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# EMF Effects on Cellular Calcium

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## Introduction

This breakout group evaluated the data addressing EMF effects on cytosolic free calcium and other ions as well as the effect of EMF on calcium signaling pathways. A literature search of papers published from 1990 to 1996 showed approximately 25 manuscripts directly related to the topic (see Table 1 and References; note these searches are inevitably incomplete). The breakout group discussed several of the manuscripts including some that report that EMF can alter intracellular $Ca^{2+}$ regulation and some that reported no effect.

We evaluated the methods used to measure calcium, the time course of any response, and if available, the dose response. Dr. Kenneth McLeod suggested that the we should also evaluate the induced electrical field. Taken together, the available data indicate that in some cell types under certain conditions, EMF may alter cytosolic free calcium; however, EMF effects on cytosolic free calcium do not appear to be a universal response. The effects of EMF are highly specific regarding both cell type, requirements for priming (e.g., ConA), and the amplitude and frequency of the applied fields.

## Questions Discussed by the Breakout Group

1. Does EMF alter cytosolic free calcium within cells?

The group concluded that there was evidence that EMF altered calcium in some cell types under defined conditions (e.g., Liburdy et al., 1993; Lindström et al., 1993), but that this was not a universal response (see Garcia-Sancho et al., 1994). The majority of studies and virtually all studies which report an effect of EMF, are performed on immune cells. In studies in which EMF effects have been described, some indicate that activation by mitogens is required (e.g., Liburdy et al., 1993) whereas others indicate that this is not necessary (e.g., Lindström et al., 1993). Alterations have been reported to occur in transformed cells, non-transformed cells that have been primed, and in non-primed non-transformed cells. In addition, different groups used different measures of calcium (e.g., fluorescent indicators vs. 45Ca) making it difficult to compare results. For example, most of the studies employing 45Ca measured changes over 30 to 60 minutes whereas studies with fluorescent indicators measured more rapid changes over the time course of 1-2 minutes. The exposure

conditions were variable. Also, in some studies cells were in suspension, and in others, cells were attached.

In discussing the literature, it was noted that EMF has been reported to both stimulate and inhibit 45Ca uptake depending on the conditions. Yost and Liburdy (1992) reported that EMF inhibits 45Ca efflux, which is induced by addition of 1-3 µg/ml of ConA, whereas Liburdy et al. (1993) reported that EMF stimulates 45Ca efflux induced by the addition of 1 µg/ml of ConA, which in this study is a suboptimal dose. As discussed by Walleczek and Budinger (1992), in some cells ConA stimulates 45Ca uptake while in other cells ConA has no effect. EMF tends to inhibit 45Ca uptake induced by ConA, but in cells in which ConA alone has no effect, addition of EMF + ConA can stimulate 45Ca uptake.

2. How do these exposure patterns relate to long-term effects within cells?

The consensus of the group was that the effects of calcium in cell biology are too complex to draw meaningful conclusions with current knowledge. However, it was noted by some members of the group that the response pattern observed (e.g., EMF altered calcium in activated transformed cell) is similar to the response pattern reported by the cellular replication breakout group. In addition, the variability of the responses also make interpretations difficult. It was pointed out by a member of the group that the question refers to "exposure patterns" and the answer refers to "response patterns."

3. What is the biological significance of "modulation and intensity windows" for EMF effects on calcium?

Although frequency dependence was observed in some cases, the explanation for this observation is unclear. "Modulation and intensity windows" have been reported, although some panel members expressed reservations about whether they are real or just experimental artifacts.

4. To what degree does this indicate strong support for an *in vivo* effect of EMF on processes mediated by cellular calcium?

Because of the complexity of calcium mediated processes and the inconsistencies in the data, there is not strong support for *in vivo* effects at this time.

5. What health effects might be associated with these types of *in vitro* effects?

Without information on how long calcium is elevated and what other signals are also altered, it is difficult to draw conclusions. However, the group noted that alterations in calcium have been associated with an increase in ß-amyloid production as well as an increase in cell proliferation, and therefore, an increase in calcium might have an effect on these processes.

6. Does EMF alter cellular pathways related to calcium's role in signaling?

There are very few studies on this topic, and because of the limited nature of these studies it is difficult to draw conclusions about *in vivo* effects. There is a study

reporting that EMF causes an increase in IP3 (Korzh-Sleptsova et al., 1995). This is an important study because it would suggest that multiple signaling pathways (e.g., calcium and PKC) are activated. There is also a study reporting that calmodulin binding of calcium is not altered by EMF (Lednev et al., 1994). This is an important study because Lednev has suggested that calcium binding to calmodulin might be altered by EMF, and given calmodulin's central role in calcium signaling pathways, this would be an important finding. However, a careful study by Bruckner-Lea et al (1992) found no effect of EMF on calmodulin binding to calcium.

7. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on processes mediated by cellular calcium?

It was suggested that one could investigate whether EMF alters ß-amyloid via changes in calcium. It was also suggested that one could investigate whether EMF has effects on rapid calcium oscillations such as those that occur in the heart or islets of Langerhans. On reviewing this report several members of the group expressed the concern that these particular studies mentioned at the session reflect the particular research interest of some of the breakout group participants.

8. At the EMF exposure levels observed *in vivo*, would cellular effects on calcium and other ions be likely to be observable *in vitro* ?

The answer depends on whether effects are due to electric or magnetic fields and the temporal characteristic of exposure (time-average or peak); these effects have not been sufficiently studied. Typical exposure conditions in the calcium experiments were in the 1G range.

**Additional Issues Discussed by the Breakout Group**

There was a discussion of defining typical "EMF exposures observed *in vivo*." Should one consider time-averaged exposures (typically low mG) or should one consider peak exposures (~1 G) as typical *in vivo* exposures? There was also a discussion of the role of magnetic vs. induced electric fields.

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**Related Studies**

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**Summary and Recommendations**

The available data suggest that EMF might cause an increase in cytosolic calcium in some, but not all cells. However, given the complexity of calcium mediated processes, the biological effect of any such increase is unclear.

Table 1

Summary of Literature Related to EMF Effects on Calcium

(Published in English 1990-96)

| **Name** | **Journal** | **Cell type** | **Does alter $Ca^{2+}$?** |
|---|---|---|---|
| 1. Yost and Liburdy | FEBS Lett. 296:117-22, 1992 | lymphocytes | yes |
| 2. Liburdy | FEBS Lett. 301:53-9, 1992 | lymphocytes | yes |
| 3. Liburdy et al. | FEBS Lett. 334:301-8, 1993 | lymphocytes | yes |
| 4. Lindström et al. | J. Cell. Physiol. 156:395-8, 1993 | Jurka/lymph. | Yes |
| 5. Lindström et al. | Bioelectromagn. 16:41-7, 1995 | Jurkat | yes |
| 6. Korzh-Sleptsova et al. | FEBS Lett. 359:151-4, 1995 | Jurkat | yes |
| 7. Lindström et al. | FEBS Lett. 370:118-22, 1995 | Jurkat | yes |
| 8. Walleczek et al. | FEBS Lett. 314:351-55, 1992 | lymphocytes | yes |
| 9. Walleczek | FASEB J. 6:3177-85, 1992 | immune cells | yes |
| 10. Rosen | BBA 282:149-55, 1996 | GH3 cells (static field) | yes |
| 11. Lyle et al. | Bioelectromagn. 12:145-56, 1991 | immune cells | yes |
| 12. Carson et al. | Am. J. Physiol. 259:C687-92, 1990 | HL-60 | yes |
| 13. Garcia-Sancho et al. | Bioelectromagn. 15:579-88, 1994 | immune/RBC/HL-60 | no |
| 14. Bruckner-Lea et al. | Bioelectromagn. 13:147-62, 1992 | calmodulin | no |
| 15. Hojevik et al. | Bioelectromagn. 16:33-40, 1995 | RINm5F | no |

| | | | |
|---|---|---|---|
| 16. Schwartz and Mealing | Bioelectromagn. 14:521-33, 1993 | frog heart | no |
| 17. Fitzsimmons et al. | Calcif. Tissue Int. 55:376-80, 1994 | bone cells | yes |
| 18. Coulton et al. | Phys. Med. Biol. 38:347-60, 1993 | lymphocytes | no |
| 19. Barbier et al. | Bioelectromagn. 17:303-317, 1996 | rat pituitary cells | yes |
| 20. Galvanovskis et al. | Sci. Total Environ. 180:19-33, 1996 | leukematic-T cells | yes with poly-lys<br><br>no w/o poly-lys |

Related Studies---but these studies do not measure cytosolic calcium

| **Name** | **Journal** | **Cell type** |
|---|---|---|
| 21. Blackman et al. | Bioelectromagn. 12:173-182, 1991 | temperature |
| 22. Karabakhtsian et al. | FEBS Lett. 349:1-6, 1994 | need extracellular Ca |
| 23. Ayrapetyan et al. | Bioelectromagn.15:133-42, 1994 | solutions |
| 24. Eichwald and Kaiser | Biophys. J. 65:2047-58 | model |
| 25. Prasad et al. | Health Phys. 66:305-12, 1994 | diatom/positive effects |
| 26. Parkinson and Sulik | Radiat. Res. 130:319-30, 1992 | diatom/negative |

Top Contents

Appendix 1

Specific Comments on Calcium Metabolism Summarized by

Dr. Kjell H. Mild from his Plenary Lecture,

"EMF Effects on Calcium Metabolism"

Many of the earlier studies of calcium and EMF were looking at calcium efflux from chick brain tissue and "frequency and intensity windows" were observed--that is, the

response of the biological system depended on particular combinations of the dc magnetic flux density and the ac frequency. By changing the ac frequency over a wide range, they observed that the active frequencies for a given dc flux density were integer multiples of a fundamental. This work provided the impetus for the subsequent development of the Cyclotron Resonance (CR) model. CR phenomena has as a basis the interaction force between a charged particle and a magnetic field. The condition for a circular movement is that the velocity or the number of turns, frequency, follows the relation: f=q B/2pm). While the model has been criticized on theoretical grounds, the "harmonic" relation observed in the data seems to be real and persists over quite a spectrum of frequencies.

A complex, but different, interrelation of the independent variables, ac flux density, ac frequency, and dc flux density was identified by the ion parametric resonance (IPR) model. (The only point of commonality between the IPR and CR models is at their fundamental frequencies. The harmonics identified by each model are inverse of each other.) Another model is the Parametric Resonance (PR) Model where interference of the vibrational energy sublevels of ions bound in calcium-binding proteins is the basis for the interaction of weak magnetic fields with the biological systems.

None of these models have full experimental support today, but the data found in the literature show that many of the EMF biological effects seem to fulfill the basic formula for the frequency and static magnetic field. Very often, a non-linear extremely low frequency amplitude response is also seen.

Changes in intracellular calcium concentration have been reported from several laboratories using different cell models. Since calcium is a general messenger molecule, this means that the possibility exists for many diverse responses from the cell system. Our own work has shown that the primary interaction site for the EMF is at the membrane level, and thus, the effect is not primarily on the calcium ion. We have used a fluorescent microscope with the possibility to study single cells and Fura-2 as an indicator for the intracellular free calcium. Using the T cells Jurkat, an acute response was observed with oscillatory increases in calcium concentration, which subsided when the field was turned off (Lindström et al., 1993, 1995 a,b). The response at 0.15 mT was over a frequency range from 5 to 100 Hz with a fairly broad peak having its maximum at 50 Hz. The result of testing with increasing flux densities at 50 Hz was a threshold response with no effect below 0.04 mT and a plateau at 0.15 mT. By using different CD45 mutant clones of Jurkat, we found that cells with a chimeric CD45 responded to the magnetic field (B field) in the same manner as when treated with anti-CD3, but when the cytoplasmic part was missing, no effect of the B field was seen. This showed the necessity for an intact signal transduction pathway for the increase in intracellular calcium to occur.

Our laboratory has also measured the inositol triphosphate (IP3) level in the Jurkat cells after exposure to B field, 50 Hz, 0.10 mT (Korzh-Sleptsova et al., 1995). The exposure resulted in significant increases of IP3 levels, and this peaked after two minutes. When cells were treated with anti-CD3, the peak came first after six minutes. Combining the two shows an additive effect.

The conclusion from our experiments with Jurkat cells is that the ability of EMF to activate Jurkat cells is comparable to anti-CD3 crosslinking antibodies, the target molecules are localized at the cell membrane and the field effect is reversible and has a threshold.

Bioelectromagnetic research has much more to learn from a basic understanding of calcium regulation. For instance, it has been seen that in stimulated cells, calcium concentration rose as predicted, but did so only transiently, and fell back to its base level within a few minutes while the cells continued to respond for hours. Another example is the recently demonstrated causal relationship between (Ca2+) transients that occur during the cell cycle. These things throw light upon the discussion of "what is exposure," as we will see magnetic fields effect the calcium concentration and thus a short term - maybe high peak - exposure might be all that is needed and the long duration might not be needed. More research along the line of different exposure regimes with respect to the timing is needed, and things like biological reset time need to be introduced into this research.

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Top

Contents

## Effect of EMF on Enzymes and Polyamines

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Rapporteurs: Martin Blank, Ph.D. and Charles N. Rafferty, Ph.D.

**Introduction**

Elevated levels of enzyme activity have been consistently correlated with cell transformation and tumorigenesis. Research reported in recent years has made it clear that this enhanced enzyme activity can have a causative role in tumorigenesis [e.g., transformation of mammary epithelial cells, Manni (1997); neoplastic transformation in prostate tissue, Magi-galluzzi (1997); tumorigenesis in mice, Moshier (1993)]. For this reason an evaluation of reports concerning the modification by electric and magnetic fields (EMF) of cellular enzyme activity (both in cell cultures and intact tissues) is important in any EMF hazard identification.

Our major goals were to establish whether or not there was sufficient credible *in vitro* evidence to determine:

1. If electromagnetic fields can alter enzyme activity,
2. If the magnitude of the change in activity is biologically significant,
3. If the fields at which these changes occur have magnitudes of importance in environmental exposures, and
4. If the *in vitr*o changes are consistent with the available *in vivo* data.

The opening question in the discussion suggested that the group address the issue of whether effects on enzyme activity at low field strengths were possible. This issue was discussed to a limited degree at various points in response to some of the questions below. However, the primary focus of our discussion was reviewing the enzymatic evidence for EMF effects. Many participants thought that the evidence for weak 60 Hz magnetic field effects was strong. Those that raised the biophysical plausibility argument felt that the experimental standard has to be higher when there is no biophysical model to explain the effect. Both physicists and biologists share the burden of advancing our understanding in the area; biologists by extending the amount and quality of the experimental data, and physicists by pursuing models of field-cell interaction that are realistic and thus appropriate for enzyme reactions.

## Questions Discussed by the Breakout Group

### *EMF effects on enzymes*

1. To what extent has it been shown that extremely low frequency EMFs can alter cellular enzymes?

A number of studies of electromagnetic field effects on cellular enzymes have been reported, including effects on ornithine decarboxylase (ODC), Na,K-ATPase, lyn kinase, cytochrome C oxidase, ethanolamine ammonia lyase, and horseradish peroxidase. The breakout group focused on ODC, Na,K-ATPase, and enzymes (ethanolamine ammonia lyase, and horseradish peroxidase) that are affected through radical pair mechanisms. There is substantial evidence from several labs that EMF can alter the activity of ODC (23 cell lines or tissues, 7 labs). Both electric and magnetic fields have been studied over a large range of exposures and frequencies [extremely low frequency (ELF) to modulated microwave fields]. At ELF the direct applied electric fields ranged from 0.1 mV/cm to 50 V/cm; the magnetic fields ranged from 2 mG to 1500 mG; the electric fields induced by these magnetic fields ranged from 0.1 V/cm to over 100 V/cm.

Both *in vivo* and *in vitro* studies have been reported. Many cell types have been investigated. Most *in vitro* studies have indicated a two to four-fold enhancement of ODC activity; one study reported a modest suppression, and one study failed to show an effect. Studies of ODC activity in animals are more variable. One explanation for this variability is that the effect of the EMF on ODC activity may be tissue dependent. In one *in vivo* study (El Kanza, 1996), examination of the brain, intestine, spleen, spinal cord, and kidney showed a two to five-fold increase in ODC activity. In another study on a different strain of rats (Mevisson, 1995), mammary and spleen tissue showed a 1.4 to two-fold increase in ODC activity, but the liver, intestine, bone marrow, and ear skin showed no effect. Studies of skin cancer in mice show no enhanced ODC activity in the epidermis after either acute (24 hr) or prolonged (weeks) exposure. No studies on ODC gene induction have shown an EMF induced effect, suggesting the possibility that the changes found above are due to alterations in the degradation rate of ODC.

The preponderance of evidence clearly supports the existence of ELF EM field effects on ODC. Efforts to replicate specific studies using the same cell types and exposure conditions have yet to be reported. It appears that *in vitro* confounders exist. It has been noted that cell sub-line, confluence, and passage number can play a role in determining whether or not there is an *in vitro* response to EMF exposure. (Table 1 a tabular summary of studies and reference list based on one prepared by Dr. Craig Byus.)

Two laboratories have investigated effects of ELF electric or magnetic fields on the Na,K ATPase activity in two different preparations, one cellular and the other consisting of vesicular membrane fragments. Consistent observations of enhanced activity were reported for high (>1 V/cm) electric fields. One laboratory has reported enzyme effects for extremely weak (< 20 mG) magnetic fields.

There is strong evidence (Grissom, 1995; Harkins, 1994; Taraban, 1997) of magnetic field effects on the activity of two enzymes, ethanolamine ammonia lysase and horseradish peroxidase, via radical pair mechanisms at high dc magnetic field levels exceeding 10 G. The specific mechanisms underlying these effects are well established; however, because of the high magnetic fields required, it is uncertain that this radical-pair mechanism is relevant to environmental considerations. Many other enzymes have also been investigated with dc magnetic fields but with no effects reported (See Table 2).

2. Are there dose-response data supporting these effects?

Dose-response data have been reported for three experimental enzyme systems. Sigmoidal magnetic field dose-response patterns have been reported for two ODC systems and one Na,K-ATPase system. ODC data from one *in vivo* study were consistent with a linear magnetic field dose-response. Complex dose-response patterns have been reported for enzymes affected through a radical pair mechanism.

- ODC *in vitro* (murine fibroblasts) effects detected over a 20-100 mG range (at 60 HZ) with a sigmoidal response and an inflection at ~40 mG.
- ODC *in vivo* (ENU treated rats) detected over a 20-2000 mG range (at 60 Hz) with an approximately sigmoidal response (i.e., linear response at low fields

but appearing to saturate at high field strengths) with an estimated inflection point at (i.e., 1/2 way to saturation) ~20 mG.
- Na,K-ATPase (micellar preparation) detected over a 2-500 mG range (at 60 Hz) with a sigmoidal response and an inflection at 4 mG.

Na,K-ATPase (micellar preparation) studied with applied electric field gave a sigmoidal response with inflection at 6 mV/m.

Na,K-ATPase (micellar preparation) studied with combined electric and magnetic field (an induced electric field together with a stray magnetic field). Since under these conditions, the electric field decreases enzyme activity and the magnetic field increases enzyme activity, it is possible to show that the result (a sigmoidal response with inflection at 35 mV/m) is approximately an arithmetic sum of the two effects.

- Ethanolamine ammonia lysase effects detected over a 100-1500 G range with a complex dose-response.
- Horseradish peroxidase effects detected over a 10-1500 G range with a complex dose-response.

It must be emphasized that for the data above where the magnetic fields are time varying (e.g., 60 Hz), there is an associated induced electric field. It is not known whether the field-cell interaction is electric or magnetic. If it is the induced electric field, then care must be taken in extrapolating the threshold results found *in vitro* with anticipated thresholds in humans. Because of geometric considerations, the induced electric fields will be larger in humans for a given 60 Hz, magnetic field and thus predicted threshold magnetic fields will be lower.

In the group discussion, concern was expressed regarding the credibility of low level effects, especially for levels of magnetic fields below 100 mG, for the ODC and Na,K-ATPase systems. The reasons given were

a. the lack of a biophysical plausibility of magnetic field effects that induce electric fields at levels less than the thermal noise level of membranes,
b. the lack of precise replication for any given set of results,
c. the preliminary nature of some of this data that may be reported only in abstract or work in progress form.

On the supportive side is:

a. the internal consistency of the individual data sets and the tightness of the reported error bars,
b. the broad range of systems studied,
c. the general agreement between the reported *in vivo* and *in vitro* results, and
d. the consistency between the reported ODC and polyamine results.

3. In what way is enzymatic activity affected?

Most *in vitro* studies on ODC activity have indicated a two to four-fold enhancement of the observed enzymatic activity; one study reported a modest suppression. Based upon his research, Dr. Craig Byus suggested that the increases in activity are due to

increases in concentration of the enzyme possibly caused by a decrease in the degradation rate. No studies on ODC gene induction have showed an effect.

Studies of ODC activity in animals have, except for those using skin models, indicated an enhancement at least in some tissues when 50 or 60 Hz magnetic fields were applied.

Experiments on Na,K-ATPase show increases of activity of up to two-fold in either electric or magnetic fields. There are some differences in the responses to the two fields, but in both cases, there is a relation between field interaction and basal enzyme activity. The differences in the effects may arise because the two fields penetrate to different extents.

$V_m/K_m$ decreases 25% when ethanolamine ammonia lysase is exposed to 1000 G dc magnetic fields and 60% when exposed to 1500 G dc magnetic. $V_m$ is not sensitive to magnetic field exposure. In this system (as well as in horseradish peroxidase) the observed kinetic rate constants are changed by the magnetic field. One of the rate constants, $k_2$, in the reaction scheme for horseradish peroxidase is increased by about 20% due to exposure to 10 G dc magnetic fields and decreases by about 60% when exposed to 750 G dc magnetic fields.

4. Are there long-term effects of EMF exposure in cells?

There is little or no experimental data to address this question depending on the enzyme system examined. Very limited adaptation studies have been carried out on ODC and Na,K- ATPase in cell systems. However, adaptations to electric and magnetic field exposure in animals have not been investigated. Long-term animal studies in which ODC has been measured demonstrate that ODC enhancement can be sustained. There are no studies on either cell or animal systems in which radical pair mechanisms have been investigated.

5. Do EMFs affect polyamine levels?

The limited evidence available suggests that EMF can affect polyamine levels. Only two relevant studies have been carried out, one *in vitro* and the other *in vivo* (Valtersson et al., 1997 in Jurkat cells; Juutilainen et al., 1996 in mice). In both of these studies, the polyamines, putrescine and spermidine, were found to increase by a factor of ~2 following ELF magnetic field exposure. The initial and rate limiting step in polyamine synthesis is catalyzed by ODC. Belief that ODC activity can be enhanced by EMF is strengthened by these experimental findings. In the *in vivo* study by Juutilainen et al. (1996) polyamine levels were higher after 24 hours of exposure whereas ODC activity was lower than the initial activity. This can be explained by assuming that (as in the *in vitro* studies) acute exposure causes a transient increase in ODC activity (peak in 4-6 hr) followed by rapid degradation even in the presence of the field. The complexity of these temporal relationships emphasizes that one must not only look at ODC levels, which may or may not appear to change depending on the duration of the exposure and the time of measurement of ODC activity, but that the amount of polyamine produced by the transient enhancement of ODC activities should also be determined.

6. Is the magnitude of the observed EMF-induced effects on enzymes and polyamines large enough to be biologically significant?

The magnitude of the observed EMF-induced effects on the enzyme ODC is large enough to be biologically significant. *In* vivo evidence shows that the magnitude of the EMF-induced increase in the enzyme ODC is large enough to increase tumorigenesis. However, these findings do not prove that these EMF-induced alterations in ODC activity actually cause either adverse or beneficial downstream effects. It simply means that EMF induced effects can not be ruled out on the basis of the magnitude of the change in ODC activity. Most of the ODC studies reviewed indicate that EMF can cause an approximately two-fold increase in ODC activity.

This appears to be a small (and possibly insignificant) change in such a highly inducible enzyme such as ODC. However, the work of Moshier et al. (1993) bears directly on this subject. They have studied the transformation of NIH/3T3 cells by ODC over-expression using an expression construct. They demonstrated that when the ODC activity levels were elevated by three - six-fold, the cells were no longer contact inhibited, exhibited anchorage independent growth, and induced tumors in nude mice more efficiently and rapidly (e.g., a five-fold increase in tumors relative to controls). The data indicate that a three-fold increase is not the smallest increase needed for enhanced tumorigenesis.

Two lines of *in vivo* experimental evidence pertain to a possible relationship between EMF effects on polyamines or ODC activity on the order of two-fold and tumorigenesis: the preliminary results of Juutilainen et al. (1996) using a UV potentiated mouse skin tumor model and the published results of Mevissen et al. (1995) using the DMBA rat mammary tumor model. Both groups report positive effects of magnetic field exposure on various measures of tumorigenesis. Juutilainen et al. (1995) reported that the measured effect of the magnetic field is the same as that of being ODC transgenic (i.e., in the presence of UV, both conditions cause an approximate doubling in the average number of tumors per animal). The work of Mevissen further substantiates the significance of a two fold increase in ODC. They found that exposure of this strain of rats to EMF caused a significant increase in the number of mammary tumors despite only a two fold increase in the activity of ODC**.** (Note: the ODC studies were done in a separate experiment on the same strain of rats as in the tumor study).

Having suggested that a small change in EMF induced ODC activity can be significant, one must offer caveats:

a. The homeostatic mechanisms of organisms will tend to minimize the magnitude and duration of such changes in enzyme activity.
b. The experimental conditions and possible biological effects of EMF exposure are quite different from the stable transfection of a cell with an expression construct containing ODC genes, as in Mosier et al. (also true for chemical carcinogens).
c. Care should be taken in inferring a cause-effect relationship between increased ODC activity and tumorigenesis because of the limited nature of these experiments and the weak statistical strength of the tumor findings (McCann et al., 1997). (See Table 1 for details.)

For **Na,K-ATPase** and **radical pair** enzyme effects, no conclusions can be drawn at this time.

7. What health effects might be associated with these types of *in vitro* effects?

There was no agreement on the answer to this question. Most discussion focused on the potential role of ODC in the promotion of cancer. It is recognized that over-expression of ODC is known to enhance tumorigenesis. (Moshier, 1993; Manni, 1997). If we accept that EM fields do enhance ODC activity by a factor of about two to four, then we are faced with two views. The traditional view is that large changes in ODC are needed when caused by the high chemical concentrations of the usual tumor promoters (e.g., TPA ). Another view is based upon the recent literature which indicates that a ~3-fold change in ODC can be sufficient to play a causative role in tumorigenesis (Moshier, 1993).

During the discussion it was emphasized that it is not the magnitude of ODC change which is important but the magnitude of the downstream polyamine changes that are crucial. A further consideration is that it is not the percentage change that is important but the absolute change in concentration; for example, if the basal concentration of ODC is high, then a two-fold increase might be significant with respect to cancer. The participants, in general, felt that based on the extent of the available information, it was premature to draw conclusions about the relationship of EMF effects on ODC to cancer. However, a number of participants felt, after reviewing the *in vivo* and *in vitro* enzyme and polyamine data, that a relationship between EMF-induced ODC and cancer has become more likely, as the data has become more extensive and refined.

8. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on enzymatic activity?

Several studies were proposed:

a. Evaluate frozen sections archived in current and recently completed long-term EMF animal studies. Samples from both promotional animal studies and chronic bioassays should be available.
b. Conduct a new rodent study in which ODC and polyamine levels are measured at 1,2,3,4 and 5 weeks of 60 Hz magnetic field exposure. Following cessation of field exposure, ODC and polyamines should also be assayed over a 0-24 hour period to evaluate reversibility of responses.
c. Repeat study by Mandeville (Kanza et al., 1996) in which ODC was evaluated in multiple tissues at multiple magnetic field levels in a rat brain cancer model in which ethylnitrosourea (ENU) was administered in utero.

9. At environmental EMF exposure levels would cellular effects on enzymes and polyamines be likely to be observable *in vitro* ?

While there was no difficulty obtaining a consensus of the discussion group that this is possible for high occupational and residential exposures, the feeling about lower exposures was divided. The differences reflected the level of comfort one felt with

the results of enzyme studies. Those actively involved in experiments with enzymes felt that examination of the data required a definite yes answer to this question.

Transients were mentioned as a possible, though fairly uncommon (especially in residential environments) source of high field magnetic field levels. However, the ODC coherence experiments suggest that EM field exposure with field constancy less than ~ 10 seconds should not cause any bioeffects.

In ensuing discussion, support of new *in vitro* studies was also suggested. One participant suggested that a cost-benefit analysis be conducted to evaluate the relative merits of animal and cell studies. There was also support for more basic research to find mechanisms to account for observed effects at low magnetic field levels (e.g., less than about 100 mG). This last point is especially important since the credibility of weak field results will depend on improved understanding in this area.

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**Summary and Recommendations**

Electric and magnetic fields can alter enzyme activity in such enzymes. The preponderance of evidence clearly supports the existence of ELF em field effects on ODC both *in vivo* and *in vitro*. The *in vitro* changes in ODC are consistent with the

available *in vivo* data. There is credible data that indicates effects on other enzymes, such as Na, K-ATPase and horseradish peroxidase. It appears that *in vitro* confounders exist. It has been noted that cell sub-line, confluence, and passage number can play a role in determining whether or not there is an *in vitro* response to EMF exposure. The magnitude of the observed EMF-induced effects on the enzyme ODC is large enough to be biologically significant. *In vivo* evidence shows that the magnitude of the EMF-induced increase in the enzyme ODC is large enough to increase tumorigenesis. However, these findings do not prove that these EMF induced alterations in ODC activity actually cause either adverse or beneficial downstream effects.

While there was no difficulty obtaining a consensus of the discussion group that effects on enzymes are possible for high occupational and residential exposures, the feeling about lower exposures was divided. The participants in general felt that based on the extent on the available information, it was premature to draw conclusions about the relationship of EMF effects on ODC to cancer. However, a number of participants felt, after reviewing the *in vivo* and *in vitro* enzyme an polyamine data, that a relationship between EMF-induced ODC and cancer has become more likely, as the data have become more extensive and refined.

Table 1

EMF-Induced Alterations in Ornithine Decarboxylase (ODC) Activity and Polyamines

(prepared by Dr. Craig V. Byus, University of California at Riverside)

| | | **ODC Response** | | **Laboratory (PI)** |
|---|---|---|---|---|
| **Cell/Tissue** | **EMF Exposure** | **E/C** | **Time** | **Reference (*abstract)** |
| Primary rat bone cells (collagenase calvaria) | Pulsed electric field (25 msec, 3 Hz repit.)<br>5 min. exposure<br>12 V/cm<br>22 V/cm<br>55 V/cm | 2<br>1.2<br>3 | 4 h<br>4 h<br>4 h | R. Korenstein (Sömjen et al., 1983) |
| Primary mouse bone cells (collagenase calvaria) | Pulsed electromagnetic field (PEMF)<br>(+ 100 msec, -2 msec,<br>15 Hz) 1 h exposure<br>Induced magnetic field 8 G<br>E-field 0.6 V/cm<br>Current 20 mA/cm | 1.7 (6 d basal)<br>5.0 (8 d basal)<br>1.5 PTH | 3.5 h<br>3.5 h<br>3.5 h | C. Cain (Cain et al., 1985) |
| Reuber H35 | 450 MHz RF-field | 1.5 (basal) | 1-4 h | C. Byus |

| | | | | |
|---|---|---|---|---|
| hepatoma | AM 16 Hz<br>1.7 watts PEP<br>1.0 mW/cm$^2$ field intensity | 2.6 (+ TPA) | 4 h | (Byus et al., 1988) |
| Chinese Hamster Ovary | SAR 0.08 w/kg<br>1 hr exposure | 2.0 (basal)<br>2.0 (+ TPA) | 1-2 h<br>4 h | |
| 294T melanoma | | 2.0 (basal) | 0-1 h | |
| CEM (human lymphoma | 60 Hz electric field | 3-5 | 1-2 h | C. Byus<br>(Byus et al., 1988) |
| P3 (mouse myeloma) | 10 mV/cm - 0.1 mV/cm | 1.75 | 2 h | |
| Reuber H35 (rat hepatoma) | (nonlinear d.r.) 1-3 h | 1.5 | 1 h | |
| L929 mouse fibroblasts | 60 Hz, 10mT$_{rms}$<br>4 h exposure<br>sinusoisal signal coherent for 5-10s; 30-90 Hz incoherent (noise) field blocks<br>ODC response | 2.1 | 4 h | T. Litovitz<br>(Litovitz et al., 1991)<br>(Litovitz et al., 1994) |
| L929 mouse fibroblasts | Amplitude-modulated RF<br>915 MHz, AM 50, 60, 65 Hz<br>S.A.R. 2.5 w/kg<br>(coherence effects similar to ELF) | 2 | 8 h | T. Litovitz<br>(Litovitz et al., 1993 |
| Jurkat (human lymphoblastoid)<br>HL 60<br>ELD<br>F9 (embryonal) | 50 Hz, 0.10 mT$_{rms}$<br>vertical polarization<br>30 min-4 h | 1.5<br>(P,SD also increase õ) | 3 h | M.-O. Mattsson<br>(*Valtersson et al., 1995)<br>(*Mattsson et al., 1992)<br>(*Valtersson et al., in press) |
| C3H/10 T 1/2 fibroblasts | 60Hz,<br>50mG<br>100mG<br>200mG | <br>0.84<br>0.53<br>0.75 | <br>3 h<br>3 h<br>3 h | C.D. Cain<br>(*Cain et al., 1993)<br>(*Cain et al., 1995) |
| Chicken embryo (26 h) | 60 Hz sinusoidal<br>4 mT | 2.0<br>0.5 | 15 h<br>25 h | T.A. Litovitz<br>(Farrell et al., in press) |
| ODC transgenic | 50 Hz MF | 0.75 | 24 h | *Juutilainen et |

| | | | | |
|---|---|---|---|---|
| (K2)<br>mouse-epidermis | 100mT continuous | (2-3 increase P & SD<br>at 24 h | | al., 1996 |
| SENCAR mouse epidermis, papillomas | 60 Hz MF<br>2 mT<br>6-7 h/d, 5-7 d/wk | 1.0<br>(3 doses TPA)<br>1.0<br>papillomas, epidermis | 1, 2, 5 wks<br><br>43 wks | Byus et al., (submitted) |
| Sprague-Dawley Rat<br>various tissues | 50 Hz MF<br>50 mT<br>24 h/d, 7 d/wk | 2.0 (mammary tissue)<br>1.42 (spleen)<br>1.0 liver, small intestine, bone marrow, ear skin) | 6 wks<br><br>6 wks<br>6 wks | W. Loscher (Mevissen et al., 1995) |
| Rats (Fischer)<br>various tissues | 60 Hz<br>2 mT<br>20 mT<br>200 mT<br>24 h/d, 6 d/wk<br>(in utero, day 13 pregnancy) | linear dose/response increase ODC, up tp 5.0<br>time/flux density<br>5, 15, 32 wks<br>liver, brain, intestine, spleen, spinal cord, kidney | | R. Mandeville (*Kanza et al., 1996) |

Table 2

Enzymes Investigated Using Strong Dc Magnetic Fields That Do Not Show Magnetic Field Effects Due To Radical Pair Mechanisms

(Provided by Dr. Charles Grissom, University of Utah)

Note: These fields do not induce an electric field and are clearly not typical of environmental fields.

- $B_{12}$-Dependent Methylmalonyl-CoA Mutase (Human)
- $B_{12}$-Dependent Methylmalonyl-CoA Mutase (Bacterial)
- Momoamine Oxidase B (Human)
- Lipoxygenase (Soybean)
- Tyrosine Hydroxylase (Human)*
- Hexokinase (Yeast)
- Catalase (Bovine)
- Luciferase (Bacterial)
- Methane Monooxygenase (Bacterial)*
- Nuclease (Staphylococcus)*
- Chymotrypsin*

* Not published, but reliable report

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Top Contents

EMF Effects on Early Signal Transduction Events

at the Plasma Membrane

Facilitator: Mats-Olof Mattsson, Ph.D.

Rapporteurs: Deborah McK.Ciombor, Ph.D. and Gayle E. Woloschak, Ph.D.

**Introduction**

There have been many studies in the literature reported by a variety of investigators using a large number of different *in vitro* systems which have suggested that cellular signal transduction pathways are affected by EMF. This finding is quite expected since the signal transduction pathways receive both physical and biological information from other cells, as well as the environment, and transmit them in a cellularly appropriate fashion to produce the biochemical signals that regulate cellular functions. The electric field is attenuated within the cell but is amplified across the plasma membrane; therefore, in the absence of a clear indication that the magnetic field alone is responsible for all observed EMF effects, we must hypothesize that recognition and amplification of the information carried in the EMF signal is the domain of the plasma membrane and its signaling systems.

There is reasonable agreement as well on which early signaling pathways may be affected. The agreement, however, does not extend to a consensus regarding whether or not EMF effects at the *in vivo* level can be directly ascribed to any one pathway. Lively discussions not withstanding, the group feels fairly strongly that the interaction of the affected pathways is a critical and potentially fruitful area of future research.

**Questions Discussed by the Breakout Group>**

1. Has EMF exposure been associated with alterations in early steps in signal transduction?

As an overall response, it was the considered opinion of the group that there was a positive association of EMF effects and signal transduction. The group also feels, however, that the earliest steps have not yet been identified, including the recognition step (or steps).

a. If yes, identify the signaling systems affected.

The following list is meant to indicate those areas the group felt were the most well accepted and well represented in the literature. The final two items on the list are in

parentheses since the group felt these were possibly the province of another breakout group; we list them only for completeness.

i. Protein kinase C (PKC, work of Dr. Richard Luben)
ii. Syk and syk-related pathways (work of Dr. Fatih Uckun)
iii. cAMP
iv. IP3
v. (Na+ / K+ ATPase )
vi. (Voltage sensitive channels)

Dr. Richard Luben suggested that the group note that *in vitro* responses were frequently dependent on cell specificity and heterogeneity and this should always be considered in evaluating data. His example: In B lymphocytes--PKC is elevated in response to EMF while exposure of osteoblasts to a comparable EMF shows little or no effect. Others noted that there is also evidence of Ca++ efflux from EMF-exposed cells.

b. At what level are these effects observed?

The following is the consensus list of the group:

i. Ligand - Receptor binding
ii. Enzymatic Activity
iii. Phosphorylation
iv. Secondary messenger

c. Are there dose-response data supporting these effects?

The group felt that little dose-response data exist in the literature. Few references could be recalled. This may be due in part to the difficulties in constructing a "dose-response." Several suggestions were put forth for promising experiments to fill the perceived gaps in the literature. They include: 1) a study of coherence to include physical and biological coherence; 2) time sequence to include intermittence and transients; and 3) amplitude, frequency and co-factors (although difficulties with interpreting the cAMP studies in the literature were cited).

d. What are the temporal characteristics of the response?

The group felt that this was an important question that has not been examined in adequate detail, particularly the definition of "early," i.e., microseconds versus minutes. Additionally, everyone expressed the belief that the characterizations were entirely dependent on the biological systems studied and that attempts to generalize across systems were unproductive pursuits. Fundamentally, however, it was believed that the current literature does not indicate that the time constants of the pathways mentioned above were changed significantly.

e. How do these exposure patterns relate to long-term effects within cells?

It was deemed biologically plausible that long-term effects would result from changes in the signaling pathways mentioned, but detailed accounts are limited. It was noted

by one attendee that ornithine decarboxylase (ODC) was to date probably the only system that could be assessed in this manner. Field effects on the epidermal growth factor (EGF) signaling pathway were also mentioned as likely to result in significant long-term effects within a given tissue, and that further study in this area, if it were to replicate the preliminary results, would be important. Two other studies were mentioned as possibly indicating the potential for these types of effects: the Litovitz coherence studies with ODC and the Schimmelpfeng study of cAMP.

f. To what degree does this indicate strong support for an *in vivo* effect on signal transduction?

There is not yet strong support in the literature for an *in vivo* effect on signal transduction. More experiments are needed to assess whether an *in vivo* effect occurs.

g. What health effects might be associated with these types of *in vitro* effects?

It was believed that it would be premature to attempt to answer this question at this time.

2. Do short-term exposures to EMF lead to long-term changes in factors involved in early signal transduction events?

Basically the group answered this question in an identical manner to the previous question; however, it was noted that experiments designed to answer this question are underway in several laboratories, including experiments with 10 T 1/2 cells treated with TPA and continued EMF exposures.

3. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on early signal transduction activity?

There are numerous possibilities and some studies are currently underway, including studies on cell signal transduction molecules/pathways known to be important in stress and mitogen responses. Since induction of signal transduction can bring about several cellular responses, the cellular effects would include:

a. cell proliferation
b. cell differentiation
c. programmed cell death or apoptosis
d. adaptive responses of differentiated cells, such as secretion of hormones, contraction of cells; induction of calcium waves between cells.

4. At the EMF exposure levels observed *in vivo*, would cellular effects on the early steps in signal transduction be likely to be observable *in vitro* ?

The group answered with an unanimous and resounding - YES.

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**Summary and Recommendations**

In general, there are several demonstrations in the literature of an effect of EMF exposure on cellular signal transduction. These effects have been shown in particular cell types, but experiments demonstrating broad applicability to other cellular systems are needed. In addition, further work to uncover specific pathways involved, as well as activation mechanisms, will be important.

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Top

Contents

EMF Effects on the Signal Transduction Cascade

Facilitator: Steven C. Miller, Ph.D.

Rapporteurs: Henry C. Lai, Ph.D. and Jeffrey D. Saffer, Ph.D.

**Introduction**

Considerable advances in research on intracellular signal transduction pathways have been made in recent years. Such pathways amplify signals initiated at the cell surface, e.g. ligand-receptor interaction, and affect cell functions via enzyme activation and affect genetic functions.

Though biological systems have evolved an efficient signal transduction system to respond appropriately to chemical, biological, and environmental stimuli, the mechanism whereby the energy in low level extremely low frequency electromagnetic fields (EMF) may be coupled to a biophysical process is unknown. There are speculations and experimental data that suggests that EMFs act through an interaction mediated at the cell membrane that affects enzyme activities, gene expression, and ultimately cellular functions. The signal transduction processes link these cellular events. Studying changes in signal transduction pathways *in vitro* can be a powerful means to understand the mechanisms of interaction of EMF and to aid in the further assessment of a possible EMF hazard to human health.

**Questions Discussed by the Breakout Group**

1. Has EMF exposure been associated with alterations in the signal transduction cascade?

Several examples in the literature were named suggesting changes in the signal transduction cascade after EMF exposure. These include: receptor-mediated tyrosine phosphorylation, second messengers, enzymes such as ornithine decarboxylase (ODC), and amplification through protein kinase C (PKC).

There was a general feeling that the signal transduction processes are dynamic and complicated, involving multiple pathways and steps, and that present knowledge is limited. The question of where to start looking for effects of EMF on signal transduction pathways was raised. Clearly, significant publications in the literature provide evidence for mechanisms of interaction that should be examined.

The literature base for discussion in this breakout group focused upon two papers. One, a paper by Uckun et al. (1995) was discussed as a significant publication with evidence for effects of a 0.1 mT 60 Hz EMF on tyrosine phosphorylation induced signaling at multiple levels. This paper was compared to another paper by the same first author (1992) on ionizing radiation in which a similar magnitude of effects was observed at 1 Gy. The papers illustrate the complex protein interactions taking place at the level of the cell membrane in response to a radiation-induced signal. The issue of the similarity of the signaling responses, which suggests a common mechanism, was raised. Getting essentially the same signaling response from a known stimulus (1 Gy of ionizing radiation) and EMF was pointed out as "curious." Since signaling responses to ionizing radiation are generally robust, the discussion centered on the question of why EMF effects are not that obvious. During discussion it was pointed out that both Dr. Steven Miller's and Dr. Richard Luben's laboratories have had difficulties maintaining the Nalm-6 human pre-B leukemia cell line used by the Uckun laboratory. Thus, an exact replication effort has not been attempted by either of the laboratories. The actual details of this effort were not discussed or data presented for

evaluation by the breakout group. It was also commented during the session that using a different human B leukemia cell line (Daudi), Miller's laboratory has been unable to detect an effect of a 0.1 mT 60 Hz field on phosphotyrosine profiles of proteins under conditions where anti-IgM treatment demonstrated significant and reproducible changes in phosphotyrosine protein profiles as compared to controls.

A question was raised on whether a model system exists with well known pathways such that each step can be dissected out for study. The Jurkat T cell leukemia model was suggested since multiple levels of evidence have now been presented.

2. Are there dose response data supporting these effects of EMF exposure on alterations in the signal transduction cascade?

Several experimental issues were raised, but no strong conclusion was made on this question. It was pointed out that no reliable dose-response data on EMF effects on signal transduction processes are available at this time. Dose-response study of bone healing was mentioned, but it was pointed out that it was not related to cancer and health risk and that unique and pulsed EMF wave-forms were used in those studies. The implication of effects of these fields relative to effects of sinusoidal fields was questioned. With regard to the time-course of response, evidence suggests that rapid changes can occur after EMF exposure (e.g., Uckun et al. reported changes in tyrosine phosphorylation of multiple proteins and protein kinase C activation within ten minutes of exposure to a 60-Hz magnetic field at 0.1 μT).

3. Do short-term exposures to EMF lead to long-term changes in factors involved in the signal transduction cascade?

It was agreed that there were no data available and therefore, not much discussion on this question was made.

The effect of exposure time was briefly discussed. It was mentioned that in ionization radiation studies it is known that the effect is more pronounced if a dose is given in one bolus than if it is fractionated into smaller ones and given over time. However, it was mentioned that this may not be true for EMF effects.

Another concern of exposure time is that the cellular re-set time may limit the responses of cells to EMF. For example, cells may be only responsive to EMF at a certain phase of their cell cycle. This could be an important issue in *in vitro* experiments.

4. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMFs on the signal transduction cascade?

Several models were proposed: (1) cells used in an *in vitro* model can be implanted in animals for an *in vivo* exposure; (2) transgenic animals with appropriate transduction pathways hypersensitive to a ligand-induced signaling can be used; (3) if a field sensitive pathway is identified, one can evaluate the significance of EMF exposure in cells initiated *in vivo*.

5. At the EMF exposure levels observed *in vivo*, would cellular effects on the signal transduction cascade be likely to be observable *in vitro*?

It is generally agreed that this question is context dependent, e.g., on the processes studied and the parameters of exposure. However, it was pointed out that cellular effects from EMF exposures could be tested by the implantation of the cells from the *in vitro* model into an *in vivo* animal model. Otherwise no answer is possible at this time.

**Additional Issues Discussed by the Breakout Group**

There was a long discussion on the logistics in the replication of an experiment. It was generally agreed that it is necessary to recognize the sources of possible artifacts in an experiment. Since this research involves studying small changes in cell functions in response to EMF, protocol design and experimental details may be critical. It was pointed out that cooperation of and sharing of important reagents and methods from the original investigator are important in an attempt to replicate an experiment. To illustrate these points, the facilitator presented a "rigor to risk pathway" (rigor, robustness, replication, requirement, response, and risk). The group expressed consensus that the study of the bioeffects of EMF begins with **rigor** to define an exposure environment, field conditions, and biological conditions that results in a **robust** biological response. To facilitate **replication** the original investigator must define the physical and biological **requirements** responsible for a given **response**. Thus, careful attention to exposure assessment and protocol design is important for an EMF-induced signaling pathway to be 1) recognized, 2) understood mechanistically, and 3) evaluated for human health **risk**.

It was pointed out that there is a significant difference between research that is hypothesis-driven versus replication-driven. The former may require flexibility in protocols whereas the latter requires the rigid adherence to predetermined protocols.

Use of simpler model systems such as yeast was suggested because they have signal transduction pathways similar to higher species and their genome has been mapped. These features would be useful in investigating the mechanisms of EMF interaction in robust signaling effects observed in mammalian systems.

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**Summary and Recommendations**

It is generally agreed that the effect of EMF on signal transduction processes is an important area of research both in the understanding of the mechanisms of bioeffects and human health risk assessment. Although definite effects are observed in experiments and therapies using pulsed EMF waveforms to initiate bone healing in recalcitrant fractures, there is not enough information at this time to conclude whether EMF exposure conditions, which are of concern for human health risk, affect signaling cascades. Therefore, more research is needed. The further assessment of the possible human health hazards of EMF exposure depends, in part, upon establishing a causal connection between the existence of robust, replicable biological effects and the precise dosimetric parameters of EMF exposure upon which these effects depend.

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Back to: **Table of Contents of Breakout Groups** | **EMF Science Review Symposium - March 24-27, 1997**

**EMF*RAPID* Home** | **NIEHS Home**
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