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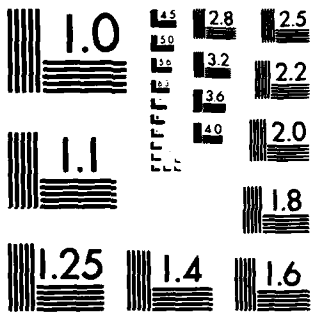
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ONR LONDON REPORT

R-8-84

(12)

OFFICE OF NAVAL RESEARCH

BRANCH OFFICE LONDON ENGLAND

BIOELECTROMAGNETICS RESEARCH IN FRANCE--AN ASSESSMENT

THOMAS C. ROZZELL

29 June 1984

UNITED STATES OF AMERICA

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER R-8-84	2. GOVT ACCESSION NO. AD-A144 305	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Bioelectromagnetics Research in France--An Assessment		5. TYPE OF REPORT & PERIOD COVERED Technical
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Thomas C. Rozzell		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Office of Naval Research Branch Office London Box 39 FPO NY 09510		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE 29 June 1984
		13. NUMBER OF PAGES 10
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Biomedicine
Bioelectromagnetics
France
Non-ionizing Radiation
Electromagnetic fields

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Over the past decade, France has played a major research role in bioelectromagnetics (BEM) (studies of the interaction of electromagnetic fields with biological systems). 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 area of the field. This report examines recently completed BEM research and discusses some work done over the past 3 to 5 years.

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ACKNOWLEDGEMENTS

I wish to thank Professor E.H. Grant for his help in supplying some of the literature and information which was left in my files in Arlington, VA. I am also grateful to my many colleagues in France who so patiently discussed their work with me in my native language rather than their own, never an easy task.

BIOELECTROMAGNETICS RESEARCH IN FRANCE--AN ASSESSMENT

1 INTRODUCTION

Over the past decade, France has played a major research role in bioelectromagnetics (BEM) (studies of the interaction of electromagnetic fields with biological systems). 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 area of the field. A wide variety of cellular and membrane work was carried out in Paris at Institute 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 2 years ago, the program at DRET was managed by Colonel G. Plurien, a military physician who fought to build the pro- gram 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 will cease by the end of this fiscal year.

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 doesn't 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 use of electromagnetic energy, on the other hand, is 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 have 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.

Except for Victor Fellus in Paris, 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.

2 RECENTLY COMPLETED RESEARCH

Dardalhon, Averbeck, Moré, and Berteaud (Paris)

These researchers have just completed their last project at the Institut Curie, where Dietrich Averbeck is chief of the cellular biology section; Michele Dardalhon works under him in that group. C. Moré and A.J. Berteaud are at the CNRS laboratory in Thiais, just on the outskirts of Paris. They provided the engineering and measurements aspects of the project, as they did with many others over the years.

This study, discussed in much more detail in *ESN* 38-8:420-422 (1984), was aimed at elucidating cyctoplasmic events in- side 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 using the method of Cercek and Cercek (1972). The method is based on polarized light excitation of fluorescein molecules following their production in the cytoplasm by enzymatic hydrolysis of nonfluorescent fluorescein diacetate (FDA). 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 (1982).

DAMB compared the results obtained by the fluorescence polarization technique with those obtained on cell viability measured by the method of trypan blue exclusion and those obtained on cell survival, i.e., on the colony-forming ability of the cells.

Using microwaves at 2.45 GHz at power densities from 125 to 200 mW/cm^2 and 30-minute treatment times, DAMB were able to show that at temperatures below 40°C there were no significant changes in the degree of polarization of the emitted fluorescence. However, a slight stimulation was seen in the enzymatic hydrolysis of FDA, which increased at higher power densities.

At temperatures below 40°C the response of samples treated by the water bath was similar to that of those treated by microwaves, except that the slight stimulation of enzymatic hydrolysis of FDA appeared to be absent. At temperatures above 40°C, the response of the samples treated by the water bath was less than that of those treated by the microwave energy. This may have happened because the amounts of thermal energy absorbed by the two techniques are somewhat different, even though the measured temperatures are the same. The water-bath samples lost no heat to the environment, whereas the microwave-treated ones were able to diffuse part of their energy. This is a characteristic problem in such an experiment. As yet there is no way to exactly mimic, and thus accurately compare, heat input due to electromagnetic energy.

DAMB measured the capacity of the cells to exclude trypan blue in an effort to determine if the observed changes were related to cell viability (i.e., the colony-forming ability of the cells). They found a close relationship between the decrease in enzymatic hydrolysis and the capacity of the cells to exclude trypan blue. This finding would seem to indicate that changes occur in the cell membrane and are accompanied by changes in FDA hydrolysis. The membrane permeability changes were examined by measuring the fraction of fluorescein that left the cytoplasm and went into the solution. Permeation of fluorescein was shown to increase when cells received an absorbed energy of about 100 J/g, corresponding to a temperature of 48°C. This increase occurred sooner after microwave treatments than after treatments with the water bath.

Cell survival was slightly lower in cultures exposed to microwaves than in those that were given hyperthermia with the water bath. The changes in cell survival were seen at slightly shorter treatment times and at slightly lower final temperatures than the changes in the degree of polarization and in enzymatic hydrolysis.

These studies by DAMB suggest 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.

<u>Quinquenet, Son, Ollivon, and Berteaud (Paris)</u>

A study to determine nonthermal effects of microwaves on enzymatic activity has just been completed at 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.

In the study just completed the variation of activity of B-D galactosidase (BGAL) was studied on the orthonitrophenyl B-D galactoside-orthonitrophenol (ONP) reaction. First, the researchers determined the enzyme, substrate, and sodium concentrations necessary to prevent deviations from the Beer and Lambert's law and Michaelis-Menten kinetic conditions. Then, enzymatic activation energy was determined; no deviation from Arrhenius law in the 25°C to 37°C range was expected. Molecular absorption of the ONP was measured as a function of the temperature in similar conditions in the presence of the thermally denatured enzyme. A quadratic law was calculated from these data to correct optical-density variations due to temperature changes.

A crossed-beam apparatus for simultaneous spectrophotometric observation and microwave exposure was specially designed and fully automated. It measures simultaneously the concentration and the temperature of ONP produced and also monitors the microwave irradiation. The electric field was amplified at the cuvette level by a rectangular resonant cavity (TE_{012} mode). With the 0.05M, pH 7.3 Tris/HCl buffer, and 0.1M sodium chloride concentrations, the quality factor of the cavity remained about 200. The temperature was measured in the cuvette by a nonperturbing fluorescence thermometer. For each experiment, enzyme activity was measured on the same mixture in order to compare enzymatic activity with and without irradiation, strictly under the same conditions. Optical densities and temperatures were recorded continuously before, during, and after the irradiation period by a microcomputer. The total time of the experiment was about 2 minutes.

At a 7-W power level the temperature increase was only a few degrees and quite below enzyme denaturation temperatures. Enzymatic activities were calculated using quadratic regression analysis from the optical densities recorded before and after microwave irradiation. They were compared with computed activities under irradiation, especially at the exact point of the irradiation exposure. No important or significant variation of the enzymatic activities has been noticed. The experimental optical densities are compared with optical densities corrected for the variations due to temperature increases. These measurements were extended to various frequencies of pulsed microwaves in order to determine possible interactions with molecular activity of the BGAL.

Mamouni, Leroy, Van de Velde and Bellarbi (Lille)

These workers (MLVB), who have been the most active French team working in microwave hyperthermia, recently 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.

The principle of CMWT requires two probes, P_1 and P_2, which are put flush on a lossy material. These are then connected to a "correlator" (Figure 1). Thermal noise is emitted by each subvolume (ΔV) of the material. Since ΔV consists of a cluster of molecules, it can be considered as an isotropic source of radiation. The noise power that can be collected by one probe, for a reduced bandwidth Δf near a frequency f, is equal to:

$$kT\Delta f \ , \qquad (1)$$

according to the Rayleigh-Jeans approximation of the Planck equation, and to a coupling parameter

$$C = \sigma E^2 \Delta V \ , \qquad (2)$$

where E is the root mean square value of the electrical field created in a volume ΔV in the active process--that is, when the probe is transmitting a signal at the frequency f.

In CMWT it is assumed that some subvolumes (such as ΔV_i) are coupled to both probes P_1 and P_2. In assessing the contribution that each such subvolume makes to the output signal S, one must take into account their position with respect to the probes, the delay times of the radiations between ΔV_i and the two probes, and the delay time introduced in the correlator by the delay line Δl. Consider three subvolumes ΔV_1, ΔV_2, and ΔV_i located in the plane of symmetry of the system, with ΔV_1 coupled to P_1, ΔV_2 to P_2, and ΔV_i both to P_1 and P_2. Then let T_1, T_2, and T_i equal the temperatures of the three subvolumes; C_1, C_2, C_{i_1}, C_{i_2} equal the coupling parameters--as in Equation (2) above--between ΔV_1 and P_1, ΔV_2 and P_2, ΔV_i and P_1, ΔV_i and P_2; ϕ equals the shift in phase corresponding to the difference of the delays between ΔV_i and P_1, ΔV_i and P_2 at the frequency f, or

$$\phi = \frac{2\pi \Delta l f}{C} \ ,$$

which is the phase shift introduced by Δl.

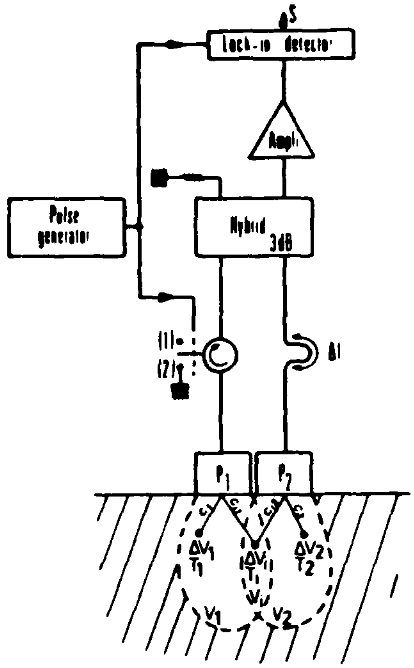

Figure 1. A system for correlation microwave thermography.

By changing the switch from position 1 to position 2, one can determine the contribution to the output signal by each subvolume. The signals radiated by each subvolume are uncorrelated, so their respective contributions to the output signal are simply added together.

The pulse generator provides a signal with a 50-percent duty cycle. Thus both the peak-to-peak amplitude of the signal applied to the lock-in amplifier and the output signal are proportional to the summation of the quantities

$$4\sqrt{C_{i_1} \quad C_{i_2}} \quad \cos(\phi_i+\phi)T_i \,, \qquad (3)$$

corresponding to the subvolumes that constitute the lossy material covered by both the probes. It is considered that the lossy material contributes to the output signal only via the thermal emission of the volume of material coupled to the probes. Here is one major difference between CMWT and MWT; in CMWT, subvolumes coupled to only one probe make no contribution to the output signal.

Another important characteristic of CMWT is that it is not sensitive to a uniform temperature in the material under test, but rather to a temperature gradient in the material covered by both probes.

MLVB have constructed a correlator 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.

3 RESEARCH DURING THE PAST 3 TO 5 YEARS

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 (<5mW/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 is moving to a new post at a naval training facility in Bordeaux. He has offered to make the tapes containing his database available to the Office of Naval Research (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 sulphate 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.

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 antennae 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 9.4, 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 intergenic 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 differencein 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.

4 CONCLUSION

The major governmental supporters of BEM research in France have decided to redirect funds for this research into new, emerging technologies. In so doing they must rely on the research results of other countries to support any new standards or develop biomedical applications. The BEM researchers, who have been very active over the past decade, are now beginning to channel their expertise in other areas of science and engineering. Unless support is found immediately in industry or in other countries, France will no longer be an actor on the BEM stage, merely a spectator in the audience.

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6 ADDITIONAL READING

Listed below is a sampling of the publications that have been derived from the French research over the past 3 to 5 years. The list is included to show the scope of the research, its distribution, and the principal participants. In searching the literature it was interesting to note that with all of the success of the *Bioelectromagnetics Journal*, not one French paper has been published in it. Whether any were submitted is not known.

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