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BIOELECTROMAGNETICS RESEARCH IN WEST GERMANY: AN ASSESSMENT

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This report highlights some of the key research that has been carried out in Germany on millimeter-wave effects during the past 2 to 4 years. In addition, the report examines other bioelectromagnetics research related to biological effects as well as diagnostic and therapeutic applications.

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BIOELECTROMAGNETICS RESEARCH IN WEST GERMANY: AN ASSESSMENT

1 INTRODUCTION

Research in bioelectromagnetics (BEM) in West Germany is centered around two major laboratories: Gesellschaft für Strahlen- und Umweltforschung (GFS) in Neuherberg, just outside Munich, and the Max-Planck-Institut für Festkörperforschung (MPIF) in Stuttgart. While there is a considerable amount of research in other parts of Germany, as this report will attempt to show, these two laboratories are by far the major centers.

For several years now a number of researchers have been stimulated to look for the frequency-dependent effects predicted by Fröhlich (1968) and suggested by the experiments of Webb in the US and by a group of Soviet workers led by Smolyanskaya and Vilenskaya (1974). Fröhlich suggested that critical oscillations exist in macromolecules that determine the activity and function of the organism. It is believed that the frequencies of these oscillations lie roughly between 100 and 1000 GHz. It is reasonable to conjecture that functional activities occurring at the macromolecular level depend on critical, and perhaps matching, oscillations being present at the right place and time for a given reaction to occur. Such oscillations might be finely tuned and "metastable," in which case small inputs of energy to one or both halves could cause disruption in the progress of the function or reaction. Fröhlich and others actually postulate that there is a type of threshold or limit cycle in metabolic excitation of large-amplitude vibrations (Fröhlich, 1980; Adey, 1981; Kaiser, 1981).

It appears that the BEM workers in Germany decided that this was an important enough area to devote considerable effort to it. This they have done, and for the last few years have produced some of the most interesting and technically precise research on this area to be reported anywhere in the world. Since some of the early work suggesting nonthermal, resonant phenomena was done at millimeter-wave frequencies (above 35 GHz), the Germans apparently decided to work in this range. Thus, almost all of their research has been conducted using millimeter waves. This report highlights some of the key research that has been carried out in Germany on millimeter-wave effects during the past 2 to 4 years, and other BEM research relating to biological effects as well as diagnostic and therapeutic applications.

2 GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG, NEUHERBERG

The research team at GFS is led by Werner Grundler and includes U. Jentzsch, Vera Putterlick, Dietrich Strube and Ingrid Zimmermann. The lab is probably most closely identified with the very exhaustive studies on the resonant growth-rate response of yeast cells exposed to low-level microwaves in the millimeter-wave range.

Yeast cells have produced the most consistent findings of nonthermal, resonant responses. The research at GFS and MPIF used diploid, homozygot, and isogene wild type *Saccharomyces cerevisiae* grown on agar plates for 3 days at 30°C, then stored at 4°C. Cells for exposure (power input from 10 to 25 mW) were taken from these plates after 10 to 16 days and placed in liquid growth medium in small glass cuvettes equipped both with mechanical stirrers and with submersible Teflon antennae for coupling in the microwaves. The simultaneous growth of two yeast cultures (one control and one exposed) was measured in a double-beam spectrometer. When the researchers reported their first experiments, they obtained the curve shown in Figure 1a (Grundler and Keilmann, 1978). They recently published the results obtained during the last 2 years; they used the same yeast system, but with two different antennae (Grundler and Keilmann, 1983). One was the "fork"-shaped antenna used in the earlier experiments, and the other was a simpler, cylindrical, "tube"-shaped antenna. The results of these experiments are shown in Figures 1b and 1c.

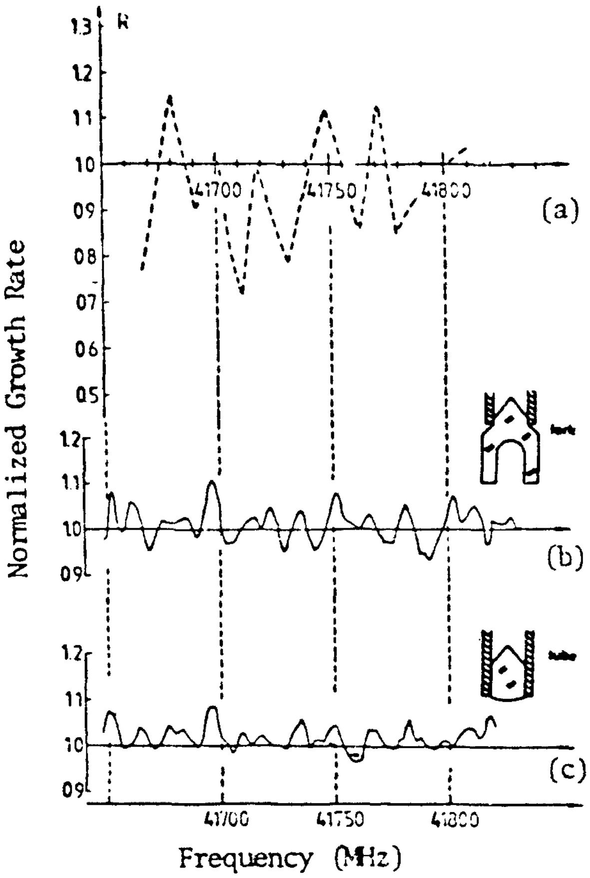

Figure 1. Effects of frequency on growth rate of yeast.

The most obvious differences between the 1978 and 1983 results are: the apparent increase in resolution; the differences in the degree of change, both positive and negative; and the displacements in frequency for some of the peaks. A cross-correlation test was used to compare the present results (Figures 1b and 1c) with those obtained originally (Figure 1a). They found that maximum correlation is obtained only at a nonzero frequency shift, $\Delta f = -11$ MHz. This indicates that a systematic calibration correction of +11 MHz has to be applied to the previous study--assuming both spectra probed a common feature, i.e., a frequency selective response in the yeast cells. Keilmann and Grundler feel that this correction would be reasonable since it is within the ±20-MHz systematic error margin originally estimated. The reality is that there was a significant increase in the ability to control the frequency of the microwave source and in the environmental control of the samples. In addition, whereas in the original study the controls and experimentals were done on different days, the recent study ran both sets of samples on the same days by using two spectrometers. Further, temperature was measured and controlled better in the 1983 study.

There is another difference between the spectra of the two types of antennae in the 1983 study. With the "fork" antenna, there was almost an equal number of positive and negative growth rate peaks (Figure 1b). With the "tube" antenna (Figure 1c), almost all of the peaks occur above the "normal" line, with very few real negative growth rates shown. However, there is excellent correlation between the two sets of curves in terms of the major peaks, and Keilmann does not know why they did not observe the same degree of growth-rate decrease with the "tube" configuration. This study, in which 331 growth experiments were conducted, seems to demonstrate unequivocally that low-level microwaves in the frequency range of 41 to 42 GHz affect yeast growth rate within resonant bands of only 8 MHz, or a total offset of about 16 MHz.

Fröhlich's "Type A" coherent excitation of a single polar mode may explain the effect on the growth rate of *Saccharomyces cerevisiae* (Fröhlich, 1983). Oscillations of this type are long range and give rise to frequency-selective interactions in and between systems (or parts of systems) with equal excitation frequencies. It seems likely that we are dealing here with a membrane phenomenon wherein access of the cell to the nutrient molecules is the key step. One must remember that the membrane has a high electric field, and it follows that the higher the inherent electric field, the closer polarizable molecules will be toward their metastable state. For most large macromolecules--such as proteins, liposomes, or even hemoglobin molecules—the amount of energy necessary to reorient the entire molecule at environmental temperatures is very large. However, intramolecular rearrangement of charge is quite likely, especially as the amount of associated water is varied.

If we assume that some "breakdown" may be occurring in the cell membrane (or polarization of internal structures of membrane proteins), it would be well to gather some information about the membrane capacitance. The capacitance would determine, for example, the charging time of the membrane.

Now that the effect has been well established with the yeast cell culture, it is necessary to take a closer look. At a symposium held near Munich in September 1983, Grundler reported that he has set up a microscopic arrangement that allows him to view single cells that are locally fixed but in a growth medium. He hopes to be able to define the distribution of the generation time for cell divisions and to analyze the effect of the millimeter waves on different phases of the cell cycle. His system uses a moving stage of a computer-controlled scanning microscope with a flexible antenna that allows continuous exposure directly to the cell chamber on the microscope stage. Such a study leads in the proper direction to attack the question of the interactive mechanism. In addition, experiments are planned at lower temperatures: 29°C instead of 30.7°C.

Once the frequency-dependent resonance effects were demonstrated and verified, Jentzsch and others at GFS set about to investigate more specific parameters in an effort to pinpoint the mechanism leading to the phenomenon. They have studied the influence of millimeter waves on cell respiration and on duration of certain cell-cycle phases. For this purpose, they developed two different exposure chambers and registration systems for detecting: (1) the oxygen uptake of exposed and unexposed cells in monolayers during one cell cycle, and (2) the time needed for synchronized cells to pass the G_1-phase.

This latter parameter was measured by the shift of a small volume distribution of early G_1-phase cells to higher values. The volume distributions of the cells were measured using a Coulter counter. The spectra were stored in a multichannel analyzer connected to a computer. The curves of the volume distributions were approximated by logarithmic normal functions.

The cells grew in a thermalized irradiation box with two positions for both control and irradiated samples. The cells formed a monolayer at the bottom of petri discs consisting of gas-permeable foils. A thermostated stainless-steel body was dipped in the medium. The temperature was measured by thermocouples and kept constant within 0.05°C. For oxygen-uptake measurements, the cells grew in a nonfermentable medium Starved cells from stock cultures were synchronized in G_1-phase within a linear ficoll gradient.

A backward wave tube was used as a microwave source. The oscillator was stabilized by means of a crystal-controlled phase-lock loop; the result was a frequency stability of 1 MHz within the range of 40 to 60 GHz. The incident and reflected powers were measured by power meters. The reflected power was minimized by an x-y tuner. At maximum microwave-input power (90 mW), an increase in temperature of 7°C was determined to be nearly proportional to the absorbed power.

Two identical stainless-steel vessels served as cell containers. The bottoms of the vessels were sapphire plates, ensuring a relatively good thermal conductivity and transmission of millimeter waves. One of these was connected to the microwave system. The cells formed a monolayer at the surface of the plates. The temperatures of the vessels were regulated by a closed water circuit within ±0.01°C. The oxygen-detecting electrode (Clark type) was introduced from the top of the vessels and dipped into the medium above the cells. The electrode currents for both control and irradiated samples were measured during the time of one cell cycle at different microwave frequencies.

The final results of these studies were not analyzed at the time of this report. They will be reported at the URSI/BEMS Open Symposium in Florence, Italy in August 1984.

3 THE MAX-PLANCK-INSTITUT FÜR FESTKÖRPERFORSCHUNG, STUTTGART

MPIF is perhaps the most well-equipped and productive BEM laboratory in Germany. The team there is led by Fritz Keilmann and includes Friedrich Kremer, Lannianti Santo, F. Drissler, and A. Poglitsch. As indicated above, there has been a close tie between this group and Grundler's group at GFS. A number of studies have been conducted jointly.

Yet another interesting example of a nonthermal, frequency-specific effect of millimeter waves has been found by a group at MPIF headed by Kremer. When his researchers swept frequencies in the range between 64.1 and 69.1 GHz and used stabilized frequencies of 67.200 ±0.001 and 68.200 ±0.001 GHz (power densities <<5 mW/cm^2), they observed a reduction in the size of the puffing of giant chromosomes of the midge *Acricotopus lucidus* (see Kremer et al., 1983). The puffing phenomenon is a highly complex process that does not depend solely on transcriptional activity--i.e., enzymatic activity of RNA polymerases. The process comprises interactions between transcription, RNA-processing, packaging of RNA with distinct nuclear proteins, and storage or transport of ribonucleoprotein products. It was concluded that the coherence of the radiant energy is decisive in causing the effects because the photon energy (about 2.9×10^{-4} eV) of millimeter waves is less than 1/200 of the thermal energy kT. It was further concluded that the observed effects might be explained by Fröhlich's theories of coherent electric vibrations in biological macromolecules--i.e., the externally applied radiation field influences the excitations within the biological system.

A point made by Kremer et al. (1983) may be questioned by some. One of their conclusions was that their "result could be of importance in the discussion of safety standards with regard to possible hazards from millimeter wave radiation." This conclusion was reached because their exposure levels were below the safety standards of most European countries and the US. However, it must be remembered that at millimeter-wave frequencies, absorption of energy by genetically important material is essentially precluded due to the very shallow depth of penetration.

In search of an influence of microwaves on living systems, Poglitsch and others in Kremer's group chose the growing root of cress seedlings, widely used as a model system for rapidly proliferating cells. The fact that the seedlings grow well in an atmosphere of high relative humidity makes them especially suitable for irradiation experiments with millimeter waves, which only have a penetration depth of 0.5 mm in aqueous solutions.

The seedlings were placed on a moist filter paper in a sample chamber and incubated for 20 hours at 24 ±0.5°C before irradiation (root length at that time, about 5 mm). The sample chamber was a sealed plexiglass container with windows of polyethylene foil (area, 14×6 cm^2; thickness, 100 μm), which is essentially transparent to millimeter waves. Inside the chamber the atmosphere was saturated with humidity due to a water bath at the bottom. The whole chamber and the measurement system were kept in a dark, temperature-controlled (24 ±0.5°C), wooden box.

A computer-controlled optical system was used to determine the growth rates of individual roots. It included a microscope with twofold magnification, four light emitting diodes (λ≈1 μm) for weak illumination of the seedlings, and a linear array of 128 photodetectors. The whole optical system was mounted on a stepper-motor-controlled dish that could be moved in three dimensions. Use of image-processing techniques in the computer allowed continuous measurements of the positions of one or more root tips with an accuracy of ±5 μm. A time of about 50 seconds was needed to measure the position of an individual root tip. Usually four roots were observed--two as controls, two for exposure.

The seedlings were exposed to millimeter waves (43 and 56 GHz) with power density ranging between 0.1 mW/cm^2 and 10 mW/cm^2. At 56 GHz a power density of 2 mW/cm^2 resulted in a 30-percent reduction of the root growth rate. The microwave-induced temperature increase was measured to be less than 0.2°C. At 43 GHz the microwave energy turned out to be less than effective. Furthermore, the researchers observed a strong dependence on the polarization of the millimeter waves, with a greater effect being seen when the E-vector of the microwave field was parallel to the long axis of the roots. Both findings can be explained on the basis of the dielectric properties of the root material. Applying infrared radiation ($\lambda >$ 2 μm) of comparable power density resulted in a similar reduction of the root growth rate. Originally it was thought that the observed effects were of thermal origin, despite the fact that the irradiation-induced temperature increase is small on a physiological temperature scale. It is now felt that the effect is nonthermal since it depends strongly on the polarization and frequency rather than on the microwave-induced temperature increase.

The kind of interaction of coherent electromagnetic waves with the root tissue is--at the moment--unclear. A single quantum effect can be excluded because of the very small quantum energy of a millimeter-wave photon hν ($2.3 \cdot 10^{-4}$ eV) compared to the thermal energy kT ($2.6 \cdot 10^{-2}$ eV) at room temperature. One possible explanation would be a piezoelectric interaction which, however, requires a quasiferroelectric system in the cell in order to raise the interaction energy above the thermal level kT. Another possibility would be the conjecture of Fröhlich (1980) of coherent electric vibrations (polarization waves) that are excited through a supply of metabolic energy and which might interact directly with the externally applied field.

Kremer, Poglitsch, and Genzel have used broadband, temperature-dependent, millimeter-wave spectroscopy on proteins and biopolymers. They have studied the frequency range from 40 to 170 GHz. Absorption measurements have been reported for lyophylized hemoglobin, lysozyme, silk keratin, and poly-L-alanine.

The measurements were extended over the temperature range from liquid helium to room temperature using the untuned cavity technique. For dried lysozyme, a nearly linear increase with frequency and an exponential increase with temperature of the absorption coefficient were observed between 50°K and 300°K. This frequency and temperature dependence could be explained by relaxation processes in asymmetric double well potentials with relaxation times in the picosecond range.

Hydration yields a nearly frequency-independent contribution to the absorption, which arises only at temperatures above 150°K. The frequency independence indicates relaxation rates for the bound water that are small compared to millimeter-wave frequencies. Thus the contribution of bound water can clearly be distinguished from the fast intrinsic processes. The investigators suggest that these picosecond relaxations can be assigned to the NH...OC hydrogen bond of the peptide backbone.

Preceding investigations by this group have shown that the frequency- and temperature-dependent millimeter-wave absorption of several proteins can be described in terms of relaxation processes, with typical relaxation times in the picosecond range. Because of these very short relaxation times and the relatively high dipole moments, an assignment to processes within the hydrogen bonds of the peptide backbone seemed most probable. A contribution of bound water to the millimeter-wave absorption, however, couldn't be totally excluded. To study the influence of absorbed water, the researchers chose lysozyme as a particularly stable protein which can be completely dehydrated, except for approximately three tightly bound water molecules per lysozyme molecule (Imoto et al., 1972).

The sample was prepared as follows: lyophilized hen egg-white lysozyme, purchased from Sigma Chemicals, was dried over P_2O_5 at 360°K for 2 days. One part of the material was kept dry, while the other two parts were brought into atmospheres of 40-percent and 80-percent relative humidity to obtain equilibrium hydration levels of 8 percent and 18 percent (w/w), respectively. This was controlled by measuring the weight increase. A comparison of the enzymatic activity before and after preparation showed no difference, thus indicating no denaturation of the lysozyme. For the millimeter-wave measurements, the sample material was compressed into discs with $4.8 \cdot 10^{-2}$ m diameter, $6 \cdot 10^{-3}$ m thickness, and a density of 1060 ±50 kg m^{-3}.

As the appropriate spectroscopic method for weakly absorbing, scattering samples at millimeter-wave frequencies, the researchers used the untuned cavity technique (Llewellyn-Jones et al., 1980; Kremer and Izatt, 1981). In a greatly oversized resonator an electromagnetic field was made approximately isotropic and homogeneous (on time average) by mode stirring. Measuring the change in the Q value of the resonator after inserting a sample enables the determination of the pure absorption of the sample regardless of reflection and scattering losses. A millimeter-wave transparent fused silica cryostat inside the cavity allowed measurements between 5°K and 300°K.

The absorption coefficient α of dried lysozyme for three frequencies exhibited a nearly exponential increase with temperature from 50°K to 300°K. Below 50°K, α dropped quickly and seemed to level off at about 10°K. In the range of the exponential increase with temperature, α was almost linear in frequency (ν), while at low temperatures α was proportional to ν^2, as previously observed for other proteins.

Santo and Keilmann have recently been studying photobacteria, which have a natural ability to emit visible light, in an attempt to monitor their activity in a simple and direct way. The researchers are exploring the thermal response of the bacterial luminescence in an effort to develop a sensitive, nonperturbing temperature probe for microwave experiments.

In their study, thin layers of *Photobacteria leiognathi* in nutrient agar were prepared on sapphire plates. The cultures showed nearly constant light emission for about 24 hours. Steps of +0.6°C were applied to the sample to study the influence of temperature changes on the bioluminescences. Strong responses occurred. Surprisingly, the size and even the sign of this response depend on the cultivation parameters of the photobacteria. This may point to the possibility of similarly complicated thermal responses in other systems.

Furthermore, millimeter-wave irradiation experiments were conducted. The thin layer of photobacteria allows a nearly homogeneous irradiation, while the sapphire provides a very efficient heat-sink--and this excludes thermal effects. In the range between 40 and 90 GHz (slowly scanned at 1 MHz per minute), no microwave effect could be observed at incident intensities of 1 to 30 mW/cm^2.

4 THE UNIVERSITY OF COLOGNE

Just as yeast and *E. coli* are favorite one-cell organisms for biological study, the fruit fly, *Drosophila melanogaster*, is by far the most-studied insect, representing a highly differentiated species that is especially well suited as a model for studies concerned with effects of various agents on genetic material. It is thus surprising that there have been relatively few millimeter-wave investigations using this insect. Because of its size, it is particularly well suited for high-frequency, short-wavelength studies. However, just as with the yeast, there are some apparent conflicts in the reported results of the work that has been done. For example, Zalyubovskaya (1974) reported decreased fertility (37 to 55 GHz, 10 mW/cm^2, adult insects), while Dardalhon et al. (1977) found an increase (17 GHz, 60 mW/cm^2, adult females only). No evidence has been found for a mutagenic effect of millimeter waves in the fruit fly.

The most recent study of millimeter-wave effects in *Drosophila*, conducted by Gunter Nimtz at the University of Cologne, is yet to be published. He did publish the results of a 2-year study in which pupae were exposed for 120 hours at an intensity of 10 $\mu W/cm^2$ and a frequency of 40 GHz (Nimtz, 1983). A few hours after becoming adults, one female and two males were crossed to start a family. Up to 15 such families were started from both control and experimental groups, and their offspring were counted. The number of offspring in the first generation represented the fertility of the parent (P) generation. The fertility of two successive generations, F_1 and F_2, were similarly determined. Analysis of the results led to the conclusion that the fertility of the P generation was strongly enhanced in the exposed insects, while their offspring appeared not to be affected. The fertility of the "grandchildren," the F_2 generation, appeared to drop by about 10 percent compared with the controls.

After completing two additional experiments under the same conditions, Nimtz recompiled and reanalyzed his data. Applying more rigorous and revealing statistical tests, he found, in fact, that in this series of six experiments with over 82,900 flies, millimeter waves had no apparent effect. One of the major reasons for the ultimate conclusion had to do with the extreme variability in the number of offspring per family--under all experimental conditions. The observed range was from 0 to 669 for one family. In fact, of 360 families, 13.3 percent had no offspring at all and 18 percent had between 0 and 50 offspring. On the other end of the spectrum, 4.4 percent of the families had between 551 and 669 offspring. There were several other analytical and statistical anomalies that a less careful and less astute investigator than Nimtz might have overlooked in the zeal to find a result. As mentioned earlier, these new findings, which negate the previous ones, will be published soon.

5 OTHER RESEARCH

Several other laboratories throughout Germany are engaged in BEM research. While I cannot highlight all of these, a few do deserve attention.

Professor Friedmann Kaiser at the University of Stuttgart is a theoretician who has published a number of papers dealing with coherent oscillations in biological systems. A paper that he published in 1979 dealt with a Boltzmann-equation approach to Fröhlich's vibrational model of Bose condensation-like excitations of coherent models. In 1982, a paper in *Radio Science* treated coherent oscillations at extremely low frequencies.

On the biomedical applications side, the Germans have not done as much as one would expect given the general level of health-care industries and research in the country. Some significant research and clinical studies are being carried out in the general area of bone stimulation and in a new area that, so far, seems to be fairly unique to Germany: the use of pulsing electromagnetic fields to treat the loosening of implanted hip joints.

A multi-center study was conducted in Germany and Austria to study whether pulsing electromagnetic fields at low frequency would cause loosened hip joints to be refitted in the joints and integrity re-established. The German portion of the study was carried out at Garmish-Partenkirchen Hospital and at the Institute of Experimental Surgery at the Technical University of Munich under the direction of Dr. R. Ascherl. (While I do not have all of the details of the study presently, a visit is planned to Germany, and a follow-up *ESN* article this fall will address this in detail.)

Dr. Ulrich Warnke of the University of Saarland in Saarbrucken has found that extremely-low-frequency pulsating magnetic fields can dilate peripheral blood vessels in humans and horses. He has also found an increase in the oxygen partial pressure measured transcutaneously in human beings. The results on humans are not convincing, suffering from large individual variations.

Another study at Garmish-Partenkirchen Hospital is directed by Dr. F. Lechner. Over 400 patients suffering from non-unions and delayed healing of fractures have been treated. Most of the fractures that became pseudoarthrotic were in the tibia. On average, the patients had had 3.6 surgical attempts to repair the fractures. The researchers used electrodes as well as inductive coils to provide 5 to 10 $\mu A/mm^2$. They report a success rate of 93 percent in these difficult cases. This is a little higher than most other investigators throughout the world have found. One difference is that Lechner does some bone transplantation in many of the cases.

In another study at the Institute of Experimental Surgery of the Technical University Munich, Drs. J. Scheiblich and O. Petrowicz have developed a high-frequency radiator for treating prostate cancer by hyperthermia. The radiator produces a deep-heated hot spot. The radiator has an outer diameter of 20 mm and an insertable length of about 175 mm. A high-frequency cylindrical slot antenna inside the applicator is cooled by water. The frequency used is 433.9 MHz, one of the frequencies allocated for medical therapy in West Germany. There is a control system regulating the power output of the radiator to avoid damage to the tissue around the prostate, especially the rectum mucosa and the tissue of the rectum and the prostate. After about 60 experiments with dogs, they have demonstrated that it is possible to locally heat the prostate without any damage to the surrounding tissue.

At Phillips GmbH Forschungslaboratorium Hamburg, Drs. Ludeke and Kohler have developed a new radiometer to determine the temperature of tissue lying beneath the surface of a body. The new microwave thermographic system solves the problem of emissivity-independent noise temperature measurements by simultaneous registration of an object's apparent temperature and its reflectivity with just one microwave receiver and real-time calculation of the object's emissivity and its actual temperature. Unlike some earlier microwave radiometers, this one uses dielectric-filled probes that contact the skin. This improves the emissivity from about 25 percent to nearly 100 percent.

6 CONCLUSION

Bioelectromagnetics research in Germany is scattered fairly widely, but two centers dominate the research area. They may be rightly considered centers of excellence as they are well equipped and staffed with very capable scientists and engineers. General support for BEM research is good in Germany and the research produced is rated among the best in the world. Much of the German effort has been centered around millimeter-wave effects, and two laboratories have been successful in demonstrating resonant phenomena in such systems as yeast cells and cress roots. A considerable amount of biomedical research is under way, but not as much as would be expected when compared with the level of health-care research in general.

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