Biochemical and Biophysical Research Communications **265,** 73–78 (1999)
Article ID bbrc.1999.1642, available online at http://www.idealibrary.com on IDEAL®

Electromagnetic Fields in Combination with Elevated Temperatures Affect Embryogenesis of *Drosophila*

Anja Michel and Herwig O. Gutzeit[1]
Institut für Zoologie, Technische Universität Dresden, D-01062 Dresden, Germany

Received October 7, 1999

The effect of electromagnetic fields (50 Hz, 100 μT magnetic flux density) on *Drosophila* embryogenesis was tested under conditions of mild thermal stress (temperatures between 34 and 37°C). When exposed to these stressor(s) for 30 min during early embryogenesis those embryos which were subjected to both electromagnetic fields and elevated temperature (costress) showed pattern anomalies more frequently than embryos subjected to thermal stress alone. Furthermore, under costress conditions development was considerably delayed in three different strains tested. The use of transgenic strains with a lacZ reporter being expressed in segmental patterns facilitated the identification and quantification of the pattern anomalies.

Key Words: **electromagnetic fields; pattern formation; rate of development; heat shock; environmental stress.**

The biological effects of extremely low frequency electromagnetic fields (ELF-EMF) have been studied in recent years in view of potential health hazards. Despite intensive research a widely accepted concept concerning the biological effects of ELF-EMF has not emerged (reviewed in 1–4). Any promising molecular approach has to be based on clear and reproducible biological effects of ELF-EMF. This precondition was, as a rule, not met and this may, in part, explain the slow progress in the field. In this unsatisfactory situation it is surprising that established animal model systems like *C. elegans* and *Drosophila* have not been fully exploited to study the cellular effects of ELF-EMF, the possible primary molecular targets, and the relevant signal transduction mechanisms.

We reasoned that it might be easier to disturb development by ELF-EMF when the embryos are at the same time subjected to a moderate stress thus making them more susceptible to the applied weak electromagnetic fields. The observation of Goodman and coworkers (5) that ELF-EMF induces the expression of the heat shock protein 70 (hsp70) prompted us to use elevated temperatures as a second stressor (costress) in addition to ELF-EMF. The hsp 70 protein is thought to act as a sensor to stress (6) and responds to stress by increasing the fitness of the animal. This can be shown experimentally by the improved tolerance of *Drosophila* larvae to stress which carry extra copies of the hsp70 gene and express higher levels of the hsp70 protein (7). In a previous series of experiments using transgenic strains of *C. elegans* we were able to show that under suitable costress conditions the induction of a lacZ reporter gene under the control of hsp70 or hsp16 control sequence can be strongly enhanced by ELF-EMF (8).

Encouraged by the success of the costress strategy we asked a more ambitious question: could costress conditions be defined which resulted in clear and reproducible developmental effects? As an animal model we chose the fruit fly *Drosophila melanogaster* because of the extensive genetic work which will greatly facilitate a biochemical and genetic analysis at a later stage. Also, transgenic strains with a patterned expression of the lacZ reporter genes are available which greatly facilitate scoring of developmental defects. The phase of embryogenesis was chosen for our analysis since the complex molecular signalling during early embryogenesis is likely to be most susceptible to stressors.

In this communication we show that ELF-EMF at elevated temperatures may interfere with development in this species in two ways: ELF-EMF exposure at early development increases the fraction of abnormally developing embryos and, in addition, delays development.

MATERIALS AND METHODS

Fly stocks. Several transgenic stocks of *Drosophila* were used in which the expression of a lacZ reporter gene is controlled by the relevant sequences of the segmentation genes *engrailed* (en), *fushi tarazu* (ftz) or *sloppy paired* (slp) The stocks will be referred to as en-lacZ (9), ftz-lacZ (10), and slp-lacZ (11). For details of the stocks

[1] To whom correspondence should be addressed. Fax: 49-351-463-7093. E-mail: gutzeit@rcs.urz.TU-dresden.de.

0006-291X/99 $30.00

like expression patterns and genetic details see original papers. The stocks were kindly supplied by Prof. W. Gehring, Basel.

Handling of embryos and exposure to ELF-EMF and heat. Flies of the respective stocks were placed on fresh agar plates supplemented with a drop of Baker's yeast. Egg deposition was allowed for 1 h followed by three egg deposition periods of 30 min each and only these three egg collections were used for the described experiments. The laid eggs were collected with a brush, washed with PBS and dechorionated using sodium hypochloride. The eggs were washed with PBS again and left to develop at 25°C for 2 h 30 min, 3 h, and 3 h 30 min. Taking the egg deposition period of 30 min into account the samples could be divided into the following age groups: 2 h 30 min–3 h (cellularization), 3 h–3 h 30 min (early gastrulation), and 3 h 30 min–4 h (midgut invagination to early germ band extension). The embryos of each age group were split into 3 samples; one served as control (25°C), the second was subjected to elevated temperatures (see Results) and the third group was kept at the stress temperature and, in addition, subjected to ELF-EMF. The stress was limited to 30 min. Afterwards the three groups of embryos were left to develop for 16 h at 18°C. The embryos were fixed in 200 μl of 3.7% formaldehyde in PBS and 200 μl heptane for 25 min. After washing the embryos in PBS twice, β-galactosidase activity was visualized by staining using X-gal as a substrate. The embryos were stained for several hours at 37°C. For each experimental condition 5 to 7 independent experiments were carried out with a total number of 400 to 500 embryos in typical experiments. Of these embryos 60 to 70% showed lacZ staining and were used to score developmental defects as described under Results.

Control of stress parameters. Defined ELF-EMF were generated by a set of Merritt coils (12, 13). The technical details to produce ELF-EMF were described previously (8). With the experimental set-up we were able to generate a magnetic flux density of 100 μT with a precision of $\pm$2%. Other magnetic flux densities were not tested. The harmonic distortion, the terrestrial magnetic field as well as stray field sources in the laboratory were measured and did not influence the results significantly. The temperature (costressor) was controlled with an accuracy of 0.1°C in specially constructed PVC chambers (for details see 8).

RESULTS

1. Induced Pattern Anomalies

A transgenic *Drosophila* strain with the characteristic *engrailed* expression of the lacZ reporter genes was used in this series of experiments so that any developmental defect resulting in segmental pattern anomalies could easily be recognised. Typical defects which were scored as embryonic pattern anomaly were, for example, incompletely formed segments, lack of segments, fused segments and, in extreme cases, patchy lacZ-expressing cells with grossly disturbed segmental expression pattern. Since the spectrum of anomalies did not differ significantly under the different experimental conditions (including the controls) we subsumed all defects in one class and just compared the frequency of anomalies scored under different experimental conditions.

Our experimental strategy was to test the effect of ELF-EMF under conditions of mild thermal stress. In preliminary experiments suitable conditions for thermal costress were defined and a temperature between 34 and 37°C met our expectations. Several experiments carried out in this temperature range were pooled and subdivided into two groups (34–35°C, 36–37°C). Controls were kept at 25°C (see Materials and Methods). Even at this stress-free temperature embryonic defects are regularly observed. The measured frequency of anomalies at 25°C was defined as 100% and the data of the experimental samples calculated with reference to the controls. Another variable which was explored was the age of the embryos at the beginning of the stress treatment. We concentrated on early embryogenesis starting from cellularisation up to early germ band formation.

Under all conditions tested (Fig. 1) the samples at elevated temperatures alone or in combination with ELF-EMF (B and C, respectively) clearly showed more pattern anomalies as the controls. Furthermore, under costress conditions more anomalies were observed than at temperature stress conditions alone (compare C to B). Of the variables tested (temperature and age) there was a clear tendency for anomalies to increase with increasing temperatures and to decrease with age when the three tested embryonic stages are were compared. Data obtained for temperatures below 34°C (not shown) confirmed this tendency. Hence the clearest effects were observed in 2 h 30 min to 3 h old embryos exposed to 36–37°C. When compared to the controls the anomalies in temperature-stressed embryos was more than 4 times as frequent and in embryos exposed additionally to 100 μT ELF-EMF about 10 times as frequent. When tested statistically by non-parametric ANOVA sample C was significantly different from A in two cases (Fig. 1, age groups 2 h 30 min–3 h, both temperature ranges) while B compared to A was not.

From these results the clear picture emerges that at the time when the zygotic pattern forming genes like *en* become activated and specify the segmentation pattern of the embryo the processes can be disturbed by thermal stress. This effect can be strongly enhanced by ELF-EMF.

2. Induced Developmental Delay

When the experiments described above were carried out we noticed that embryos subjected to EMF treatment developed somewhat slower than control embryos kept at the same temperature. To quantify the effect of ELF-EMF we used embryos of three different genotypes. As before, embryos of the desired stages were collected and subjected to thermal stress (36°C) for 30 min with or without ELF-EMF (100 μT). After incubation of the embryos for 16 h at 18°C the embryonic stages were determined and classified into 3 categories (larva, extended germ band and short germ band). Depending on the stress conditions the number of undeveloped or grossly abnormal embryos whose stage could not be determined was high and these cases were scored as "not classifiable". The results

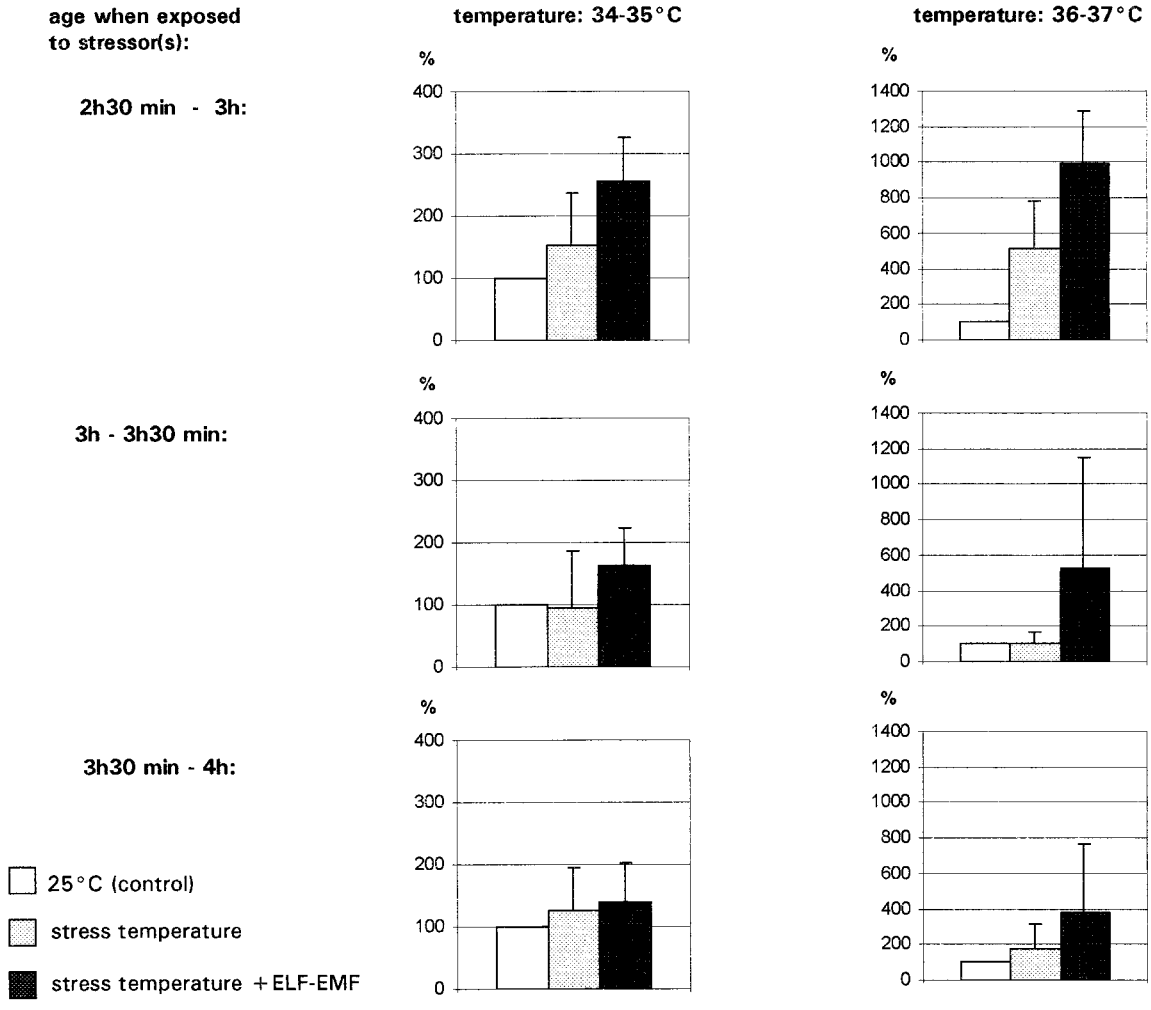

FIG. 1. Effect of heat and ELF-EMF on the formation of embryonic pattern anomalies. The number of anomalies scored in the controls (25°C) was used as reference and set as 100%. The incidence of anomalies under stress conditions was calculated in % with reference to the controls. The standard deviation is indicated. In all costress experiments (black columns) the magnetic flux density was 100 μT.

which are summarized in Fig. 2 illustrate the following points:

(i) in all tested genotypes the fraction of control embryos (25°C) and temperature-stressed embryos (36°C) are predominantly in the short germ band stage while the embryos which were in addition to the temperature stress also subjected to ELF-EMF were predominantly in the long germ band stage.

(ii) The age difference between temperature-stressed and ELF-EMF costressed embryos increased when the stress was applied late (2 h 30 min–3 h < 3 h–3 h 30 min < 3 h 30 min–4 h).

(iii) The sensitivity to the stress conditions varied between the different genotypes. Highest sensitivity appeared to have the ftz-lacZ strain in which a high percentage of (co)stressed embryos did not develop properly (category "not classifiable"). In fact, in the youngest age group no ftz-lacZ embryo at all reached the germ band stage under costress conditions. In accordance with the observations reported in the previous section the sensitivity to stress was generally highest in the 2.30 min–3 h age group and declined in older embryos (Fig. 2).

The stressor(s) were present in these experiments for only 30 min and yet a developmental delay was observed which clearly exceeded this time-span since the phase of germ band contraction alone takes 1 h 30 min at 22°C (14).

DISCUSSION

Embryonic development of invertebrate and vertebrate species can be disturbed by a variety of chemical and environmental stressors and it is, therefore, not surprising that the possible influence on ELF-EMF on embryogenesis was subject of several studies (e.g. 15–17). In our experiments with *Drosophila* embryos ELF-EMF alone at control temperature (25°C) or tempera-

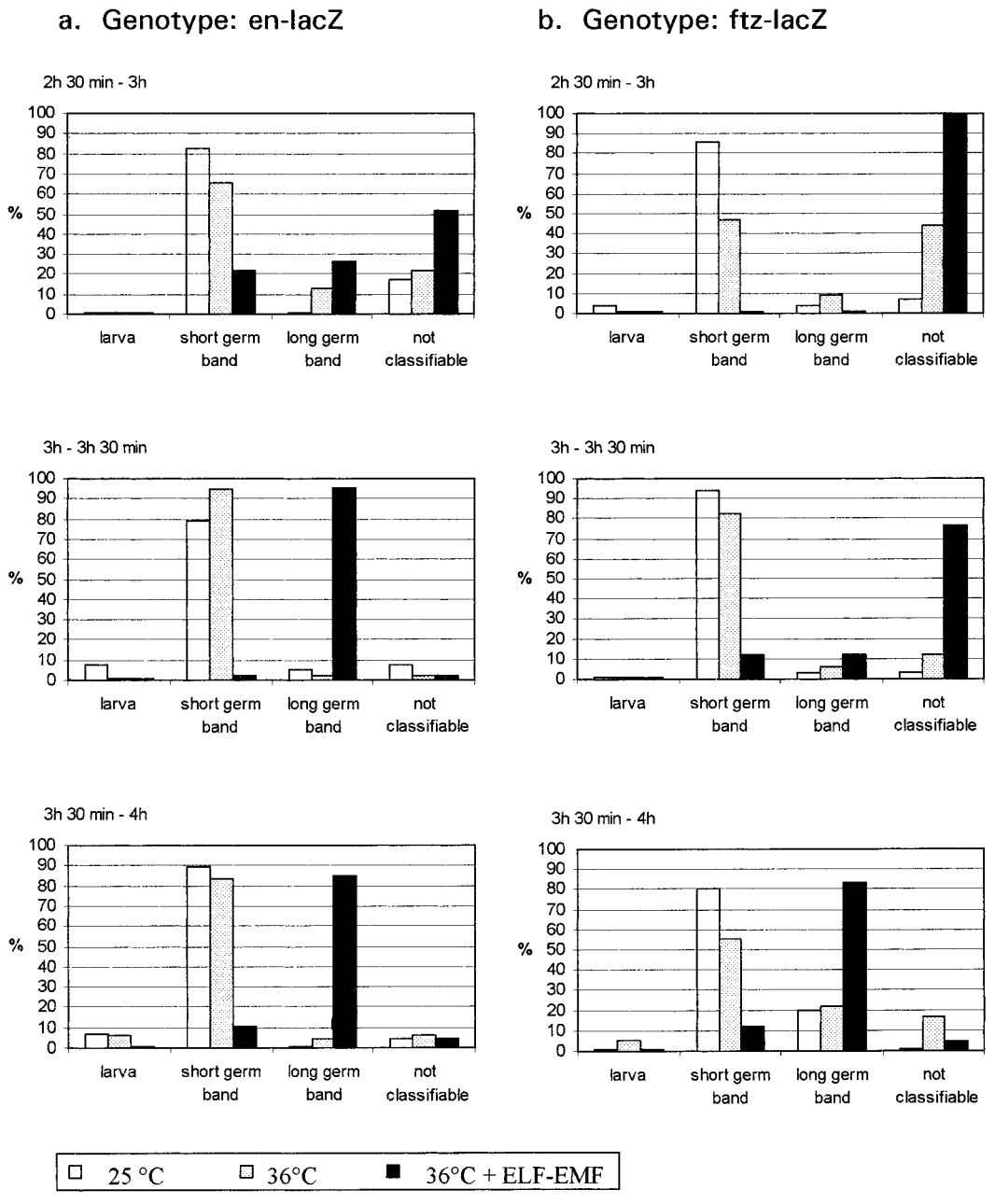

FIG. 2. The effect of heat and ELF-EMF on rate of embryogenesis. The effect of heat and ELF-EMF was studied in three different genotypes (a–c) using the same three age groups of embryos as in Fig. 1. The developmental stage of the embryos after 30 min exposure to stress conditions followed by 16 h development at 18°C is shown. The embryos were grouped into three developmental stages (larva, long and short germ band, respectively) or scored as "not classifiable" (not developed or grossly abnormal). For each experimental condition, i.e., control temperature, stress temperature, and stress temperature plus ELF-EMF, the percentage of embryos in each of the four categories is shown (adding up to 100%). In all experiments involving ELF-EMF the magnetic flux density was 100 μT.

tures below 34°C had only a small (if any) effect on pattern formation and rate of development. Our results under these conditions were variable and inconclusive. Similar observations were made by Ma and Chu (18) who studied the effect of ELF-EMF on *Drosophila* embryogenesis at 26°C.

Recently we showed that ELF-EMF (same exposure conditions as in the experiments reported here) increases the hsp response to elevated temperatures in transgenic nematodes (8). Interestingly, a similar observation was made by Tsurita and co-workers (19) who found that ELF-EMF potentiates the effect of thermal stress in normal and malignant human cell lines. Hence any effect that may be caused by elevated temperatures alone could be expected to be potentiated by ELF-EMF. In this communication we provide direct evidence for this notion.

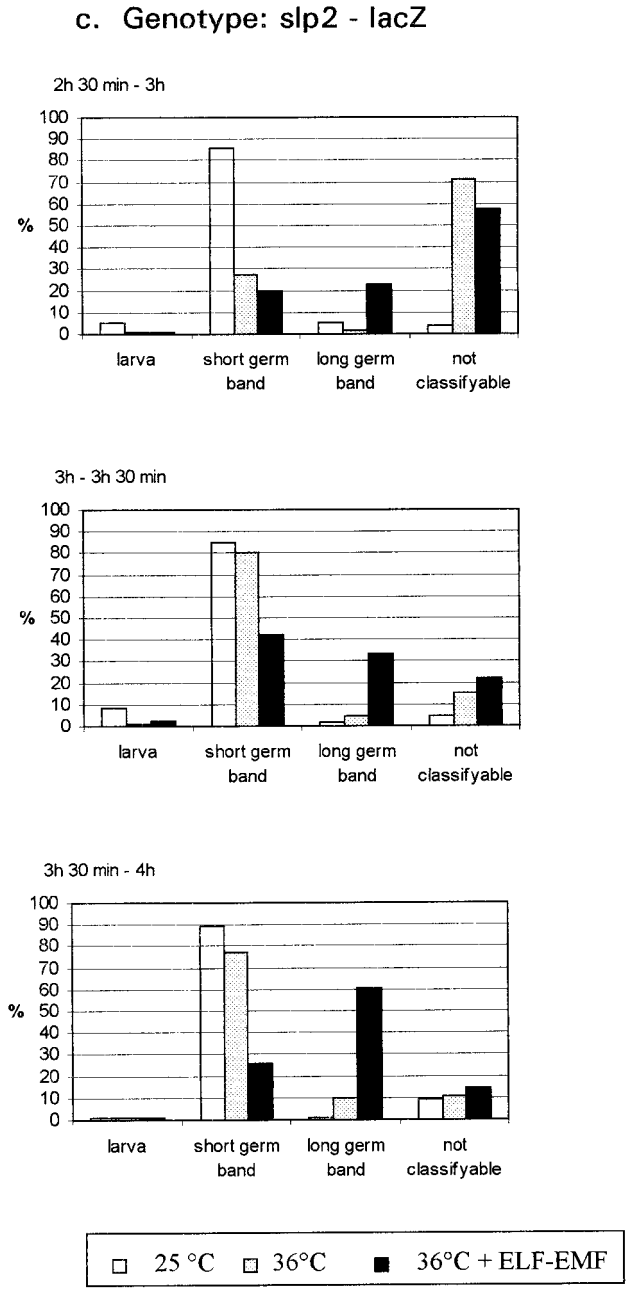

FIG. 2—*Continued*

Before considering the combined effects of both stressors it is useful to be aware of the effects of heat treatment alone on development. The stress response to heat and the signal transduction pathways that lead to the expression of hsp has been analyzed in great detail. Apart from heat a number of different physical and chemical stressors are known to induce hsp and, concomitant with transcriptional control, also translational control is exerted. For this reason it is not surprising that heat (or other stressors that induce the hsp response) may interfere with critical developmental processes. At sublethal temperatures phenocopies of, for example, dominant mutations in the *fushi tarazu* gene can be produced (Welte *et al.*, 1995). The cellularisation stage when the segmentation is specified is the most sensitive stage for phenocopy induction in keeping with the interpretation that the heat stress interferes with the signalling in the relevant developmental processes. Maas (21) found that at the blastoderm stage pattern anomalies in the abdomen and the metathorax of the *Drosophila* imago can be induced by heat (35°C for 4 h). Some of the observed morphological defects of the metathorax may be regarded as phenocopies of the mutations *bithorax* or *bithoraxoid*.

The observed stage differences concerning the sensitivity to the stressors is in keeping with observations made by Bergh and Arking (22) who subjected *Drosophila* embryos to mild heat stress (40 min at 37°C) and observed that preblastoderm embryos were extremely sensitive to this stress and development was arrested or embryogenesis was abnormal while embryos at the stage of gastrulation survived the heat stress well and almost reached control levels.

The same authors noticed in their experiments that the heat stress of 40 min at 37°C results in a developmental delay which is considerably longer than the duration of the heat shock itself. This agrees well with our observations reported here. We showed in our experiments that this heat-induced delay is further increased by ELF-EMF to a surprising extent considering the fact that ELF-EMF alone is a very weak stressor under a variety of experimental conditions. The molecular basis for this effect is unknown but an influence of the stressors on the regulation of the cell cycle is indicated. Soon after the onset of gastrulation a number of mitotic domains form in the *Drosophila* embryo at more than 50 distinct mitotic trigger sites (23). The observation that the delay of development was more pronounced in embryos past the gastrulation stage (between 3 h and 4 h) than at the cellularisation stage (2 h 30 min–3 h) provides indirect evidence for an effect of ELF-EMF on cell cycling.

The experimental strategy to study the biological effect of a stressor in the presence of a second stressor is not only applicable to detect small and alone insignificant effects. The approach may also aid in the study of the relevant signal transduction pathways which apparently interact to produce the combined effect. Furthermore, the biological effects in a costress situation of physical and/or chemical stressors may lead to a more realistic appreciation of potential health hazards. A case in point is the finding of Löscher and Mevissen (24) that the incidence of DMBA induced mammary tumors of rats increases strongly when the experimental animals are, in addition to the chemical agent, also exposed to 50 Hz pulsed magnetic fields with a flux density of, for example, 50 or 100 μT.

In our previous experiments with *C. elegans* (8) we noticed that the temperature which acted as efficient

costressor to ELF-EMF had to be defined with an accuracy of 1°C. In the experiment reported here the temperature range between 34 and 37°C produced the desired effect with the most efficient costress temperature being 37°C. Since in contrast to *Drosophila* the nematodes are hermaphrodites it is conceivable that there is less genetic variation in *C. elegans* than in *Drosophila* laboratory populations. While a number of observations support this suspicion (e.g. the "background" of developmental defects even in unstressed wildtype *Drosophila* embryos or the variability of protein patterns analysed by two dimensional gels, unpublished observation) quantitative data are lacking.

The genotoxic potential of ELF-EMF has been studied intensively but clear evidence for such effect has not been presented. However, circumstantial evidence suggests that the genotoxic potential of ELF-EMF in the presence of a second stressor needs to be addressed in further studies (reviewed in 25).

Since the experimental conditions have now been defined under which ELF-EMF has a strong effect on gene expression and development in two model organisms, a molecular analysis of ELF-EMF-induced gene expression is called for. This approach is likely to enhance our knowledge on the interaction of the combined stressors and may well change our views on the risk assessment of "weak" stressors.

ACKNOWLEDGMENTS

We thank Dr. S. Scholz for his help with the statistical analysis and for critically reading the manuscript. Dr. H. Bauer′s help and expertise in maintaining defined ELF-EMF exposure conditions is appreciated. Mrs. C. Arnold contributed to the final layout of the figures. This work was supported by the DFG (Grant Gu 208/11-1).

REFERENCES

1. ELF-EMF European Feasibility Study Group (1997) *Scand. J. Work Environ. Health* **23,** 5–14.
2. Goodman, E. M., Greenbaum B., and Marron, M. T. (1995) *Int. Rev. Cytol.* **158,** 279–338.
3. Lacy-Hulbert, A., Metcalfe J. C., and Hesketh, R. (1998) *FASEB J.* **12,** 395–420.
4. Valberg, P. A., Kavet, R., and Rafferty, C. N. (1997) *Radiat. Res.* **148,** 2–21.
5. Goodman, R., Blank, M., Lin, H., Dai, R., Khorkova, O., Soo, L., and Weisbrot, D. (1994) *Bioelectrochem. Bioenerg.* **33,** 115–120.
6. Voellmy, R. (1996) *in* Stress-Inducible Cellular Responses (Feige, U., Morimoto, R. I., Yahara, I., and Polla, B. S., Eds.), pp. 121–137, Birkhäuser Verlag, Basel.
7. Feder M. E,. Cartano N. V., Milos L., Krebs, R. A., and Lindquist, S. L. (1996) *J. Exp. Biol.* **199,** 1837–1844.
8. Junkersdorf B, Bauer, H, and Gutzeit, H. O. (1999) *Bioelectromagnetics,* in press.
9. Kassis J. A. (1990) *Genes Dev* **4,** 433–443.
10. Hiromi, Y., Kuroiwa, A., and Gehring, W. J. (1985) *Cell* **43,** 603–613.
11. Grossniklaus, U., Pearson R. K., and Gehring.(1992) *Genes Dev.* **6,** 1030–1051.
12. Merritt, R., Purcell, C., and Stroink, G. (1983) *Rev. Sci. Instrum.* **54,** 879–882.
13. Kirschrink, J. L. (1991) *Bioelectromagnetics* **13,** 401–411.
14. Wieschaus, E., and Nüsslein-Volhard, C. (1986) *in Drosophila*—A Practical Approach (Roberts, D. B., Ed.), IRL Press, Oxford and Washington DC.
15. Cameron, I. L., Hardman, W. E., Winters, W. D., Zimmerman, S., and Zimmerman, A. M. (1993) *J. Cell Biochem.* **51,** 417–425.
16. Levin, M., and Ernst, S. G. (1995) *Bioelectromagnetics* **16,** 231–240.
17. Ubeda, A., Trillo, M. A., Chacón, L., Blanco, M. J., and Leal, J. (1994) *Bioelectromagnetics* **15,** 385–398.
18. Ma, T.-H., and Chu, K.-C. (1993) *Mutat. Res.* **303,** 35–39.
19. Tsurita, G., Ueno, S., Tsuno, N. H., Nagawa, H., and Muto, T. (1999) *Biochem. Biophys. Res. Commun.* **261,** 689–694.
20. Welte, M. A, Duncan, I., and Lindquist, S. (1995) *Genes Dev.* **9,** 2240–2250.
21. Maas, A.-H. (1948) *Wilh. Roux′s Archiv. Entwicklungsmech. Organismen* **143,** 515–572.
22. Bergh, S., and Arking, R. (1984) *J. Exp. Zool.* **231,** 379–391.
23. Foe, V. E. (1989) *Development* **107,** 1–22.
24. Löscher, W., and Mevissen, M. (1997) *in* The Melatonin Hypothesis. Breast Cancer and Use of Electric Power (Stevens, R. G., Wilson, B. W., and Anderson, L. E., Eds.), pp 555–584, Battelle Press, Columbus.
25. McCann, J., Dietrich, F., Rafferty, C., and Martin, A. O. (1963) *Mutat. Res.* **297,** 61–95.