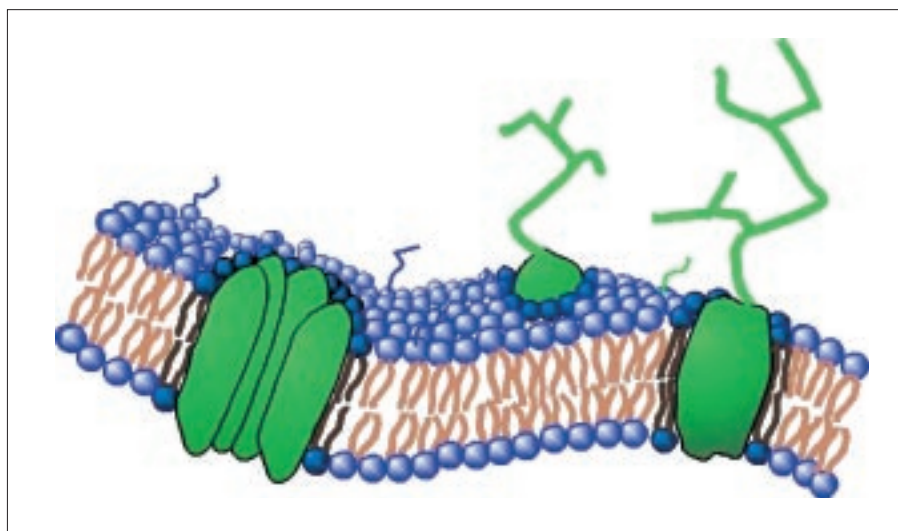


Fig. 3: Simplified sketch of the molecular structure of the biological cell membrane (top = outside/below = inside the cell). The proteins (green) and the head groups of the lipids (blue) show the highest degree of polarisation capacity. The fat residues of phospholipids (brown), however, are comparable with the dielectric property of oil. Membrane proteins are surrounded by a ring of lipids (darker color) essentially determining protein functions. Further inwards (not shown) is a network of proteins (so-called cytoskeleton). The appendices reaching outwards are charged glycoproteins. The whole system is surrounded by a layer of bound water (not shown).

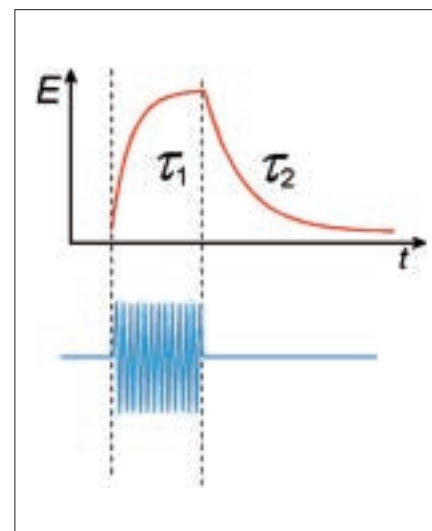


Fig. 4: At applying a short HF pulse the energy input locally increases at time constant τ_1 , then decreases sharply at time constant τ_2 .

the high-frequency range. Nevertheless, the frequency-dependent functions of these substance-specific dielectricity constants physically are easier to understand than those of complex tissues. The reason for this is that during evaluation of impedance measurements in tissues for purely pragmatic considerations the electronically highly complex RC parts of the system are conceived as a parallel connection of a single capacitor (c) to a single resistance (R). Of course, the result is a very complicated frequency-dependence of the complex impedance parameters. Looking at the substances themselves, dispersion curves seem more homogeneous. For example, pure water shows a single dispersion area at 18,7 GHz. Up to a frequency range of approximately 10 GHz the dielectricity constant at first lies at a value of 80 (T = 293 K), than decreases sharply in this dispersion area to a value of 5 (Bernardi and D'Inzeo 1989). In view of molecules this means that the water dipoles can follow the oscillations of the electric field only up to frequencies of approximately 10 GHz. In bound water, this dispersion area can alter up to the range of 0.1 GHz. In this context, Liu and Cleary et al. (1996) pointed to high local SAR values occurring in a layer of bound water outside and inside the membrane at exposure to a frequency range of 2.45 GHz, though not at 27 MHz. These calculations initiated discussions about the meaning of these intensified HF absorption.

Contrary to the small water molecule, the conditions of frequency-dependence of the complex dielectricity constant of biological macromolecules, above all proteins, proves much more complicated. Here, because of varying polarisation capacity and motility of different molecule compartments several dispersion areas occur. Until now, it is still not possible to calculate frequency-dependent SAR values at submicroscopic level. Newer approaches,

such as introduced by Liu and Cleary (1995) or Sebastián et al. (2000), are merely rough tries to solve the problem.

What is it we learn from these considerations? In summary, the examination of the submicroscopic diversity of the complex dielectric properties of the cell membrane and its environment leads to the conclusion that either the local electric field caused by HF field radiation as well as the intensity of energy absorption is extraordinarily heterogeneous. Of course, the same is true for all other cellular structures such as mitochondria, ribosomes, nuclear membranes, chromosomes, the golgi apparatus etc., which here can not be dealt with in detail. Further, we found out that each excitation of molecular structures in the range of quantum energy of HF frequencies is ultimately to be seen as thermal. The question remains which processes occur during the transfer of absorbed energy from one molecular or supramolecular level to another showing different dielectric properties. Macrophysically this phenomenon may be treated as heat conduction; however, one must never neglect time-dependence, as short as the characteristic periods may be. Fig. 4 illustrates this by the example of HF pulse absorption. Two time parameters are crucial: T1 - the time of heating, und T2 - the characteristic time of energy deduction, corresponding to the assessed values in figure 1 concerning a heated sphere in a water bath. Laurence et al. (2000) too discussed these time constants and calculated the temperature curve for a thin layer of a radiated cell culture.

These conclusions enable us to more expertly analyse the possible effects of high-frequency fields listed in table 1 occurring without being accompanied by a measurable heating of the whole system. But we need new, more exact models to gain better knowledge of spatial und temporal parameters of potential temperature gradients and of the processes occurring

during the alignment of differently absorbed HF energies. Maybe it would be of use to exceed existing boundaries and to consult biophysicists dealing with proteins for whom temperature jump experiments, f.e. using laser flashes, are a well-established methodical approach to gain knowledge of the folding dynamics of macromolecules (f.e. Nölting 1998).

There is quite convincing experimental evidence of the fact that heterogeneous field absorption at submicroscopic level plays an important part in the impact of high-frequency electromagnetic fields. Based upon the already cited calculations of Liu and Cleary (1995), Cleary et al. (1996) explained the differences of field exposure impact at 27 MHz compared to 2450 MHz by means of the varying energy absorption of bound water and membrane. Sheppard and Balzano (1995) criticised this hypothesis, rightly pointing to the peculiarities of macromolecular dipoles. In this context, the studies of La Cara et al. (1999) must be mentioned claiming that the heating of a solution of β -galactosidase from a thermophile bacterium by means of high-frequency (10,4 GHz cw) - contrary to normal heating - leads to a significant decrease of enzyme activity. Apparently, a heterogeneous energy absorption of the protein and its environment occurs leading to a measurable temperature rise only following heat alignment. Theoretical considerations on this aspect were already presented by Albanese and Bell (1984). Recently, Bohr and Bohr (2000) showed in solutions of β -lactoglobulin that 2.45 GHz fields can cause conformation changes through oscillation resonance. This effect was defined as 'non-thermal'. since it occurred much faster than the heating of the whole system.

Apparently, by use of microdosimetry considering results and methods of molecular biophysics we will not only gain insight into mechanisms of so-called 'non-

thermal' effects of high-frequency fields, but also provide a firm basis for a scientific discussion on limit values. This is especially true for the dosimetry and for limit values of HF pulses, a topic increasingly gaining importance.

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