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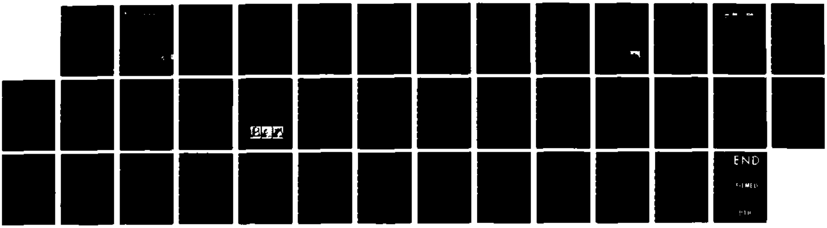

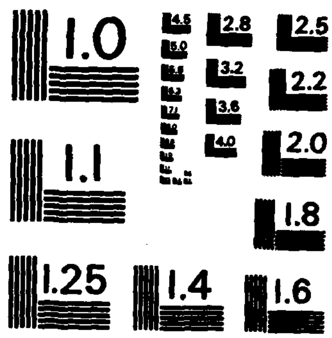

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Biological Sciences and Bioelectromagnetics in Europe: Summary Report

Thomas C. Rozzell

26 August 1985

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This report examines in detail bioelectromagnetics research in Europe and provides an overview of several aspects of general biomedical, biomaterials, and biotechnological research.

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CONTENTS

BIOLOGICAL SCIENCES AND BIOELECTROMAGNETICS IN EUROPE: SUMMARY REPORT

1 INTRODUCTION

During my 2-year stint at the Office of Naval Research, London (ONRL), I attempted to focus on several aspects of biological science research in Europe. Since my background is multidisciplinary, encompassing chemistry, industrial hygiene engineering, radiation physics, radiation biology, and bioelectromagnetics (BEM), I was able to actually delve into a broader range of scientific and technological issues than some of my predecessors. A casual reading of the titles of my *ESN* articles, Newsbriefs, and reports will serve to illustrate this (see the appendix, page 33).

One of the reasons I chose to range widely in the biological sciences is that this area has been neglected at ONRL for such a long time. To my knowledge, there has not been a liaison scientist in the biological sciences since the departure of Dr. John Bateman in the mid-seventies. The many issues and problems that face the Navy, and indeed the entire military, and the tremendously high level of activity in medical and biomedical research in Europe, would seem to make it absolutely essential to have someone in this area at ONRL at all times.

As can be seen by the list in the appendix, I have looked into biotechnology issues and spent considerable time on research related to trauma care and to biomaterials. This was all done in addition to my primary area of interest, BEM. The main portion of this report will deal with the BEM research in Europe, but first I would like to address several aspects of general biomedical, biomaterials, and biotechnological research that I have encountered over the past 2 years.

2 BIOTECHNOLOGY

During my first year at ONRL, there was no one else here with an interest in this subject. This was remedied when Dr. Claire Neurath arrived in 1984. Before she arrived, I attempted to look at some of the research and technical developments taking place in the UK and continental Europe. One of the reasons that I chose to deal with this area is that ONR, Arlington (ONR), Code 441 was just tooling up a basic research program designed to support some leading-edge science in molecular biology and genetic engineering.

Early in 1984, I reported on the International Center for Genetic Engineering and Biotechnology (ICGEB) (*ESN*, 38-1:5 [1984]). In that article, I pointed out that the ICGEB will be a valuable research and resource center for genetic engineering and biotechnology and will primarily serve the developing countries of the world. I also pointed out that the US has not taken a proper interest in the development of the ICGEB, which may be a mistake in the long term.

The very first *Science Newsbrief* published at ONRL in 1983 was one that I prepared which dealt with the production by bacteria of a unique thermoplastic polyhydroxybutyrate (PHB) (*Science Newsbrief* 1-1-83). This was a major breakthrough in biotechnology in the UK and has since engendered a tremendous amount of interest in the US, where the PHB is being used in a host of applications relating to medicine. Such products as sutures, wound dressings, and implants can be made from this material, which can be used as a biomaterial and is nontoxic and biodegradable. In fact it is a biomaterial synthesized by a biological organism.

The priority that is being given to biotechnology by European countries is exemplified by the position that France has taken in increasing its research budget and agreeing to support the International Biotechnology Training Network (*ESN* 38-2:66 [1984]). Under a recent law passed in France, its research budget must rise to 2.5 percent of the gross national product, with biotechnology having a high level of priority. Further evidence of the government's commitment is seen in the expansion of the Institut Pasteur in Paris, where a new building is now under construction. The new six-floor facility will be devoted entirely to biotechnology and will cost the government about $7.75 million. The Institut Pasteur--which is private, but about 50 percent state-supported--will spend an additional $3 million to purchase equipment. The building will have approximately 3000 m^2 of laboratory space. Construction is slated for completion in 1986. The new building will be operated by the Institut Pasteur, which has pledged to coordinate its research policies with the Centre National de la Recherche Scientifique, Institut Nationale de la Santé et de la Recherche Medicale (the medical research council), and Institut National de la Recherche Agronomique (the agriculture research institute). A number of users will share the laboratory; the research will be both fundamental and applied, and oriented to health and to industry. When completed, this new addition to the Institut Pasteur, the new attitude of the state, and involvement in the new International Biotechnology Training Network should soon put France in a position to compete as a world leader in biotechnology, as it has done in a number of other areas of biological research.

Another example of national support for biotechnology was found in Scotland. There the Scottish Development Agency (SDA) launched a very ambitious program to boost biotechnology (*ESN* 37-12:439 [1983]). To carry out its mission, SDA formed the Health Care and Biotechnology Division (HCBD), which has a specific charge to support the development of the health-care industry in Scotland, and to foster the medical, industrial, and process uses of biotechnology.

The work of the HCBD is guided by four interconnecting objectives. The first is to explore ways to help develop existing companies in Scotland. The second objective is to encourage diversification by Scottish companies into health care and biotechnology areas. The third objective is to stimulate relevant R&D activities in academic and research institutions. Finally, the HCBD is actively marketing Scotland as a base for foreign research and manufacturing corporations.

To help meet these objectives, the SDA is underwriting new companies. For example, in 1982 a new pharmaceutical company, Drug Development (Scotland) Ltd., was launched. SDA provided about $325,000 of start-up capital, and $160,000 came from the Bank of Scotland. The SDA apparently believes that such venture-capital activity is complementary to the broader grants-in-aid available from other Scottish governmental agencies.

These examples serve to illustrate the emphasis and methods being used by continental European countries and the UK to bolster their role in biotechnology. Several countries, especially the UK, realized very late that they missed the boat in biotechnology and are now trying desperately to catch up to the US and Japan (*ESN* 38-8:451 [1984]).

3 BIOMATERIALS

The interactions that occur at the interface between a biomaterial and the tissue of the host in which it is placed are at once complex and little understood. Scientists, engineers, and clinicians engaged in the study of such phenomena are drawn from materials science, biochemistry, physiology, pathology, immunology, pharmacology, molecular biology, bacteriology, and toxicology. It is little wonder that a major task of a biomaterials workshop, held in 1983 in Washington, was to define exactly what is meant by a "biomaterial."

A biomaterial is considered to be any material or substance, either natural or synthetic, that is introduced into a human (or other animal) body for the purpose of treatment or for otherwise modifying some function of the body. Thus, any artificial limbs, joints, organs, or tissue substitutes and all of their constituents are biomaterials. So too, blood taken from one person and given to another becomes a biomaterial for the recipient. All types of wound dressings, sutures, implants, drugs, artificial teeth, etc., are biomaterials. Items constructed from biological material (plant or animal) but meant for use in ways other than introduction into the body are not biomaterials.

There are certain general criteria that all substances must meet if they are to be considered biomaterials. First, and most importantly, the material must be biocompatible. That is, it must not be toxic to the body, nor cause an allergic (immune) reaction, nor cause irritation. Second, the material should be stable if it is to last for a long time, or if meant to be temporary, it must degrade into products that are biocompatible. Finally, in the case of materials used as replacements for tissue, the strength and other properties of the material should be as close as possible to that of the tissue being replaced.

The biomaterials research area is very active in Europe and that fact, combined with some recent ONR Code 441CB program interest, led me to attempt to cover a broad range of biomaterials research during my stint at ONRL. The use of biomaterials in military medicine is quite broad, and there is a significant need for basic research in this area.

I found that the Federal Republic of Germany (FRG) was one of the leading countries in biomaterials research; accordingly, I concentrated much of my effort there (see ONRL report R-3-85). In the FRG, research in biomaterials seems to be concentrated in several medical school-university complexes, with little or nothing being done in such places as the Max Planck institutes or the institutes of the Fraunhofer Society. The scope of the research was examined under the following general topic areas: wound healing and tissue repair, drug delivery, prosthetics, and miscellaneous. As one of the leading countries in Europe for research in biomaterials, the FRG has developed a number of new techniques and methods utilizing a wide range of synthetic and natural materials for use in medicine. Exotic ceramics, metals, and plastics are being used both in animals and in humans for such things as tissue repair, wound coverings, drug delivery, and prosthesis. The FRG scientists, bioengineers, and clinicians, as well as those in other countries of Europe, are very agressive in attacking new problems related to the use of biomaterials in therapy. There are fewer roadblocks between development and application of such materials in Europe than in the US.

A number of interesting developments are currently under way in biomaterials research in other countries of Europe. Over the past 2 years, I highlighted as many of them as time permitted, and will mention several here; again, see the appendix for the complete list.

Notable among the ceramics research in biomaterials was the Free University of Amsterdam, where the use of biomaterials is studied in the Dental School but applications go beyond their use in dentistry (*ESN* 39-6:247 [1985]). The group there is actively seeking a substitute for bone as well as a new drug-delivery system based on polyphosphazenes. They are typical of other research groups that have their hands in many pots.

At the opposite end of Europe, I found and reported on a broad range of biomaterials research at the Faculty of Medicine of the University of Aix-Marseille, France (*ESN* 39-6:244 [1985]). This is a program that could be a model for the training of military physicians who will be assigned to field hospitals or MASH units.

I found the UK to be a very active player in the biomaterials arena. Most noteworthy was the University of Strathclyde in Glasgow and a few industries in Scotland and in England. The British Technology Group and the University of Strathclyde recently joined forces and invested £250,000 in Polysystems, Ltd. This new company was formed at Strathclyde to manufacture and license hydrogels for drug delivery (*ESN* 39-5:187 [1985]).

Hydrogels are polymeric materials that have the ability to swell to a high degree in contact with water, yet do not dissolve in water. They have been the subject of intensive research for several years by a team led by Professor Neil Graham in the Department of Pure and Applied Chemistry at the University of Strathclyde and by Dr. Robert Howells in the Liverpool School of Medicine in London. The polymers have a number of unique properties that make them potentially suitable for delivery and controlled release of a number of drugs. Many of the techniques that are being explored could be of significant value in several areas of military medicine.

Taking another tack, the Technion, in Haifa, Israel, has a very active biomaterials laboratory which is heavily oriented towards the chemical aspects of biomaterials surfaces, artificial organs (especially related to the cardiac system), and conformational transitions in biomolecules. Located in the Julius Silver Institute of Bio-Medical Engineering Studies, this department derives great support from industry as well as the Technion Foundation. They have a special interest in molecular electronic devices, a task area in the ONR/NAVAIR Special Research Initiative in Molecular Biology.

4 GENERAL BIOMEDICAL RESEARCH

The biomedical needs of the military are many and varied. Many of the needs are quite unique due to the circumstances under which they arise. The environment of military medicine poses many special conditions that often can only be met through innovation and resourcefulness. I have tried to remain alert to new research that, while not motivated by a military need, might be adapted to that need. This is especially relevant to combat casualty care, so I have assessed and reported on a number of laboratories and conferences that are or were involved with trauma care.

As examples of these, leaving aside for the moment BEM research, attention is called to such articles as "Biomedical Research in Sweden" (*ESN* 38-7:356 [1984]), "Cardiostem-84 Meets in Monaco" (*ESN* 38-10:526 [1984]), "Eliminating Radioisotopes in Immunoassays" (*ESN* 38-11:567 [1984]), "German Groups Conduct Broad Range of Research on Post-trauma Treatment" (*ESN* 38-11:569 [1984]), "Wireless Medical Telemetry in Europe" (*ESN* 39-4:141 [1985]), and "UK Lab Provides Defenses Against Chemical and Biological Warfare" (*ESN* 39-3:82 [1985]).

In general, it must be said that biomedical and biological research in Europe is exhibiting some of the same syndromes as in the US. A lot derives from the fact that, unlike so-called high technology research involving fancy electronics or awe-inspiring physics, biomedical research is slow to show results, and when it does, it does not have the bells and whistles that cause the public to sit up and take notice. Thus, often in the battles for precious basic research dollars, biological research is easily pushed into the background. This despite the fact that it deals with and most affects our greatest resource--human health, without which the bells don't get rung nor the whistles blown.

While attending the Third International Conference on Water and Ions in Biological Systems, in Bucharest, I had the unique opportunity to visit the University of Bucharest and assess its program of training and research in biophysics. This was reported in *ESN* (39-1:4 [1985]), but because of its unique nature, I digress to repeat some of the highlights of that report.

Romania has a long history of innovative biophysical research; one of the first laboratories in Europe to use radioisotopes in clinical research and investigation was set up there just after World War II. During the last 30 to 40 years, there has been a high level of activity primarily in the following areas of research: reception and conduction of information in the nervous system, radiobiology and photobiology, membranes and cell biophysics, and the state and role of water in biological systems.

Medical and biomedical training has been under way at the University of

Bucharest for more than 100 years. Located in the heart of the city, the university has 6000 medical students. Since the course of study is 6 years, this means about 1000 students are admitted each year (the dropout rate is less than 5 percent). This number includes dental as well as general medical students. The medical students are divided into two groups--pediatrics and general medicine. The faculty numbers 120 in general medicine, 80 in pediatrics, and 50 in dentistry. This is a ratio of one faculty person for every 24 students.

The medical students come from many countries of the world; in fact, a number come from the US. About 30 to 40 percent of the students are actually from outside Romania. The school also trains physicians for the military, for unlike the US there is no dedicated school of medicine for the training of military physicians. Approximately one-half of the students are women, and a high percentage of all the graduates go into clinical practice as opposed to research and teaching.

Of particular interest is the biophysical research conducted in the medical school of the university. I was introduced to the programs there by Professor V. Vasilescu, MD, PhD, DSc, who is head of the Biophysical Department and Laboratory. Vasilescu heads a team of about 30 researchers, who have been very active and very productive despite a severe lack of modern, state-of-the-art equipment.

The Biophysical Laboratory has had extensive international ties over the past decade or so. A number of the faculty members have taught and consulted in France, England, and the US as well as in the Soviet Union and other East European countries. Representatives of this laboratory have participated in a number of international meetings and particularly those organized by United Nations Educational, Scientific, and Cultural Organization (UNESCO). Certain of the research projects in the laboratory are included in the UNESCO Program for Biophysics, the Third Working Group of which is presided over by Vasilescu.

Essentially all of the support for the research programs is derived from the Ministry of Education. However, there are some "outside" contracts from an organization that appears to be equivalent to the National Science Foundation in the US. These are competitive and awarded annually. The support for research appears to cover salaries and a minimum of equipment, but no travel. While scientists are allowed to go to selected foreign meetings, they must pay their own expenses. I saw only one or two computers in the labs and no photocopying machines. (In fact, it was quite evident that copiers were needed at the conference I attended.)

5 BIOELECTROMAGNETICS RESEARCH

As the program manager for BEM at ONR for 12 years prior to coming to ONRL, I naturally concentrated my attention in this area.

BEM is a relatively new research area and one which I am proud to have helped shape in my position at ONR. In fact, I coined the name for this research area in 1978. ONR, and indeed the Navy and the DoD, can be proud of their contributions to BEM research in the US and throughout the world.

A multdisciplinary area, BEM encompasses biology from micro to macro, physiology, psychology, immunology, biophysics, physics, engineering, etc. Though the area is new in terms of organization, BEM may actually be traced back almost 100 years. The patron scientific saint of the field is now accepted to be Arsene d'Arsonval of France, whose research on electrophysiological activity of muscles and nerves in the last quarter of the 19th century led him to explore the effects of low- and high-frequency currents, which led, in turn, to his development of radiofrequency generators and applicators for use in the clinic. This modality is known now as "diathermy" but earlier was known as "d'Arsonvalisation." The physician d'Arsonval was the first to use field-induced hyperthermia in the treatment of cancer (Justesen and Guy, 1985).

Much of the research in BEM over the past two decades has been driven by an intense desire to determine the nature and degree of biological hazard posed by exposure to electromagnetic (EM) fields. By far the largest majority of the research has been concentrated in the frequency range of about 300 MHz to 100 GHz, the so-called microwave portion of the electromagnetic spectrum (see Figure 1). In the US, the DoD is probably the largest single user of EM energy in the form of radar or radio waves. It is this use that has caused the DoD to spend large sums on research designed to answer questions regarding the hazards to personnel due to working in the environments of EM fields. Microwaves, generated in great abundance by radar equipment, have been the "mother ship" of the BEM research community, with at least 80 percent of the research centered around one frequency--2450 MHz. That this came about was due primarily to the availability of equipment, for one thing, and the early assumption that extrapolations could be made to other frequencies if certain parameters were adjusted.

The primary effect of the interaction of EM fields, and especially microwaves, and biological systems is the production of heat. The energy of the fields is absorbed by the target system resulting in molecular motion. The EM energy in this part of the spectrum does not cause ionization of atoms as x-rays and gamma rays do. For this reason, it was felt for a long time that in the absence of heat there was no hazard from short-term exposure. It is now generally accepted that this is not so, that there are field-specific effects that can occur at levels that do not produce heat. In the past 5 to 7 years, the most exciting research has been conducted in this area. It is here that the quest continues for the elusive interactive mechanisms that are responsible for effects at the level of the cell membrane and intra-cellular components. Early studies generally concentrated on the organism and looked for phenomena such as changes in behavior, or in growth and development. Now the search has turned to the cell and to macromolecules. Such scientific probing calls for a substantial increase in the precision of measurement of biological responses.

The quest for adverse biological effects has paid dividends in ways not originally anticipated. As more was learned about the responses of biological systems, it was found that some of the responses were not detrimental but were indeed beneficial. On even closer

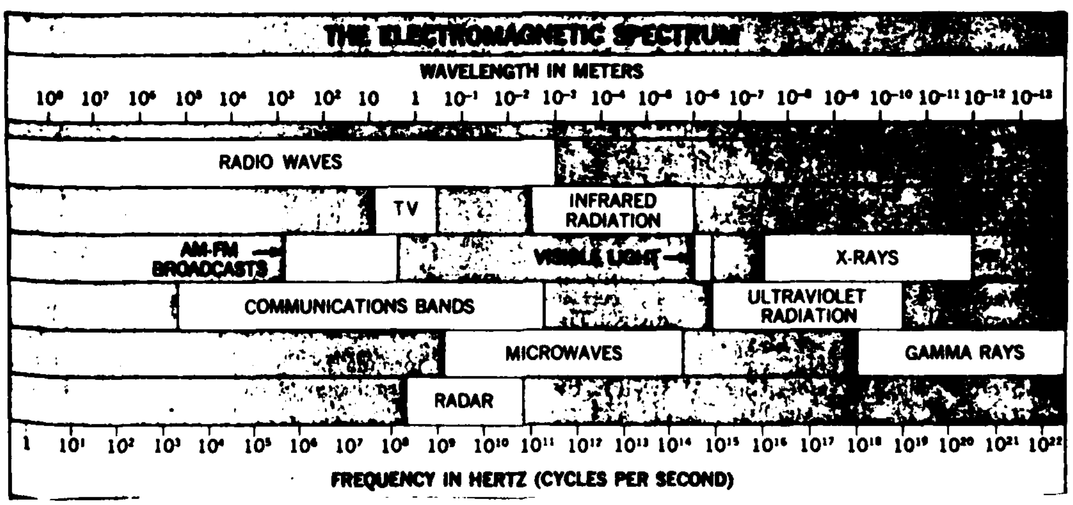


Figure 1. The electromagnetic spectrum.

examination, it was found that almost all living systems have bioelectric components, such as nerve activity or muscle conduction, and that many such as birds and other species, use EM information for navigation. We now can use EM energy in an ever-increasing number of diagnostic and therapeutic modalities. Witness such techniques as nuclear magnetic resonance (NMR), bone healing by EM field stimulation, cancer treatment by hyperthermia, and microwave imaging, among others (Rozzell and Lin, in press).

With the realization that extremely low levels of EM energy are capable of eliciting a response from a biological system, attention is now being turned to further understanding of exactly how and where such sensitive receptors reside and how they react in the presence of such weak fields as those generated by the Earth or other natural and synthetic sources.

It is difficult to categorize European research in BEM. It does not have the support of the military as is the case in the US, and tends to be supported in a number of different ways. In some countries, France is an example, there has been much national support centered around one agency (ONRL report R-8-84). In other countries, support is split between a number of agencies and there is little or no coordination among them.

A large portion of the European research in BEM relates to medical and biomedical applications. This may be due in part to the ease with which medical devices can be clinically studied and brought to market. There is some very unique research ongoing in Europe, and I shall try to highlight some of it in the balance of this report.

Italy

While this is not one of the "high" science countries in Europe, there is a burgeoning bit of research in Italy in the medical uses of EM fields. Italy is one of the countries in Western Europe where it is exceptionally easy to experiment with drugs and medical devices, and there seems to be little in the way of regulation of clinical uses. According to the information that I have been able to gather, a whole host of human ailments are being treated by the same therapeutic modality--low-frequency pulsing magnetic fields. Ailments such as muscle sprains, contusions, psoriasis, migraine headaches, motor-function impairment, surgical complications, neuralgias, venous and arterial diseases (including artherosclerosis and Raynaud's disease), arthritis, rheumatism, ankylosing spondylitis, osteoporosis, pseudoarthrosis, and nonunions can apparently all be treated in this manner. At least this is the impression given during the First International Meeting of the Association for Biomedical Applications of Electromagnetism, (A.B.A.EM.) held in Venice, Italy, in February 1985. The meeting, entitled "Biological Effects and Therapeutic Applications of ELF Electromagnetic Fields," was a mixture of basic science (one-third) and clinical presentations (two-thirds); it was well attended by Europeans and Americans.

The impetus for the meeting was the increasing use, especially in Italy and to a much lesser extent in Germany and France, of magnetic or electromagnetic fields to treat a wide range of human diseases such as those listed above. There are two principal methods for producing the treatment fields. In the first an electric current is run through a solenoid to create a magnetic field of up to 350 Gauss at a frequency usually less than 100 Hz. There are at least eight companies in Italy and one in Germany producing equipment in this class. Characteristic of the equipment in this group are cylinders of approximately 45- to 50-cm diameter. In some cases the solenoid cylinder is placed around the patient, who sits during the treatment; in others the patient lies supine on a narrow bed and the cylinder slides continuously from the head to the foot during the treatment or, depending upon the area being treated, remains stationary (see Figure 2). In either configuration,

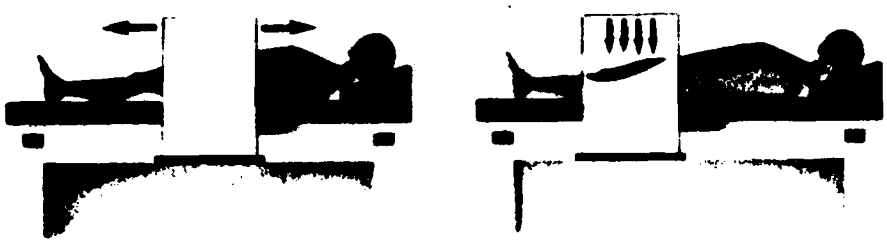


Figure 2. (a) The cylinder is moved ither manually or by program control back and forth along the platform, or remains stationary; (b) current flows through the coil in a circular fashion, inducing a magnetic field which in turn induces current flow in the patient.

the patient is subjected to a pulsating magnetic field, the frequency and intensity of which are variable from 1 to 1000 Hz and zero to 350 Gauss (depending on the model). The field parameters in most of these devices are determined by punch cards for the ailment being treated. If there is an effect, it can be assumed that electric current of varying magnitude is thus induced in the body of the patient.

The second class of equipment producing electric and magnetic fields generally uses parallel plates, or as in the case of the sole US entry, Diapulse, an irradiating antenna. Five Italian and one French device compete with Diapulse in this class. No information is available on the maximum electric or magnetic field intensities in the bodies of patients, but frequencies range from 50 Hz up to 20 GHz. The frequency is usually fixed, however, in any given device.

It is recognized by at least some of the manufacturers of both classes of equipment that full acceptance and credibility will come only when reputable scientific and clinical evidence substantiates the therapeutic claims that to many seem speculative. It is to the credit of several of the companies that a number of laboratory studies have been initiated with the actual generators used clinically or with generators that simulate the field characteristics of the clinical devices.

Since the level of energy imparted to cells using low-frequency, low-intensity magnetic fields is insufficient to give rise to quantum phenomena or cause changes comparable to the level of the thermal energy (kT), some new models and hypotheses must be invoked to explain biochemical or biophysical mechanisms that may occur in and around exposed cells. Giovanna Morgavi and Sandro Ridella (Istituto per i Circuti Elettronici, Genova, Italy) reported at the Bioelectromagnetics meeting in Boulder, Colorado, in 1983 a strong frequency-dependent behavior of sodium current through cell membranes, and Arthur Pilla (Mt. Sinai School of Medicine, New York) has suggested that modifications of intracellular ion concentrations may be controlling factors that trigger cell modifications. There are also those who feel that nonlinear molecular vibrations in the form of "soliton" waves may convey energy imparted by low-frequency electromagnetic fields. Whether such energy transport can trigger chemical reactions from one site to another is still clear only in the minds of some theoreticians.

We are seeing more and more examples of biological sensitivity to very weak electric and magnetic fields. Abe Liboff, working at the Naval Medical Research Institute, has shown that human fibroblasts exposed to low-frequency magnetic fields at amplitudes comparable to ambient geomagnetic levels consistently showed enhanced tritiated-thymidine uptakes. This may mean that such magnetic fields can increase DNA synthesis. Jose Delgado and coworkers (Madrid, Spain), on the other hand, have shown interference in the early organic development of chick embryos (see *ESN* 38-6:297-301 [1984]).

Luiggi Zecca, G. Dal Conte, G. Furia and P. Ferrario (Milan, Italy) used a sinusoidal magnetic field at a frequency of 50 Hz and intensity varying between 0 and 58 Gauss to study survival time in mice subject to Erlich's tumor. This magnetic field was generated by a Ronefor therapy unit, one of the largest sellers. An 11-percent increase in survival time was found in the mice exposed to the magnetic field before and after injection of the tumor cells. This, they feel, indicates a possible enhancement of the immune response of the mice. They have also seen a decrease in the rate of acute inflammation in rats injected with the irritant carrageenin after 2 and 4 hours of exposure when compared with controls. Sergio Curri (Center for Molecular Biology, Milan, Italy) substantiated these observations by studying the morphological and histochemical changes that occur in the rat paw after injection of carrageenin. Does magnetotherapy thus have anti-inflammatory capability?

Another example of increased DNA synthesis, as evidenced by increased uptake of tritiated thymidine, has been demonstrated at the Laboratory of Molecular Endocrinology of the Catholic University-Rome. Researchers there used the Ronefor therapy unit to expose 3T3 cells, of fibroplastic origin, three times a day, at 2-hour intervals, for 30 minutes each. Cell growth rate was also higher in treated cultures than in the controls. In both cases, the significance of the effect increased with time.

Unlike laboratory studies, in which controls can be tightly managed and observations made in a double-blind fashion, the reported clinical investigations leave significant room for doubt. This is especially true for treatment of disorders that have a high degree of associated pain and where the dependent variable is alleviation of the pain. Many patients suffering from such diseases as rheumatoid arthritis or chronic back pains invariably report some degree of additional relief from any new treatment modality. There is a significant amount of psychological input in the pain process. On the other hand, treatment of a long-term pseudoarthrosis that has not responded to other types of treatment for 18 to 24 months provides fairly hard evidence in favor of electric or magnetic field therapy, or both. (Pseudoarthrosis is deossification of a weight-bearing long bone, followed by bending and pathological fracture, with inability to form normal callus leading to existence of a "false joint" that gives the condition its name.)

Another area that has received increased attention for this type of therapy, especially in Italy and Germany, is sports trauma. Problems such as acute lesions (muscle tears), sprains, contusions, and inflammations have all been treated with extremely low frequency pulsating magnetic fields. Here it is very difficult to evaluate the results since the problems occur in young, healthy individuals who generally tend to heal rapidly. There are no controls with which to compare rates or efficacy of the treatment regime. Comparisons must depend on the clinician's general knowledge of the average course of healing of each disorder. There is the additional complication of tremendous individual variability. Thus, regardless of the claims made by clinicians, there is ever-present doubt as to the exact extent to which the therapeutic modality contributed to the healing of the ailment. What is needed are some good studies with double-blind controls and using, for example, standardized wounds or sprains in animals. If indeed the fields enhance wound healing there should be cellular changes that can be quantified.

While there is a tendency for clinicians and manufacturers, driven by economics, to make sweeping claims for new methods of treatment, there is an equal tendency for the scientific community to toss stones at new ideas. Somewhere in the middle there must be a common platform of understanding. The A.B.A.EM. and the scientists in Italy are laying a good foundation on which to construct this platform.

Germany

BEM research in the FRG 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 functions 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 in 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 decided to work in this range. Thus, almost all of their research has been conducted using millimeter waves.

Using yeast cells, German workers led by Werner Grundler at GFS in Neuherberg and Fritz Keilmann at MPIF in Stuttgart have produced the most consistent findings of nonthermal, resonant responses to microwaves. Essentially, their research 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 antennas for coupling in the microwaves. The simultaneous growth of two yeast cultures (one control and one exposed) was measured in a double-beam spectrometer. They reported their first experiments in 1978 (Grundler and Keilmann, 1978). They recently published further results, obtained using the same yeast system, but with two different antennas (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 represented by the curves b and c in Figure 3.

Keilmann is not entirely satisfied with Fröhlich's model, believing that it does not adequately explain the very low energy transitions that lead ultimately to higher order biological responses. Therefore, he is developing a completely new interactive model that he feels will be more comprehensive and will better explain the nonthermal resonance behavior that has been observed at

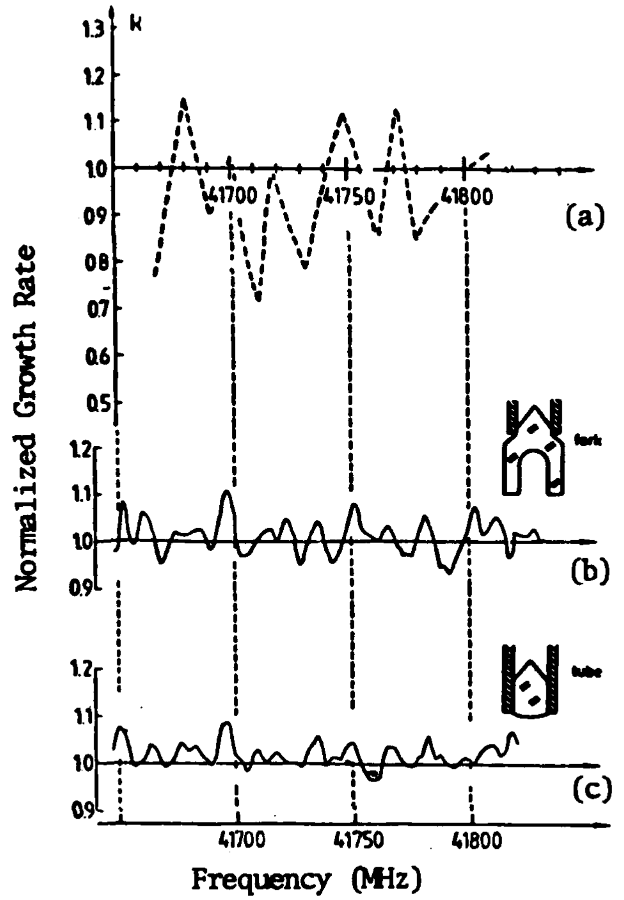


Figure 3. Results with different antennas.

millimeter-wave frequencies. He presented this model at a meeting in Florence, Italy, in August 1984, and I outlined it in *ESN* 39-1:6 (1985).

Keilmann bases his model largely on the well-accepted concepts underlying electron spin resonance spectroscopy and borrows heavily from the work of El Sayed (1974), McGlynn et al. (1969), and others. He disregards free radical formation but takes into account molecules or intermediate complexes that have two or more unpaired electrons which, in addition to exhibiting paramagnetic resonance in high magnetic fields, offers the possibility of microwave transitions in the absence of an external magnetic field.

Keilmann claims that his model predicts a rather specific behavior of the observed bioeffects in the presence of external DC magnetic fields of approximately 100 Gauss. He feels that both resonant microwave fields and DC magnetic fields might, through consideration of these highly specific interactions, offer possible analytical and diagnostic applications in that they could serve as a "fingerprint" to identify the target molecule in the biochemical pathway.

Is Keilmann on to something? I don't know; that remains to be seen. He indicates that he is going to attempt to fine tune this model and conduct experiments that he hopes will strengthen it. He will publish it soon in the *IEEE Transactions on Microwave Theory and Techniques* so that others can consider experiments to test it. He is also going back over past experiments and reanalyzing them in light of this new thinking. This line of research bears watching closely.

The findings of this group have significantly altered the thinking of the BEM research community in terms of coherent oscillations in biological systems and essentially given further credence to some of Fröhlich's theories.

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 see whether pulsing electromagnetic fields at low frequency would cause loosened hip joints to be refitted in the joints and integrity reestablished. 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.

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 nonunions 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 a 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 FRG. 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: the system allows 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.

A recent study of millimeter-wave effects in *Drosophila* was conducted by Gunter Nimtz at the University of Cologne, but the results are 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 be be affected. The fertility of the "grandchildren," the F_2 generation, appeared to drop 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 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.

There is another area in which the FRG seems to be leading all other European countries as well as the US. That is in electrofusion and electroporation.

Electroporation is the reversible formation of a pore in a biological membrane or lipid bilayer when electric impulses cause transient structural changes.

Following the opening of the pore, there can be flow of material through the opening. If two membranes are in contact, they may fuse (electrofusion). Professor U. Zimmermann, formerly of the Institute of Medicine in the Nuclear Research Center (Kernforschungsanlage) of Jülich, now at the University of Würzburg, Bavaria, has led the world in the study of electrofusion.

Cell-to-cell fusion is generally achieved *in vitro* by chemicals (such as poly(ethylene glycol)), by inactivated virus, by freezing and thawing, or by other drastic physical methods. In each case, the membrane is disrupted. Even though promising results have been obtained with these methods, science is still a long way from understanding the underlying molecular mechanisms of the fusion process. The current fusion techniques have a number of drawbacks that make it desirable to find a better way to achieve the same result.

Most cells in suspension, being electrically neutral, will not tend to come into close membrane contact with other cells. More than 25 years ago, an ONR contractor, Herman P. Schwan, noticed that individual cells in a nonuniform field form chain-like aggregates and align themselves adjacent to each other along the direction of the field--much in the manner of pearls on a string (Schwan, 1957). This was called the "pearl chain" effect and has been much observed and reported in many BEM studies.

One of the most interesting aspects of the movement of polarizable cells toward the region of high field intensity is that the cells will tend to contact each other as they form the pearl chains. Cells not only become polarized and form pearl chains, but also rotate in response to the field. This overall behavior of cells to a nonuniform electric field has come to be called dielectrophoresis (Pohl, 1978; Pethig, 1979). The forces of attraction arising from the dipole generation in each cell overcome the electrostatic repulsion between the apposed membrane surfaces bearing net charges and the repulsive hydration force. It is this critical contact between the membranes of cells under the influence of dielectrophoresis that has led to an innovative application of electric fields--that of fusing of cells without changing their overall function or without irreversibly degrading the membrane.

Zimmermann has patented a method of using electric field pulses to rapidly and gently fuse like cells to form giant cells, and unlike cells to form hybrids. Further, he and his group have been able, using electric fields, to open cell membranes and encapsulate substances such as drugs within the cytoplasm and have the cells reseal themselves with no apparent detrimental effect on the cell membrane.

The electrofusion technique requires, first of all, very close membrane contact between cells. Thus, in the first stage of the process, a low-intensity, alternating-current field (e.g., 10 to 100 V/cm, depending on cell size) is applied to the cell suspension until the pearl chain aggregates are formed. Point-to-point contact is achieved between adjacent cells in a pearl chain, as shown in Figure 4a. As a general rule, sufficient membrane contact for subsequent fusion is achieved when one can observe a flattening of the membrane contact zones under a microscope, as in Figure 4b.

Once the cells are in close contact, the actual fusion process is triggered by one or more high-intensity, direct-current field pulses of 3- to 50-μs duration (depending on the cell types). This type of pulse causes a temporary breakdown in the membrane of the cells. The field strength required for breakdown is about 0.5 to 10 kV/cm, depending on cell size. The actual breakdown voltage at the level of the cell membrane for living cells is on the order of 1 V. The electrical breakdown of a viable cell membrane causes no irreversible changes in the cell membrane or the cell itself, if the pulse length is less than about 100 μs (Zimmermann et al., 1980 and 1981).

Professor E. Neumann, University of Bielefeld, has done stochastic modeling of the fundamental process of electroporation and electrofusion and treated it in terms of a periodic lipid block model, a block being a nearest-neighbor pair of lipid molecules in either of two states: (1) the polar head group in the bilayer plane, or (2) facing the center of a pore (or defect site). He takes the number of blocks in the pore wall as the stochastic variable of the model describing pore size and stability. Neumann has gone beyond the theoretical aspects and actually been successful in transferring genes into mouse lyoma cells by electroporation.

In spite of the enormous implications for electroporation and electrofusion in areas such as plant biology, drug delivery, genetic engineering, mammalian reproduction, etc., I have found little evidence of much activity in Europe outside these two German groups. There is enormous potential for these processes, a fact that came out quite clearly in a Ciba Foundation special symposium held in London in 1983 (Evered and Whelan, 1984). As a consequence of the growing interest in this area and the now scattered research efforts, I am planning a symposium at the AAAS Annual Meeting in Philadelphia in 1986. There is a chance that the BEM program at ONR may begin to play a role in the stimulation of this research.

Much of the BEM research throughout the world is in direct support of

Figure 4. Cell fusion as viewed under a microscope, from initial contact through complete fusion.

efforts to establish safety standards for the exposure of humans to EM fields. I did not find a lot of activity actually devoted to establishing standards in Europe, however. One exception was in the FRG, where the Institut für Strahlenhygiene des Bundesgesundheitsamtes (Institute for Radiological Health of the Federal Health Office [ISB]) estimates that there are about 50,000 workers potentially exposed to electromagnetic fields (EMF) in FRG industries. It is further estimated that there are 3000 to 10,000 generators operating at power levels above 10 kW in industrial activities such as curing, gluing, drying, and thermosetting. Until recently, however, there had been no official human-exposure guidelines or standards to which industry must adhere in order to avoid possibly overexposing workers. Instead, the FRG has relied on the standards and guidelines developed by other countries, specifically those issued by the American National Standards Institute (ANSI).

Recently the FRG published its own standard, "Hazards by Electromagnetic *Fields--Protection of Persons in the* Frequency Range From 10 kHz to 3000 GHz," (see *ESN* 38-10:530 [1984]). The German standard differs from the ANSI standard in the frequency range covered as well as the exposure levels permitted within certain frequency bands. ANSI C95.1 applies to frequencies from 0.3 MHz to 100 GHz, while the German standard covers the range from 0.01 MHz to 3000 GHz. Thus the German standard is much broader. Figures 5 and 6 show how the electric and magnetic field strengths vary with frequency for the German standard.

Like the ANSI standard, the German standard is to apply to the general public as well as to the industrial labor force. In the US, the Environmental Protection Agency is busy formulating an additional standard specifically for the general public. In the meantime, those in the US who are concerned use the ANSI standard; the military, being essentially an industry, has adopted it.

As the FRG standard goes into effect, three groups are conducting surveys to determine if there are any problems now. One survey of a majority of industries is being conducted by the Federal Health Office. A companion industrial survey is being conducted by Berufsgenissenschaft der Feinmechanik und Elektrotechnik, and a third is being done by Siemens (Berlin) of all of its plants in the FRG. The Federal Health Office has already surveyed the environment around all broadcasting stations, and only a few cases were found in which the levels exceeded those called for in the standard. Home microwave

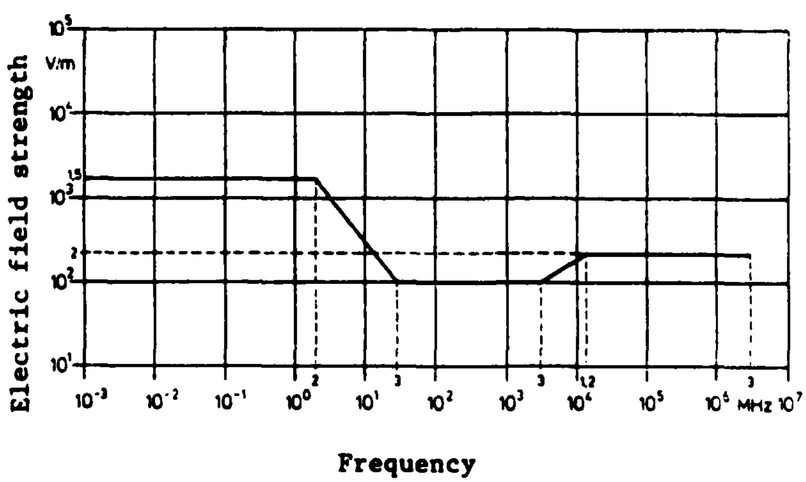


Figure 5. Boundary values of the electric field strength in the West German standard.

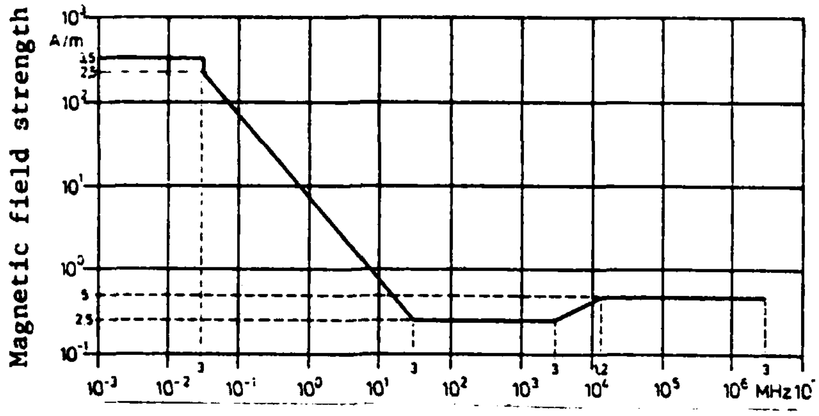


Figure 6. Boundary values of the magnetic field strength in the West German standard.

units were surveyed by the Post Office, and approximately 10 cases were found in which the levels exceeded 5 mW/cm^2 at a distance of 5 cm from the surface of the unit. These were ovens that were more than 10 years old.

One area that might cause a problem is in-plant communication systems that operate in the 60- to 65-GHz range. Mercedes-Benz and other companies use this frequency band because of its limited range and the need for security. Power levels, on the other hand, might be significant--especially from hand-held walkie-talkies. In this frequency range the eyes become critical organs and therefore possible targets for damage.

France

Over the past decade, France has played a major role in BEM research. While the total French program was moderate compared with those of other countries, the contributions it made were highly significant and had an impact on almost every aspect of the field. A wide variety of cellular and membrane work was carried out in Paris at Institut Curie and Centre National de la Recherche Scientifique (CNRS); nonperturbing temperature probes were developed in Toulouse; instruments and exposure systems were designed and built and electric properties of materials studied at CNRS; animal behavior and electroencephalogram (EEG) changes were investigated at Toulon; and hyperthermia methods were developed at Lyon, Lille, and Toulouse--just to mention a few accomplishments of the French research program.

Much of the support for the research came from the military via the Direction des Études et de Recherche Technique (DRET) and CNRS. With the exception of a few scattered projects, all of the BEM research was funded by these two agencies. Until approximately 3 years ago, the program at DRET was managed by Colonel G. Plurien, a military physician who fought to build the program and defended it at the highest level of the Defense Department. He was replaced in normal rotation by a manager who has given BEM research such a low priority that all funding has essentially ceased.

According to several of the current and past researchers with whom I spoke, CNRS, observing the decision of DRET, decided to do likewise. Instead of filling in the support void, they apparently decided not to be a loner in support of this area of research. In all fairness, I should note that part of the rationale for the decisions of these two agencies lies in the present general French economic situation and the demand from new scientific and technological areas, such as biotechnology and microelectronics, on R&D funds. Thus, in the reordering of priorities it was apparently decided that France could afford to "piggyback" onto other countries in this research area and thus free funds for other R&D that does not lend itself so readily to dependence on outside sources, or that will have a greater industrial and economic impact.

Another reason behind France's decision, according to Mde. A. Duchene (Institut de Protection et de Sûreté Nucléaire, Fontenay-aux-Roses), is that the government does not perceive a significant health hazard to the general public due to electromagnetic energy. If one examines the usage and application statistics, one sees that this is a reasonable conclusion. Occupational uses of electromagnetic energy, on the other hand, are increasing, and new standards for human exposure are being developed. These standards may be even more stringent than those of the US, because the French take occupational health very seriously. However, this industrial activity is not unique, and research findings of other countries are quite adequate to support the database necessary to write the standards.

In attempting to assess the BEM research in France, I looked at some of the recently completed research, which because of the decisions discussed above is being terminated, and at some research carried out over the last 3 to 5 years. The latter will help demonstrate the breadth and scope of the research program as it was and the level of competence of the investigators--many of whom now must redirect their energies to other areas of research if they are to remain in the French establishments where they now work. Since the job market in France is tight, it is fairly safe to say that most will follow this course. The BEM research community thus loses the talents of a small but highly skilled and productive group.

The French have not put much effort into the type of clinical applications that the Italians and Germans are currently pushing--magnetotherapy. There has been, and still is, a considerable amount of work involving hyperthermia devices for cancer treatment. Professor Y. Leroy at Université des Sciences et Techniques de Lille, Alan Priou at the Aerospatial Center of Toulouse, and Michel Gautherie of Laboratoire de Thermologie Biomédicale, Strasbourg, are among those who have pioneered in this area. Priou's group developed a nonperturbing temperature sensor at about the same time (mid-1970s) that I developed the liquid crystal optic fiber temperature probe. However, they have apparently gone on to industrial applications and no longer are concerned with biomedical applications. Leroy's group is still very active and will probably enjoy support for years to come; the support for hyperthermia research is a little different from the usual BEM support, coming as it often does from the cancer research agencies.

A good portion of the French BEM research was summarized in ONRL report R-8-84. One of the major researchers in cellular effects has been Dr. Dietrich Averbeck and associates at the Institut Curie in Paris. Over the years Averbeck has worked closely with Dr. A.J. Berteaud and his team at the CNRS laboratory in Thiais. The two groups published a major paper on what was their last federally funded BEM project.

This study, discussed in much more detail in *ESN* 38-8:420-422 (1984), was aimed at elucidating cytoplasmic events inside a line of lung tumor cells (V79) of the Chinese hamster. As has always been the case with investigations in Averbeck's laboratory, this set of experiments was well thought out, meticulously executed, and clearly presented.

Since more than one cellular target, including cytoplasmic organelles and membranes, is likely involved in hyperthermia-induced cell death, Dardalhon, Averbeck, Moré, and Berteaud (DAMB) decided to investigate: (1) changes in the degree of fluorescence polarization related to changes in the microviscosity of the cytoplasm and mitrochondria, and

(2) changes in enzymatic hydrolysis and cell permeation of an intracellular fluorescent marker. The method is based on polarized light excitation of fluorescein molecules following their production in the cytoplasm by enzymatic hydrolysis of nonfluorescent fluorescein diacetate. The preferential excitation of the fluorescein molecules serves as a probe for the physical state of the cytoplasmic organization. The fluorescence and the kinetics of enzymatic hydrolysis were determined by an automatic and computer-controlled spectrofluorimeter specially designed by Moré and Berteaud.

These studies by DAMB suggested that the microwave energy acts on enzymes, or membranes, or both. The notion that membranes are affected is also supported by the close correlation between the decrease in enzymatic hydrolysis and the decrease in cell viability.

A study to determine nonthermal effects of microwaves on enzymatic activity was carried out in Berteaud's laboratory in Thiais. The laboratory is one of many CNRS labs in a large complex; each laboratory in the complex has its own director. Berteaud is director of the laboratory of molecular and micromolecular structure. This laboratory has consistently worked cooperatively with biologists in other laboratories in France, providing design, engineering, and measurement support for BEM research. In fact, Berteaud has, in one way or another, contributed to almost every study on microwave or radio-frequency effects conducted in France during the last 10 years.

The team of Mamouni, Leroy, Van de Velde, and Bellarbi (MLVB) in Lille has been the most active French team working in microwave hyperthermia. Recently, however, they have turned part of their attention to microwave thermography (MWT). This research is a natural outgrowth of the group's studies of new methods of heating tissues. MWT has been used by only a few researchers to diagnose cancer by localization of hot pockets of subcutaneous tissue. The technique takes advantage of the relative transparency of tissue at or near microwave frequencies, and measures the "Black-body" radiation given off in this frequency range.

MLVB have proposed a new process, called correlation microwave thermography (CMWT), that brings to biomedical applications the long-proven phase-switching two-aerial interferometer used in the observation of weak radiostars. The researchers claim that their process performs better than the previous one in localizing thermal gradients in the body.

MLVB have constructed the appropriate equipment and tested it extensively. Using different thermal structures, they have been able to demonstrate that CMWT:

1. Gives a new type of information in that the shapes of the diagrams given by CMWT and MWT are different;
2. Enhances the determination of the thermal gradients by giving a more selective output signal than MWT.

However, in their preliminary observations they obtained CMWT signals with smaller amplitudes than the MWT signals. If further research goes as anticipated, CMWT should provide an improved method for detecting thermal gradients in tissues. This will not only lead to better efficiency in detecting abnormal structures, but also can control hyperthermia treatment when it is important to know precisely the temperature profile of the heated tissue.

The breadth and scope of French research in BEM over the past few years can be illustrated by examining the work of some of the key researchers.

B. Servantie. For many years, Dr. Servantie was the torchbearer of the French BEM community. A navy physician stationed at the naval hospital and research laboratory in Toulon, Servantie has carried out a broad range of studies. Most of the work was done at 2.45 GHz. He was most recently involved in research on the effect of low power levels (<5 mW/cm^2) on the efficacy of drugs and on behavior in rats and mice. He used both pulsed and continuous-wave fields at 2.45 GHz, 3 GHz, and 9 GHz. Servantie has observed and reported on the effects of microwaves on EEG patterns in rats. He has also studied some cases of human exposure at levels above the recognized safety standards and quantified the apparent damage to health resulting from these exposures.

Servantie has recently compiled an extensive computer database of BEM literature. With the termination of BEM research support, he has moved to a new post at a naval training facility in Bordeaux. He has offered to make the tapes containing his database available to ONR for use by Information Ventures, Inc. (Philadelphia), an ONR contractor. It is possible that Servantie has included some literature that ONR has missed. At least it would be good to compare the two literature databases.

H. Francois. Thermoluminescent techniques for monitoring microwave fields are being studied at the Institut de Protection et de Sûreté Nucléaire, Fontenay-aux-Roses, a suburb of Paris. Dr. Francois believes that lithium or calcium sulphate, long used as thermoluminescent materials in ionizing radiation dosimetry, may provide a means to measure microwave power density.

When either of these sulphur-based compounds is exposed to a high dose of ionizing radiation--x- or γ-rays, for example--it emits photons of a characteristic wavelength that can be detected by a photomultiplier tube coupled to an amplifier and spectrum analyzer. The intensity of the spectrum in the region of the characteristic photons is proportional to the dose of radiation that originally impinged upon the sulphate. Francois believes that a decrease in the luminescence will occur if the sulphate compounds are exposed to microwaves after being irradiated with a known dose of ionizing radiation. If he can correlate this with the microwave power density or with the specific absorption rate, he may have the basis for a microwave dosimeter.

R. Santini. Dr. Santini (Institut National des Sciences Appliqué, Villeurbanne), who is not extremely well known outside France, is interested in the biological effects of both high-frequency microwave fields and magnetic fields. His work has centered around the nature of possible hazards and the methods of quantifying such hazards and the methods of quantifying such hazards.

In recent years, Santini has been investigating the effects of electromagnetic fields on tumor development in animals, especially melanoma B_{16} of black mice. He has also been interested in the effects of electromagnetic energy on both bone ossification and bone-fracture repair, and the effects of such energy on digestive-tract physiology. In one study he exposed rats to a 2.45-GHz field at 3 to 4 mW/cm^2 for periods of 4 to 8 hours. He saw no effect after 4 hours, but after 8 hours he observed an acceleration of the transit time in the gastrointestinal track. This persisted for more than 25 hours. There was no increase in the rectal temperature of the exposed compared to the controls.

Y. Leroy. Only a few people have attempted to noninvasively measure subcutaneous temperature using microwave radiometry, and Leroy (Centre Hyper-frequences et Semiconducteurs, Université des Sciences et Techniques de Lille, Villeneuve d'Asq) is the only one, to my knowledge, in France. His laboratory has been generally concerned with developing several noninvasive temperature-measuring techniques. He has used contact radiometer probes that operate between 1 and 10 GHz and that are in contact with the skin. These probes are much like the devices used by Myers and Barrett of the Massachusetts Institute of Technology to detect breast tumors. In addition, Leroy has used remote-sensing probes with focused antennas that operate in the millimeter range.

More recently, Leroy has developed the technique of correlation microwave thermography described above for measuring subcutaneous local temperatures. This technique is based on a coherent detection of noise and helps improve the localization of thermal gradients in tissues.

A.J. Berteaud and D. Averbeck. The laboratory headed by Berteaud belongs to

CNRS, as noted above. Dr. Averbeck is actually at the Institut Curie in the heart of Paris. However, he and his group have worked so closely with Berteaud's laboratory over the years that we can easily look upon the two laboratories as being one.

Over the years, this very active group has conducted research in several different areas of BEM. They have studied the action of millimeter waves at 70 to 75 GHz and power levels of 5 to 100 mW/cm^2 on bacterial growth. They have also used procaryotic and eucaryotic cell systems to study the genetic effects of microwaves at 94, 17, and 70 to 75 GHz at power levels up to 60 mW/cm^2. They found no effect on cell survival or on mutation induction.

They went on to look for effects on other cellular targets, other than DNA. Effects on the cytoplasm and membranes have been studied, as have the effects on growth of *E. coli*. The latter was investigated at 17 GHz, 50 mW/cm^2 in combination with x-rays and ultraviolet irradiation. The researchers determined the survival of wild-type and repair-deficient mutants of *E. coli* as well as the induction of mitotic recombination in the yeast strain D_5. Their results suggested that 17-GHz microwaves at 50 mW/cm^2 exert a small but significant effect on the biological endpoints studied.

Berteaud and Averbeck have also looked at the thermal action of 2.45-GHz microwaves on the yeast *Saccharomyces cerevisiae*. They compared the effects with those brought about by water bath hyperthermia and concluded that for specific absorption rates between 20 and 100 w/kg there was no evidence for specific field effects due to the microwaves. The group also performed studies at 434 MHz. It also was concluded in this study that there was no difference in the thermal action of the electromagnetic field when compared to classical methods of heating.

Finally, these laboratories did not miss the most studied of all insects, *Drosophila melanogaster*. They looked for the induction of lethal and sublethal mutations but were forced to conclude that microwaves do not induce irreversible changes in genetic material--at least at reasonable levels of intensity.

Because these researchers were so active, it was almost natural that they would be among the last to finish following the demise of research support in France. Their latest work was described earlier in this report. There is little doubt that if French support for BEM research is renewed, this laboratory will be among the first in the starting blocks. Even now, Berteaud reports that his laboratory has a proposal in to the US National Science Foundation for a joint project with some American investigators to conduct research in microwave imagery. This should prove an interesting collaborative effort if it is funded.

A. Duchene. Even though she is not conducting research, Mde. Duchene (Institut de Protection et de Sûreté Nucléaire, Fontenay-aux-Roses) has been synonymous with BEM in France for a number of years. She is a French representative to the Union Radio Scientifique Internationale and to the International Radiation Protection Association, and is playing a leading role in the development of a French occupational-exposure standard. She effectively represents the government to consumer groups and to industry, and has served on a number of international committees and working groups dealing with electromagnetic standards. The reduction in the research program is unlikely to affect Duchene's activities.

Spain

Three years ago, Spanish researchers found that extremely weak, pulsed magnetic fields (repetition rate of 100 Hz and 1.2 μT) caused malformations in developing chick embryos. That report (Delgado et al., 1982) at once stunned and perplexed the BEM research community. This type of biological response had been seen with low-level electric fields but never before with magnetic fields at such low levels--much less than the Earth's.

What Dr. Delgado and his team at the Departamento de Investigacion, Centro 'Ramon y Cajal,' Madrid, Spain, reported was that a 48-hour exposure of fertilized eggs from white Leghorn hens to the weak magnetic fields resulted in consistent and significant inhibition of embryogenesis as revealed by gross morphological and histological analysis. Auditory pit, foregut, brain vesicles, neural tube, heart, vessels, and somites were not developed. They postulated that the alterations in growth may be due in part to disruption in production and disturbances in the structure of glycosaminoglycans (GAGs), which are essential elements in differentiating cellular activity and in cell migration.

It was immediately evident that such biological sensitivity to extremely low-frequency and low-intensity magnetic fields could have far-reaching implications for a number of military and industrial operations. Several individuals began trying to obtain enough information to replicate this study in an effort to substantiate the findings. However, when attempts were made using either frog or hen eggs, only negative results were found, and all exposed eggs were apparently no different from the controls. These findings began to cast doubt on the reported results of the Spanish group. Thus, I visited the laboratory in Madrid to find out exactly why follow-up studies have been so uniformly negative in the face of continuing reports of positive effects from their laboratory. By the time of my trip, Delgado and his coworkers had published another paper (Ubeda et al., 1983) in which they examined the importance of waveform in determining the biological response of the developing embryo.

It appeared in this set of experiments that there was a "window" for the effect of the magnetic fields, the intermediate conditions of 100 Hz and 1.2 μT being more effective in production of all abnormalities than either the higher or lower frequencies or higher or lower intensities.

Follow-up studies by Dr. Jocelyn Leal in Delgado's laboratory shown even more astonishing aspects of this very sensitive magnetic field effect. First, placement of the egg and its orientation appears to be very critical. Second, the orientation of the egg with respect to the Earth's magnetic field may be important. Third, the wave form of the pulsed magnetic field may be critical, though this has been challenged and suggestions made that the sensitive variable is the rise time of the pulse. Recently, the Madrid team discovered that there was an error in their original determination of the rise time. What was thought to be 42 μs was only about 7 to 9 μs. The full implication of this has not been determined.

The egg-placement details are apparently very important and were not mentioned in the original paper in 1982. This appears to be one of the key reasons why efforts to duplicate the Delgado experiments have been unsuccessful. In fact, Leal and her coworkers have made another observation that further illustrates the critical nature of the orientation of the embryo with respect to the magnetic field.

When the magnetic-field-treated eggs were opened at the end of the 48-hour exposure/incubation period, it was observed that some of the embryos had changed their position in the egg to lie approximately perpendicular to the long axis. Surprisingly, all these embryos were normal, whereas almost all that did not reorient themselves, and thus remained parallel to the direction of the magnetic field, were abnormal. This raises the question of whether the reorientation was a behavioral mechanism that allowed a smaller cross section of the embryo to be presented to the field, thus reducing the amount of absorbed energy. None of the control embryos changed their position in the egg during the 48-hour incubation period.

To rule out possible differences due to the ambient Earth's magnetic field, all eggs were placed so that the long axis was in an east-west direction. It is not known exactly how much shielding of the Earth's magnetic field was provided by the incubators.

A number of past studies have reported that wave form is critical in eliciting biological effects using either pure electric fields or capacitively coupled electric and/or magnetic fields. The most striking of these have been studies that show osteogenesis to be highly dependent upon the wave form of the applied field (Pilla, 1974; Bassett, 1982). However, the importance of the wave form is being questioned quite closely, and more and more studies are being reported that tend to show that the shape of the applied wave is not nearly as important as are pulse characteristics such as frequency and rise time. There was considerable discussion of this point at the First International Meeting on Biological Effects and Therapeutic Applications of ELF Electromagnetic Fields (Venice, Italy, 23 through 25 February 1984).

There can only be speculation about whether these abnormalities result from metabolic changes or whether they are due to direct action of the field on cell membranes, on GAGs components, or on both. It is evident that the developing chick embryo is very sensitive to extremely low frequency, extremely weak magnetic fields. Discussion at the Venice meeting and evidence in a recent paper (Liboff, 1984) suggests that the critical parameter may be the instantaneous rate of change of the energy input, and that the actual frequency or wave form may have very little impact on biological responses of these weak fields. There is general agreement that much is still unknown and a lot of work remains.

Mike Marron (ONR, Code 441CB) and I have spearheaded an international cooperative effort aimed at answering some of the many questions raised by the Madrid study. This project, dubbed "Project Henhouse," involves the original laboratory in Madrid (with Leal, who did the original work, as principal investigator), a laboratory in Sweden (Dr. Kjell Mild), and two laboratories in the US. The US laboratories are at the National Center for Devices, Food and Drug Administration (Dr. Mays Swicord) and the Nonionizing Radiation Branch of the Environmental Protection Agency (Mr. Richard Tell and Dr. Ezra Berman). ONR has funded the study in Madrid and is the coordinator of the project. Each of the principal investigators has been brought into the BEMNet, an electronic mail network which I established before coming to ONRL.

During Project Henhouse, each of the laboratories will attempt to duplicate the original experiment but using equipment that is completely uniform, with coils all made by the same person and identical incubators, etc.

As far as I could determine, there is only one other BEM-related research project in Spain. It is a study of the enhancement of bone healing by a magnetic field. The study is being carried out in Madrid at the Departmento de Traumatologia, Centro de Rehabilitacion MAPFRE. The investigators are P. Guillen Garcia and A. Madronero de la Cal.

They used rabbits to show that the absence of the Earth's magnetic field causes bone to lose calcium. (The US astronauts showed this in the extended space flights). When the Earth's field is restored, there is generalized recalcification. They feel that this points to the possibility that the Earth's field might be used as a "magnetic vaccine" for the treatment of consolidation delays in bone healing. These workers have studied the effects, on bone calcification, of magnetic fields produced by electronically generated signals of sine and square waveform, over a range of frequencies from 0 to 250 kHz. The bone under test was placed inside a coil and thus in the magnetic field.

Garcia and de la Cal feel that if patients with nonhealing bones are placed in a shielded room in which the Earth's magnetic field has been removed, they will loose some calcium from their bones--osteoporosis. If after a few days the patients are removed from the room and returned to their normal environment, there will be a generalized mineralization throughout the skeleton, and this general calcification might reasonably be expected to cure the unhealing bone. Thus the notion, "magnetic vaccine." This seems a little far removed and is certainly an area that needs more work.

Sweden

There has been a moderate level of BEM research in Sweden for some time. Being very health and safety conscious, Swedish research in this area tends to be driven by concerns for health hazards. One of the most recognized names is Kjell (pronounced, "Shell") Hansson Mild of Umea. Dr. Hansson Mild is with the National Board of Occupational Safety and Health.

Recent reports of epidemiological studies suggesting that prolonged exposure to power line frequencies may influence the development of cancer guided Hansson Mild and his team to investigate genetic and reproductive hazards due to working in and around electric lines and substations. In one recent study, they examined the chromosome-breaking ability of power frequency electric fields and transient electric currents, both *in vivo* and *in vitro*. For the *in vivo* investigation peripheral lymphocytes from 20 switchyard workers were screened for chromosome anomalies. The rates of chromatid and chromosome breaks were found to be significantly increased compared to the rates in 17 controls.

Exposure of human peripheral lymphocytes, *in vitro*, to a 50-Hz current with 1 mA/cm^2 current density did not induce any chromosome damage. Exposure to 10 spark discharge pulses of 3-μs duration, with a peak field strength in the samples of 3.5 kV/cm, however, resulted in chromosome breaks at a frequency similiar to that induced in lymphocytes *in vitro* by ionizing radiation--i.e., about a fivefold increase. It is considered essential in studies of induced chromosone aberrations to score only cells which are in their first cell division (48 to 50 hours).

In this study, however, the investigators chose a culturing time of 72 hours, when a portion of the cells may have passed through more than one cell cycle. This was done because many of the samples were sent to them by mail and had a reduced proliferation rate. It was thus often difficult to score enough cells from earlier cultures. This may not have been a major problem since they did make their comparisons with control cells cultured for the same period.

In both studies, cells with polyploidy, endoploidy, and premature chromosome condensation were rather frequent. Although these anomalies may arise in the cell cultures due to faulty cell divisions, another possible mechanism is cell fusion. Exposure of mammalian cells *in vitro* to electric field has been shown to cause cell fusion (Zimmermann, Vienken, and Pilwat, 1980).

Genetic damage in somatic or germ cells may cause malignancy, genetic illness, malformations, or reduced reproductive capacity. As yet, however, there appears to be no dose-response relationship between the rate of chromosome aberrations and, for example, the risk of developing cancer. High levels in one individual may either indicate increased susceptibility or extreme exposure to breaking agents. Concerning *in vivo* exposure to discharges, it is not elucidated to what extent a tissue is affected by the pulses. Much depends on the current density at "the point of contact" and on how rapidly this declines within the tissue. Theoretically, the decrease is very rapid and thus only a small volume of tissue material may be affected--e.g., small blood vessels in a fingertip. It is thus possible that chromosome aberrations of switchyard workers may be restricted to the peripheral blood cells and not to other cells and tissues. Depending on the work situation other parts of the body, however, may be exposed to spark discharges.

Drs. S. Nordstrom, I. Nordenson and Hansson Mild also have conducted a retrospective study of the progeny of 542 male employees at Swedish power stations. They analyzed data from questionnaires and hospital records and found an increased frequency (P<0.001) of congenital malformations, as well as an increase (P<0.025) in the frequency of couples with difficulties in having children when the males worked at high voltage substations. The differences could not be explained by any of the confounding factors analyzed, such as smoking habits of the mothers or their use of medications. Again, these results suggest, but do not prove, that chromosomal aberrations may be caused by spark discharges.

Hansson Mild has also studied abnormalities in the chick embryo. He and his colleages, Monica Sandstrom and Soren Lovtrup, set out to replicate the study done in Madrid by Delgado and associates (see discussion under Spain, above). They actually found abnormalities in embryos exposed for the same period of time to pulsed magnetic fields that they thought were similiar to those used in the Madrid study. As mentioned above, the rise time in the Madrid study, originally thought to be about 42 µs, was only 7 to 9 µs. Hansson Mild found his effect, however, at the 40-µs rise time. Hansson Mild is now participating in Project Henhouse, in which these studies will be replicated more precisely.

Another interesting study is currently being conducted at the University of Göteborg by Dr. Hans-Arne Hansson of the faculty of medicine. Within the last 3 years, Hansson has seen about 35 radar maintenance technicians who were long-time (15 years or more) employees of the school. These men all had symptoms of central nervous system damage. Many were unable to perform their jobs properly and had a number of complaints that led Hansson to send them to the Department of Neurology for a more thorough examination, which included an analysis of the protein patterns in their cerebrospinal fluid (CSF). He decided to do this as a result of some observations that he had made in rabbits whose heads had been exposed to high levels of microwaves.

The analysis of the CSF of the radar workers showed the same changes in the protein patterns as was found repeatedly in the rabbits. See *ESN* 39-2:40 (1985), where I have discussed some details of the procedure.

CSF plays a role in maintaining stability in the central nervous system. It has a characteristic crystalloid and colloid composition. Changes in the composition of the CSF reflect changes in the metabolism of the brain, in the cerebrovascular state, and in the CSF hydrodynamics. Proper analysis of CSF can indicate pathologically degenerative brain diseases. The majority of the proteins normally present in CSF are derived from serum, and the protein content in CSF is about 1/200 of that in serum. This difference is maintained through the relative impermeability of the blood-brain barrier (BBB) to large macromolecules like proteins. Passage through the BBB seems to depend on both filtration and vascular transport.

While it is certainly not clear at this time exactly what the changes in the CSF of the radar workers mean, it does point to a possible new effect of high-level microwave exposure. Unlike the situation with the rabbits, there are no brain tissue samples to study in the humans. Recently, the radar school in Göteborg has instituted substantial changes in its maintenance procedures. A number of safety measures and devices are in place that should prevent anyone from being exposed to operating radar at levels above the human exposure standards. These measures include new standards as well as new interlocks that prevent activation of radar units while personnel are working near the antennas. This study is one that bears close watching as the implications are far-reaching for all military operations involving repair of radar equipment.

A dramatically new medical application of electric current is being tried in Stockholm. There, Dr. Bjorn Nordenstrom, Professor of Radiology at the Karolinska Hospital, is obtaining good results in the treatment of cancer by direct current electrocoagulation. Nordenstrom has demonstrated that his technique has a good chance of avoiding

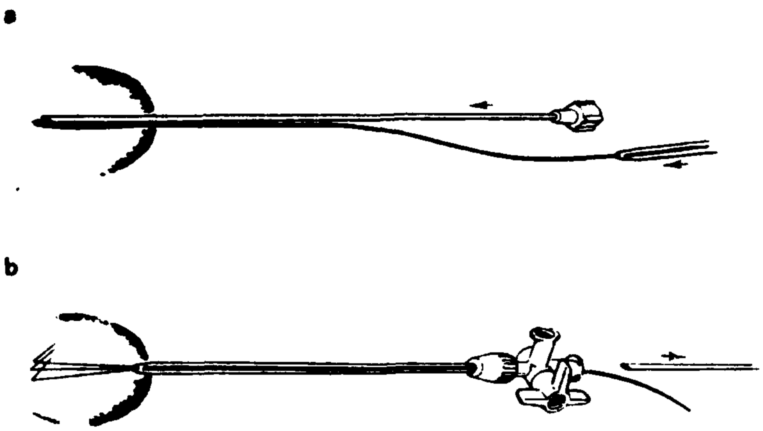


Figure 7. One type of electrode used by Nordestrom to effect direct current treatment of deep-seated tumors: (a) insertion of the hooked ends of the electrodes; (b) after the cannular is removed, a teflon tube with stopcock is passed over the platinum electrodes to the edge of the tumor. The tube serves both as an insulator and as a channel for removal of gas produced at the electrodes during treatment.

mastectomy in a high percentage of the breast cancers he treats.

The technique Nordenstrom uses is based on delivery of direct current into the tumor via a platinum electrode. Since many of the tumors he treats, especially those of the breast and lung, are deep lying, he inserts the electrode with a long needle. The needle is then removed and a plastic tube inserted to remove gas that forms at the anode as the tumor "cooks" (Figure 7). Histological examinations of tissue treated in animals have shown that a primary ionization apparently takes place at the electrode-tissue interface. Nordstrom says that while the tumorous tissue is destroyed by the direct current, healthy tissue seems to be "healed," or at least not harmed. There may also be field-induced changes in the environment of the tumor which are destructive to it. Whatever the mechanisms, the number of women who have had breast tumors cured by this method provides good testimony for it.

The Netherlands

A number of Dutch clinicians and physicists have been involved for years in the use of microwaves in treating cancer--hyperthermia. Microwave hyperthermia is considered to be one of the best treatment modalities for improving the local effectiveness of radiation and chemical therapy. The leading investigator, and one who seems to work with a number of hospitals, is Dr. H.S. Reinhold of the Department of Experimental Radiotherapy, Erasmus University, Rotterdam. Reinhold and his many associates have studied the dose-effect relationship for hyperthermia given alone and in combination with radiotherapy. They have looked, among other things, at changes in the pH of human tumors following hyperthermia and radiation therapy. The pH tends to rise following local application of hyperthermia, probably due to changes in tissue oxygenation and blood flow (Wike-Hooley, et al., 1984). They have also looked at cerebral temperature and epidural pressure during whole body hyperthermia in dogs (van Rhoon and van der Zee, 1983).

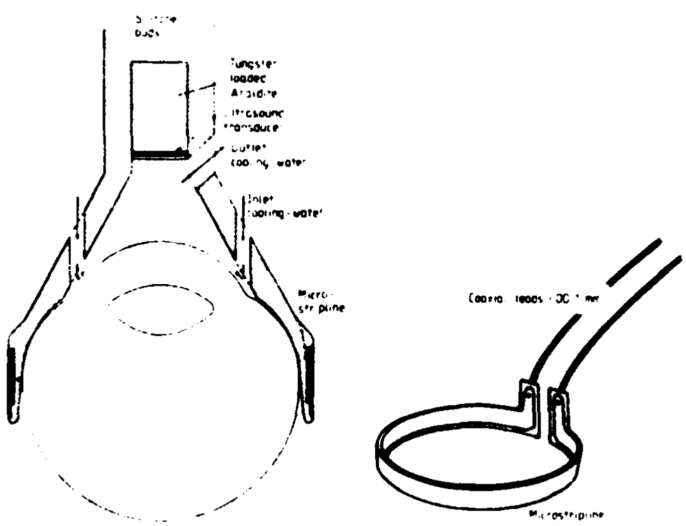
In addition to the research at Erasmus University, there is both collaborative and independent research at the Rotterdam Radio-Therapy Institute, at the University of Delft, at the

Radio-Therapy Institute in Amsterdam, and at the University Hospital in Utrecht. At the last-named institute, Dr. Jan Lagendijk, a physicist, has spent several years studying and developing treatment strategies to improve the therapeutic efficacy of radiation therapy through the use of microwave hyperthermia. Much of his work has been centered on methods to deliver 2450-MHz microwaves to the eye for the treatment of retinoblastomas and to other types of subcutaneous tumors.

Retinoblastoma is a malignant tumor that arises from the nuclear layers of the retina and which predominantly affects young children. The growth originates from single or multiple foci in one or both eyes. Retinoblastoma is the most common intraocular tumor in childhood. It is an extremely interesting tumor in several respects. It may occur either sporadically or may be inherited. Patients with the hereditary type of retinoblastoma may have a particular susceptibility to develop other malignant tumors. Retinoblastoma is sometimes found to be associated with a partial long arm deletion of chromosome 13. Furthermore, it is one of the few tumors known to undergo spontaneous regression.

Retinoblastoma is sensitive to relatively moderate doses of radiation, doses which the normal retina can apparently withstand. The lens of the eye, however, is much more sensitive to damage by irradiation and, in spite of attempts at shielding or precise direction of the irradiation beam, radiation cataract is often the price of cure of the tumor (such a cataract can be removed if necessary).

The retinoblastoma presented a special challenge to the researchers in Utrecht when they attempted to combine microwave hyperthermia with radiotherapy. They designed a special micro-stripline applicator to deliver the microwaves to the eye without exposing other parts of the head, particularly the brain. A schematic representation of Lagendijk's applicator is shown in Figure 8a. The microwave part of the device consists of a low impedance stripline (Figure 8b), which encircles the eye at a level just posterior to the lens. The line is 0.25-mm thick and 4.5-mm wide. The applicator body and the dielectric of the integrated stripline were manufactured from a flexible silicone rubber. The conductors of the stripline were made of thin (25 μm) copper foil or



a. b.

Figure 8. (a) Cross section of Lagendijk's micro-stripline applicator for producing hyperthermia in the eye; (b) the microwave part of the appliator.

silver painted stainless steel gauze (75-μm thick). The advantage of stainless steel is mechanical strength and flexibility, but it has the disadvantage that it has a higher loss in the line compared to copper. Twenty percent of the microwave power that enters the stripline is absorbed in the eye, and 80 percent leaves the stripline and is absorbed in the load. Lagendijk says that this guarantees a nearly uniform power absorption in the eye (Lagendijk, 1982a).

One of the most important aspects of microwave hyperthermia is the measurement and control of temperature. Lagendijk, realizing that it is not practical to invade the eye to place temperature measuring devices, studied the distribution of temperature using mathematical models and devised computer control based on the finite difference technique. Thus the temperature distributions inside the eye during the hyperthermic treatment can be calculated, using the measured boundary temperatures and the measured absorbed power intensity.

Lagendijk's research with the micro-stripline applicator has led him to develop other clinical applicators for microwave heating of tumors, particularly breast tumors (Lagendijk, 1983). Much of his research time in recent years has gone to the study of temperature distributions in tissue, especially considering blood flow. He has a number of papers on this subject, some of which are unique in the BEM literature (see, for instance, Lagendijk, 1982b; Lagendijk et al., 1984). Lagendijk has collaborated extensively with several people during the last few years in building up a rather impressive program there in Utrecht. Most notable among his associations has been the Stuchly team at the University of Ottawa. Lagendijk has made a great contribution to the field of BEM, and especially in the area of medical applications.

The only other BEM activity that I found worth pursuing in The Netherlands was related to possible exposure levels due to radio-frequency (RF) generating equipment.

The Laboratory of Electronic Developments for the Armed Forces in The Netherlands recently conducted a survey of RF generating equipment in the country. The survey was commissioned by the Ministry of Health and Environmental Hygiene and included most of the civil RF sources in the frequency band from 0.5 MHz to 18 GHz. In addition to doing an inventory of the range of transmitters, the project had as its objective the development of a computer model that will be capable of predicting the RF environment from a knowledge of certain of the parameters of the transmitter.

This project was carried out by a military institute for a civilian agency because no agency within the civilian part of the government had the expertise needed for such a survey. It was interesting that no consideration was given to military sources of RF energy. There is no legal standard in The Netherlands for human exposure to electromagnetic energy, although the American National Standards Institute standard is highly regarded and generally followed by anyone wishing to use some sort of guideline. The military, however, seems a bit isolated from such considerations, and apparently no guidelines are actually followed by military operators--except for keeping exposures as low as possible. This probably works quite well given the size and complexity of the military force in The Netherlands.

The project team did not set out to survey every installation that had RF-generating equipment as this would have been impossible in the time allotted. Instead, a representative sampling was made, and, using literature sources about industries, the team was able to extrapolate data for the whole of The Netherlands. Several classes of generators were evaluated in the laboratory as well as being measured on location and in actual use. Three types of medical diathermy equipment were studied: shortwave (27.12 MHz), UHF (433.92 MHz), and microwave (2450 MHz). RF heaters and sealers--such as those used for sealing plastic materials, gluing wood, and drying potato chips--were evaluated at inplant locations.

A computer program was developed under the project to roughly predict the RF environment around different types of emitters using the transmitter parameters. This is the first time, to my knowledge, that an attempt has been made to develop a computer program that can predict pulse flux density around an RF radiator used in industrial and medical environments. Surveys have been made on board ships and around commercial broadcasting antennas by various groups in the US and other countries. An approach like that used by the Dutch group might have application to the problem of predicting levels of exposure to Navy personnel on the decks of ships, particularly in the multifrequency environment where it is often difficult to integrate the pulse flux density due to transmitters operating at different frequencies. For more information on this study, see *ESN* 39-3:81 (1985).

The UK

There is a fair amount of BEM research in the UK, most of it in England. One very active group is, however, operating in Scotland. Located outside Edinburgh, the Institute of Bioelectrical Research, Ltd. (IBR) is one of the first research organizations to be formed exclusively for studying biomedical applications of electromagnetic fields. IBR was conceived and is headed by Dr. Richard H.C. Bentall, whose work in wound healing and cellular effects is known worldwide.

Essentially all the work at IBR is centered around a simple RF current-inducing device developed by Bentall. The induction treatment coil (ITC) is manufactured with the trade name PORTIC. As shown in Figure 9, the ITC consists of a loop antenna, RF signal generator, and associated electronics sealed in a silicon housing. A miniature battery provides power for about 4 weeks of continuous use. The frequency is approximately 27 MHz and is square wave pulsed at 950 pulses per second. The device produces a low intensity RF field of approximately 3 $\mu W/cm^2$. The configuration of the ITC allows it to be placed either on the surface of the body or around an animal or sample container, as shown in Figure 10.

The major clinical study at IBR involves a pool of 27 hospitals in six cities throughout the UK. Bentall is examining how the induced currents produced by the ITC affect the healing of recalcitrant decubitus ulcers (bedsores) and leg ulcers (of varicose veins). Bentall has also conducted several pilot studies aimed at establishing techniques and protocols for additional full-scale clinical studies. The first of these was on the use of induced electrical currents for the treatment of nonunion fractures in bone in humans and fresh fractures in rabbits. Both gave good

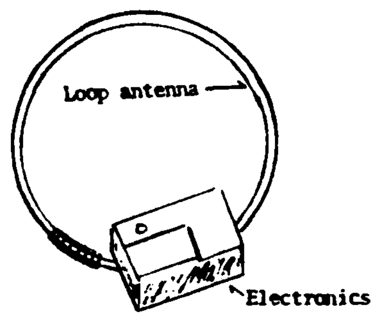


Figure 9. Bentall's ITC for inducing current in tissue and biological samples.

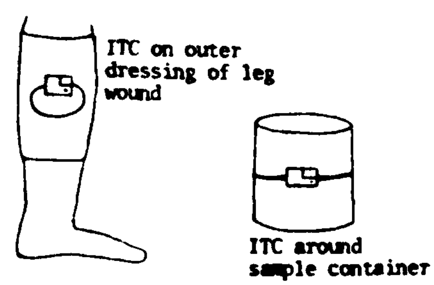


Figure 10. Examples of Bentall's ITC in use in human and laboratory studies.

evidence of accelerated healing, and soon the studies will be expanded.

Another pilot study was carried out to determine whether the locally induced currents would have any effect on reduction of post-operative bruises and edema. This study, limited as it was to only a few patients, has indicated that adverse local reactions are reduced. In other studies, Bentall has also shown that there is a 100-percent increase in the rate of repair in the water barrier of injured skin after epidermal injury. In these pilot studies, a commonly used skin-wound model was used: epidermal skin stripping was performed on the forearms of human volunteers. Bentall even has inflicted wounds on each of his legs for epidermal wound healing studies. This type of research is common in certain parts of Europe and Great Britain because the restrictions for the use of human volunteers are much less severe than in the US and Canada.

The major laboratory study at IBR is one involving wound healing. Using the developing chick embryo as a model, the researchers are using the shell-less culture technique and have developed a standardized wound made by controlled burning of the embryonic membrane. A number of parameters related to the wound healing process are being examined as a function of exposure to the RF fields. Professor E.H. Grant in London has been collaborating with Bentall in measuring the dielectric properties of the healing wound tissue. Grant has found that the conductivity and permittivity of the tissue changes as it heals.

Some of the most fundamental research in BEM is concerned with the electrical properties of materials. Professor Grant, who until 1984 was at Queen Elizabeth College in London but is now at Kings College following a merger of the two institutions, has done more work in this area than anyone else. His former student and now colleague, Dr. Rodney Sheppard, has also worked for years in dielectrics. Between them, they have characterized biological tissue at frequencies up to about 70 GHz and at a wide range of temperatures. They have studied the dispersion of water in bound and free states, and have elucidated the absorption characteristics of the different types of water in biological tissue. They continue to excel in this area and between them have several graduate students and post-doctorates working on various aspects of dielectrics.

Dr. D.M. Taylor and coworkers at the University College of North Wales has been involved in electrical properties of thin biological films grown by the Langmuir-Blodgett (LB) technique. They have studied the capacitance and conductance of thin films of dipalmitoyl phosphatidyl ethanolamine grown by the LB technique. Measurements have been made over a wide frequency range, from 10^{-3} to 10^{5} Hz. The films show a low frequency dispersion which is field-dependent. The dispersion was believed to be due to the presence of water in the films because its magnitude was sensitive to ambient conditions. When the films were put in a vacuum and stressed for a long time with an electrical field, the value of the conductivity stabilized and became dependent upon applied voltage and very weakly on temperature.

Dr. Ron Pethig, also of the University College of North Wales, has developed a computer simulation technique which predicts specific absorption rate distribution within the human body resulting from the applicaton of RF electromagnetic energy. The method uses an extension to the principle of over-relaxation of electric potentials and the basis of the simulation is a realistic three-dimensional model derived from both dielectric and anatomical data. Two of the principal means of applying radio frequency hyperthermia, namely the use of capacitive electrodes and inductive coils, have been provided for. The accuracy of the simulation has been favourably tested using an agar split-phantom and an infrared thermograph camera. The simulations can be used to assist the design and clinical use of RF applicators, and Pethig has applied the simulation to both an inductive coil and to switched capacitive electrodes to heat the thorax.

Also very active in dielectrics, Pethig and his students have studied the electrical properties of a wide range of materials, including proteins (biopolymers, lysozymes, etc.); DNA; and other types of membrane structures. His is a very strong program and well balanced in its approach. He is continually looking at ways the dielectric properties of these materials can be influenced by such things as water content, sodium chloride content, electron acceptors, etc.

The capabilities of the departments at the University College of North Wales and King's College London provide a unique strength to the overall BEM program in the UK, for these labs are constantly called upon to assist others in obtaining information about the dielectric properties of biological material under investigation.

On the more practical side should be mentioned the work of Dr. John Kemshead of the Institute of Child Health in London; he is treating a rare and often fatal type of cancer in children, neuroblastoma. Kemshead has developed "magnetic antibodies" that he uses in a unique way to fight the disease.

A neuroblastoma is a solid tumor of primitive nerve cells that forms on the outer surface of the brain. The disease almost always strikes children (only one case has ever been recorded in a person over 20 years old). The tumor soon spreads to other parts of the body, especially to the bone marrow. Normal therapy for this type of cancer involves the administration of low doses of potent antitumor drugs coupled with radiation and surgical removal of the primary tumor. This is then followed by a second phase of treatment with higher doses of drugs.

The drugs and chemicals used to kill the tumor cells are also toxic to normal cells and especially to bone marrow cells (BMCs). Killing BMCs, the basis for the body's immune system, leaves the individual highly susceptible to infection. One way around the dilemma is to remove the bone marrow before the high dose treatment. Once the tumor cells are killed, the bone marrow can be returned to the patient.

In order for this technique to be effective, all tumor cells must be removed from the bone marrow before the normal BMCs are returned to the patient--otherwise the tumor cells will continue growing and spreading. Here is where the new technique comes into play.

First, Kemshead prepares monoclonal antibodies against neuroblastoma cells. When mixed with bone marrow cells, the antibodies attach themselves to any tumor cells that are present. The next stage uses 2-μ polystyrene beads developed by Dr. John Ugelstad of Trondheim University in Norway. The core of each bead contains a small amount of magnetite. These small spherical beads are coated with a second antibody that is specially made to recognize the first. These microspheres, with magnetite and antibodies, are then mixed with the BMCs that already contain the original monoclonal antibodies now attached to the tumor cells. The first antibody and the second antibody (or anti-antibody) now merge, holding the tumor cells to the magnetic microspheres. In other words, the tumor cells adhere to the first monoclonal antibody, the first antibody to the second anti-antibody, and the second antibody to the bead.

The entire complex--normal BMCs tumor cells, antibodies, anti-antibodies, and magnetic microspheres--are now passed through a glass column surrounded by electromagnets that hold the tumor cells and magnetic beads against the sides while the normal marrow cells pass through. Several passes are made through the column to finally obtain a pure collection of normal BMCs that is then returned to the patient.

Kemshead is conducting clinical trials at the Hospital for Sick Children in London and currently has a number of children under treatment. If this method proves successful for neuroblastoma, it will surely be adapted for other types of cancer.

While this is not BEM in the truest sense of the word (i.e., it does not involve transmitted EM fields), it does use the properties of a static magnetic field to effect a therapeutic regime. Thus it must be considered somewhat within the purview of BEM.

6 CONCLUDING REMARKS

The BEM research in Europe is varied and extensive. I have not attempted to cover all of it since I put some of my effort on other areas as well. Some of the countries tend to be more interested in fundamental research, while others give increased emphasis to medical and biomedical applications. The latter uses are often out in front of those in the US, as it is much easier in Europe to obtain governmental permission to experiment on humans with EM-field generating equipment. Thus many innovative clinical techniques involving microwave and RF equipment are already in use in many parts of Europe.

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APPENDIX:
PUBLICATIONS, AUGUST 1983 TO AUGUST 1985

ESN ARTICLES

1983

37-10/11:395	*Scottish Institute Studies Biomedical Applications of Electromagnetic Fields*
37-12:439	Scottish Development Agency Boosts Biotechnology

1984

38-1:3	Cancer Therapy With Magnetism
38-1:5	The International Center for Genetic Engineering and Biotechnology
38-1:6	A Biotechnological Route to Polyphenylene
38-2:66	France Backs Biotechnology Research
38-2:67	New Hepatitis Vaccine
38-3:118	A New Method of Assessing Biocompatibility of Materials
38-3:119	Bioelectromagnetics at Millimeter-Wave Frequencies
38-4:170	Biointeractions '84 Conference Examines Interaction of Tissues and Materials
38-4:175	Phospholipid Polymers Form Basis for New Biocompatible Material
38-5:240	German Research Center Leading Cell-Electrofusion Research
38-5:243	The Biozentrum at the University of Basel
38-6:297	Extremely Low Frequency Magnetic Fields Affect Chick Embryos
38-7:356	Biomedical Research in Sweden
38-7:360	Frequency of Magneto-Therapy Increasing in Italy
38-8:420	French Use Fluorescence Polarization to Measure Microwave-Induced Hyperthermic Effects
38-9:468	European-Community Action Program for Biotechnology
38-9:470	New Reports Assess BEM Research in France and Germany
38-9:471	The Ciba Foundation--Promoting International Cooperation in Medical and Chemical Research
38-10:526	Cardiostim 84 Meets in Monaco
38-10:530	West Germany Publishes EMF Exposure Standard
38-11:567	Eliminating Radioisotopes in Immunoassays
38-11:569	German Groups Conduct Broad Range of Research on Post-trauma Treatment
38-12:606	Electromagnetic Waves and Neurobehavioral Function: An International Workshop

1985

39-1:4	Medical and Biomedical Research and Training in Romania
39-1:6	New Theory of EM Interaction Developed by German Researcher
39-2:40	Changes in Cerebrospinal Fluid Proteins
39-2:42	Third International Conference on Water and Ions in Biological Systems
39-3:79	Dutch Military Lab Surveys RF-Generating Equipment
39-3:81	UK Establishes National Collection of Animal Cell Cultures
39-3:82	UK Lab Provides Defenses Against Chemical and Biological Warfare
39-4:139	Medical Oceanography in France
39-4:141	Wireless Medical Telemetry in Europe
39-5:187	Hydrogels Form New Basis for Drug Delivery in Systems Being Developed by Scottish Firm
39-6:244	Biomechanics and Biomaterials Training and Research in Marseille
39-6:247	Bone Replacement and Drug Delivery at the Free University of Amsterdam

39-7:307	Biological Ultrastructure Research at ETH-Zurich
39-7:310	Electromagnetic Compatibility Conference Features Biological Interactions
39-8:359	International Low-Temperature Biological Microscopy and Analysis Meeting
39-8:362	AGARD Lecture Series on the Impact of Proposed Radio Frequency Radiation Standards on Military Operations
39-9:414	Workshop on Destruction of Bacterial Spores Held in Brussels

NEWS AND NOTES

1984

38-4:223	Yet Another New Journal
38-5:285	International Symposium on Hyperthermic Oncology
38-5:285	Eighth International Biophysics Congress
38-5:285	Scotland to Host Meeting on Medical and Biological Engineering
38-6:342	French Companies Pursue Biotechnology Research
38-6:342	Third World Congress for Microcirculation
38-6:343	Biomechanics and Biomaterials Conference
38-7:407	Cod-Liver Oil is Good for You
38-7:407	Sweden Builds Saturation Diving System
38-7:408	Stage Set for European Standards for Biomaterials
38-7:408	British Association for the Advancement of Science
38-8:451	*New Scientist* Says, "Britain Missed Biotechnology Boat"
38-8:457	Biotechnology in Ireland Highlighted at Biotech '84
38-8:457	International School of Biophysics Invites Applications
38-11:589	Long-Distance Diagnostic System
38-12:629	Commission of European Communities to Fight Diseases With Computers

1985

39-1:29	Conference on Electric and Magnetic Fields in Medicine and Biology
39-1:30	Symposium on Electromagnetic Compatibility
39-2:67	New Report on EM Energy and the Nervous System
39-2:67	Third International Meeting on Low-Temperature Biological Microscopy and Analysis
39-2:68	Immunocytochemistry Meeting Set for July
39-4:176	Colloquium on Medical Oceanography
39-4:176	Conference on Biothermodynamics
39-8:398	Eighth Colloquium on Microwave Communication
39-8:399	URSI International Symposium on Electromagnetic Theory
39-9:447	Review of Radio Science Published
39-9:447	Eighth International Wroclaw Symposium on Electromagnetic Compatibility
39-10:486	VDTs No Risk, Says Swedish Medical Study Group
39-10:487	Meat-Spoiling Bacteria Identified

REPORTS

1984

R-8-84	Bioelectromagnetics Research in France--An Assessment
R-9-84	Bioelectromagnetics Research in West Germany: An Assessment
C-6-84	Electromagnetic Waves and Neurobehavioral Function: An International Workshop

1985

C-3-85	Electromagnetic Compatibility Conference Features Biological Interactions
C-6-85	AGARD Lecture Series on the Impact of Proposed Radio Frequency Radiation Standards on Military Operations
R-3-85	Biomaterials Research in West Germany--An Assessment
R-4-85	Biological Sciences and Bioelectromagnetics in Europe: Summary Report

SCIENCE NEWSBRIEFS

1983

1-1-83	Bacteria Produce a Unique Thermoplastic-Polyhydroxy-Butyrate (PHB)

1984

2-1-84	Phospholipid Polymers Form Basis for New Biocompatible Material
2-4-84	Europe's Largest Cell Bank Slated for UK
2-14-84	A Call for Papers on Electric and Magnetic Fields in Medicine and Biology

1985

3-1-85	Ion-Interaction Workshop To Be Held in Athens
3-3-85	Bioelectromagnetics Mini-Symposium To Be Held in Bologna
3-6-85	Low-Temperature Microscopy Meeting Set for April in UK
3-11-85	Scottish Firm Set Up To Develop New Controlled-Release Systems Based on Hydrogels

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