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Exposure of Sprague-Dawley Rats to a 50-Hertz, 100-μTesla Magnetic Field for 27 Weeks Facilitates Mammary Tumorigenesis in the 7,12-Dimethylbenz[*a*]-anthracene Model of Breast Cancer[1]

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ABSTRACT

We have shown previously (W. Löscher *et al.*, Cancer Lett., *71:* 75–81, 1993; M. Mevissen *et al.*, Carcinogenesis (Lond.), *17:* 903–910, 1996) that 50-Hz magnetic fields (MFs) of low [50 or 100 μTesla (T)] flux density enhance mammary gland tumor development and growth in the 7,12-dimethylbenz[*a*]anthracene (DMBA) model of breast cancer in female Sprague Dawley rats. In these previous experiments, groups of rats were given 20 mg of DMBA (four weekly gavage doses of 5 mg each) and were MF- or sham-exposed for 13 weeks. The objective of the present study was to examine whether the use of a lower dose of DMBA (10 instead of 20 mg per rat), MF exposure of the rats before DMBA injection, and the increase of the MF exposure period after DMBA application to 26 weeks enhance the effect of MF on tumor development and growth. A group 99 rats was exposed to a homogeneous, horizontally polarized 100-μT MF of 50-Hz for 24 h/day for 7 days/week; another group of 99 rats was sham-exposed under the same environmental conditions as the MF-exposed rats. The exposure chambers were identical for MF-exposed and sham-exposed animals. The age of the rats was 45–49 days at the onset of exposure; duration of MF or sham exposure was 27 weeks. DMBA was administered p.o. at a dose of 10 mg/rat after 1 week of MF or sham exposure. The animals were palpated once weekly from week 6 onwards to assess the development of mammary tumors. At the end of the exposure period, the animals were killed for the determination of number and volume and histological verification of mammary tumors. All of the recordings were done in a blinded fashion; *i.e.*, the investigators were not aware which animals were MF- or sham-exposed. Mammary tumor development and growth was significantly enhanced by MF exposure, the most marked effect on tumor incidence (190% above sham control) being observed 13 weeks after DMBA administration. At the time of necropsy, *i.e.*, 26 weeks after DMBA administration, the incidence of histologically verified mammary tumors was 50.5% in controls and 64.7% in MF-exposed rats, the difference being statistically significant. More marked intergroup differences were recorded when tumor incidence was separately evaluated for each of the six mammary complexes, the most pronounced MF effect on tumor incidence being seen in the cranial thoracic complex. The data substantiate that, at least under the experimental conditions used in our laboratory, 50-Hz, 100-μT MF exposure significantly facilitates the development and growth of mammary tumors in the DMBA rat model of breast cancer.

INTRODUCTION

Two products of electric power, light-at-night and electromagnetic fields, can decrease the production of melatonin by the pineal gland and thereby perhaps increase the risk of breast cancer (1–3). This electric power/breast cancer hypothesis, also known as "melatonin hypothesis," has attracted a great deal of interest, in part because it is a plausible explanation for the increased tumor growth upon 50-Hz MF[4] exposure previously seen by two independent groups in chemical models of breast cancer in rats (4–6). In a large series of experiments in female SD rats (cf. Ref. 6), we recently found that, consistent with the melatonin hypothesis, prolonged exposure to 50-Hz MFs at flux densities in the μT-range decreases nocturnal melatonin plasma levels, increases the activity of ODC in breast tissue, impairs immune surveillance, and enhances mammary tumor development and growth in response to the chemical carcinogen DMBA. However, our experiments have been criticized because we did not use a conventional DMBA protocol with one application by gavage but administered DMBA four times at single doses of 5 mg/rat at weekly intervals. This resulted in an incidence of grossly observed mammary tumors of about 40–60% after 13 weeks of sham exposure. MF exposure at 100 μT significantly increased the incidence of mammary tumors observed grossly in female rats by 50% above sham control (5, 7). This finding was reproduced in a subsequent replicate experiment in our laboratory (8). For further evaluation of this acceleration of mammary tumor development and growth by MF exposure, we undertook the present study with a more conventional DMBA dosing protocol, *i.e.*, one intragastric dosing with 10 mg/rat. To enhance the potential of MF exposure to interact with DMBA-induced tumorigenesis, rats were MF-exposed for 1 week before DMBA application. Furthermore, the exposure period was increased to 26 weeks after DMBA application. In addition to evaluating the total number of mammary tumors per animal, we examined whether MF exposure differentially affects the carcinogenic response of glands in different topographic areas. This was prompted by the known differences in susceptibility of different parts of the rat mammary complex to the carcinogenic effect of DMBA (9) and the recent observation that MF exposure of female rats increases ODC primarily in the thoracic glands (10).

MATERIALS AND METHODS

Female SD outbred rats, 39–42 days of age, were obtained from Charles River (Hagemann, Extertal, Germany) and were acclimatized for 6–10 days before used for the experiments. Care of the animals was in accordance with institutional guidelines. After acclimatization, groups of 9 rats/cage were placed into the exposure chambers (for details see Refs. 7, 8) and MF or sham exposure was started for 24 h/day (minus time for weighing, tumor palpation, cage cleaning, cage rotation) 7 days/week for a total duration of 27 weeks. For the MF-exposed rats, field characteristics were 50-Hz, horizontal linear polarization, 100 μT root-mean-square (100 μT = 1 Gauss). The size of groups was 99 for MF exposure and 99 for sham exposure. After 1 week of exposure, when all of the rats were at the age of 52–59 days, each rat received an administration of 10 mg DMBA by gavage, dissolved in sesame oil (1 ml/rat). The two groups of rats (*i.e.*, sham-exposed and MF-exposed) were housed in the same room under controlled conditions of temperature (23–24°C), humidity (about 50%),

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[4] The abbreviations used are: MF, magnetic field; DMBA, 7,12-dimethylbenz[*a*]-anthracene; ODC, ornithine decarboxylase; SD, Sprague-Dawley; TEB, terminal end bud; T, Tesla.

and light (12-h dark/light cycle; light off at 5 p.m.); food (Altromin standard rat diet) and water were available *ad libitum*. Light intensity produced by the artificial white light in the room with the exposure system varied between 30 and 38 lux (measured by a luxmeter in the exposure and sham chambers). In the dark period, the room was weakly illuminated by a dim red light (using four Phillips 15-W darkroom lamps), which produced a light intensity below 1 lux in the exposure and sham chambers. In this respect, it is important to note that dim red light exposure at night, which itself does not inhibit pineal melatonin production, seems to be a necessary predisposing factor for MF to inhibit the melatonin-forming ability of the mammalian pineal gland (11, 12) and has, thus, been used in all of our experiments on the melatonin hypothesis.

No difference between exposure and sham coils regarding noise, vibrations, temperature, or light was evident. The MF in the exposure chambers was measured once a week with an EMDEXC instrument (Electric Field Measurement Co; West Stockbridge, MA) to ensure homogeneity of the field during the course of the experiment. The 50-Hz stray fields in the sham-exposure coils were around 0.1 μT. The static earth MF, measured with a Bell 610 Gaussmeter (F.W. Bell, Inc., Orlando, FL), was about 40 μT, with the generated 50-Hz MF being horizontal and parallel to the horizontal component of the earth's north/south MF (see Ref. 8). Measurement of the electric field in the exposure and sham-exposure chambers with the EMDEXC together with a M115EB handle did not disclose any significant differences between exposed and sham-exposed locations, the electric field varying from 20–60 V/m. All of the field measurements were performed by a person not involved in the animal experiments. In other words, the scientists and technicians involved in handling and treatment of animals and subsequent necropsy and pathological examination of rats were not aware of which group of animals was exposed or sham-exposed, to ensure "blind" conditions during the experiment until all of the results were in.

The exposure parameters described above and several additional parameters important for the exposure conditions were validated and verified at regular intervals by a physicist from another institution (Niedersächsisches Landesamt für Ökologie, Hildesheim, Germany) not involved in the present study. In addition to the verification of the values given above by the use of other instruments, 24-h measurements showed that, under the conditions of the experiment, the MF exposure system produced a stable flux density of 100 μT and stable frequency of 50-Hz with negligible harmonics and no power spikes.

Animals were weighed once a week; cage cleaning was done three times a week; and cage rotation in the exposure chambers was done once a week. Five weeks after the application of DMBA, the animals were palpated once weekly to assess the development of mammary tumors. Each rat was palpated by two observers (S. T. B., M. M.) and only those tumors that were recorded by both observers were used in the final data analysis. The size of palpable tumors was estimated by a rating scale as recently described (13). Furthermore, the location of each tumor among the six mammary complexes of the rat was recorded. Specific mammary glands were identified by site as L(left)1 through L6 and R(right)1 through R6, with 1 being the most cranial and 6 the most caudal gland.

After 27 weeks of MF- or sham-exposure, all of the rats were killed for necropsy. One rat died and four rats had to be necropsied before the end of the exposure period because of large bleeding tumors. These rats were included in the pathological examination. The weight of liver and spleen was recorded in all of the animals before fixation. For preparation of the mammary glands, the skin was opened by a midline incision to expose the six pairs of mammary glands extending from the salivary glands to the perianal region. All of the grossly observed (*i.e.*, macroscopically visible) mammary tumors were recorded, excised, trimmed, and saved for further histopathological analysis. The size of macroscopically visible mammary tumors was measured by a caliper after dissection, and tumor volume was calculated from the length, width, and depth of tumors on the basis of an ellipse. The mammary tumors were then fixed in 4% phosphate-buffered formalin (pH 7.3). The fixative was changed after 24 h. Small tumors were fixed in total or cut in two halves. For large tumors, one to two sections were cut vertically to the surface and to the midline. These tissue samples were embedded in Paraplast, sectioned at 3–4 μm, and stained routinely with H&E. Neoplastic lesions of the mammary glands were classified according to Bader *et al.* (14) and Russo *et al.* (15). The histopathological evaluation was done "blind."

Differences between groups in tumor incidence were determined using the χ^2 test and in the mean number, size, and latency-to-onset of tumors by the Mann-Whitney *U* test. Volume of tumors was calculated as median with range from the first to the third quartile (interquartile range); differences were calculated by the *U* test. Differences in the cumulative proportions of animals with tumors (incidence curves) were calculated by the product-limit method, in which animals that died or were killed without tumors were included as censored, and the difference between groups was tested for statistical significance by product-limit-survival analysis [generalized savage (Mantel-Cox) test]. Differences between groups in body weight and organ weights were calculated by Student's *t* test. For all of the calculations, the SAS and BMDP programs were used. All of the statistical tests were used as two-sided tests; a $P < 0.05$ was considered significant.

RESULTS

Development and Growth of Mammary Tumors. The cumulative proportion of DMBA-treated animals that developed mammary tumors during the period of MF or sham exposure is shown in Fig. 1. The first mammary tumors could be palpated in the MF-exposed group at 7 weeks of MF exposure, *i.e.*, 6 weeks after DMBA application. During the subsequent weeks of exposure, tumor incidence in MF-exposed rats was always above that of sham-exposed rats. Individual differences in incidence of palpable tumors between the two groups were statistically significant at 13 ($P = 0.029$), 14 ($P = 0.003$), 15 ($P = 0.012$), 17 ($P = 0.035$), and 18 ($P = 0.021$) weeks of exposure. In terms of the magnitude of differences between groups, the largest percent difference was seen after 14 weeks of exposure (*i.e.*, 13 weeks after DMBA application), at which time tumor incidence in the MF group was 190% higher than that in the sham group. The percent differences became less marked during

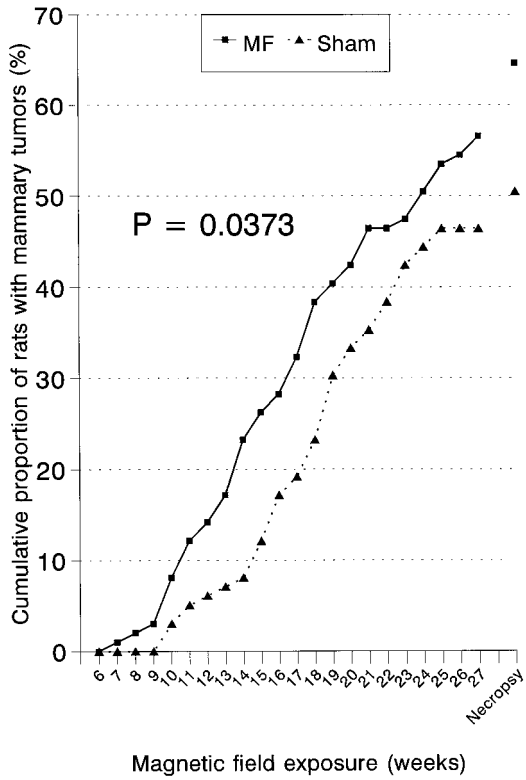

Fig. 1. The cumulative proportion of rats with mammary tumors as a function of duration of MF exposure (incidence curves). DMBA was administered p.o. at 10 mg/rat after 1 week of MF exposure. Group size was 99 rats/group. In addition to the data from palpation (weeks 6–27), the percentage of rats with macroscopically visible (and histologically verified) mammary tumors at necropsy (*i.e.*, after 27 weeks of exposure) is shown. With respect to the tumors palpated before necropsy, only the neoplasms that were subsequently histologically verified as mammary tumors are shown. Statistical evaluation of data from the palpation period by the product-limit-survival analysis gave a *P* of 0.0373, which indicated that the two incidence curves differ significantly.

subsequent exposure. At time of necropsy, *i.e.*, 26 weeks after DMBA application, 64 MF-exposed and 50 sham-exposed rats had developed macroscopically visible (and histologically verified) mammary tumors, the difference being statistically significant ($P = 0.044$). Statistical evaluation of the cumulative proportions of animals with tumors in MF- and sham-exposed groups over the whole period of MF exposure yielded a P of 0.0373 (Fig. 1), which indicated that the two groups differed significantly.

During the 26 weeks after the application of DMBA, palpable mammary tumors were not equally distributed among the six pairs of mammary glands, but most tumors occurred in the thoracic (L/R1-L/R3) glands. The thoracic glands, particularly L/R1 and L/R2 were also those glands in which in most rats the first tumor developed. When the incidence of the first palpable tumor was compared between groups (Table 1), significantly more MF rats (23 of 99) developed their first tumor in the cranial thoracic complexes (L/R1) than sham-exposed rats (7 of 99), the difference being highly significant. Significant intergroup differences were also found in terms of mammary tumor incidence in L/R1 in that more rats of the MF-exposed group than of the sham group developed tumors in that mammary complex, which was significant (P at least < 0.05) at weeks 14–19, week 21, and weeks 24–27 of the exposure period (not illustrated). A similar trend was seen for L/R2, with significantly higher incidence in the MF-exposed group at weeks 11–15 (not illustrated).

Fig. 2 illustrates the cumulative number of mammary tumors in the two group of rats during the 27 weeks of exposure. As could be expected from the incidence curves (Fig. 1), a higher number of mammary tumors was observed in the MF-exposed groups throughout the period of tumor development and growth. At the time of necropsy, a total of 116 mammary tumors appeared in the group exposed to DMBA only, compared with 166 grossly recorded mammary tumors in the MF-exposed group. Again, the most marked intergroup difference in numbers of tumors during MF exposure was seen in the cranial thoracic glands (L/R1), which is illustrated in Fig. 3. During the first 20 weeks of exposure, there were also many more tumors in L/R2 in the exposed *versus* sham-exposed group, but the difference became smaller during subsequent exposure (not illustrated).

The data on both the incidence (Fig. 1) and the cumulative number (Fig. 2 and 3) of mammary tumors may suggest that MF exposure decreased the latency to tumor onset. Thus, after the delay of tumor appearance in the sham exposure controls, tumors developed at virtually the same rate as the MF-exposed group. Calculation of the mean latency-to-onset of the first palpable mammary tumor in each rat for the MF and sham exposure groups resulted in the following findings (Table 2). When latency was calculated independently of the mammary complex in which the first tumor appeared, no significant difference between groups was determined. However, when latency was calculated separately for each of the six mammary complexes in which the first tumor appeared, the tumor latency in rats with first tumor in L/R2 was significantly shorter in MF-exposed rats. Because

Table 1 *Incidence of the first palpable mammary tumor in the six mammary complexes after the application of DMBA in sham- and MF-exposed rats*

Data are from mammary tumors that were subsequently verified histologically at the time of necropsy.

Number of rats with first mammary tumor in	Sham-exposed controls ($n = 99$)	MF-exposed animals ($n = 99$)
L/R1	11	23[a]
L/R2	15	19
L/R3	12	9
L/R4	2	3
L/R5	9	11
L/R6	3	2

[a] Significantly different from control ($P = 0.0382$).

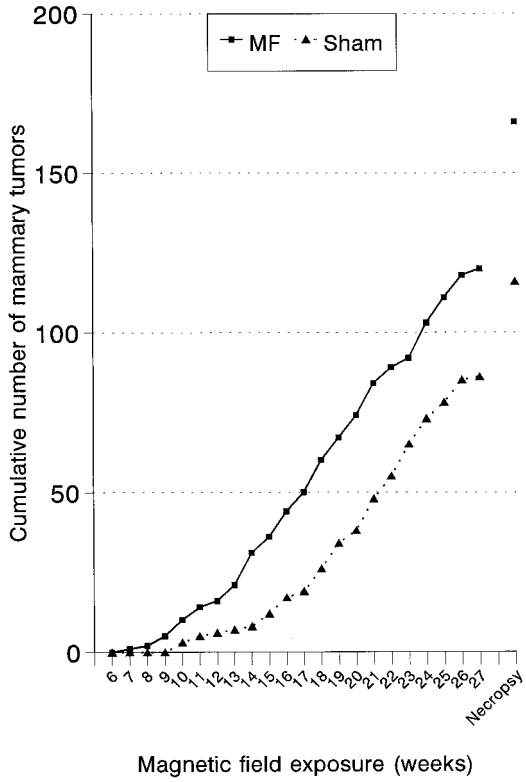

Fig. 2. The cumulative number of mammary tumors as a function of duration of MF exposure. DMBA was administered at 10 mg/rat after 1 week of MF exposure. Data from weeks 6–27 are from palpation, whereas data shown for necropsy relate to numbers of macroscopically visible (and histologically verified) mammary tumors at the time of necropsy (*i.e.*, after 27 weeks of exposure). With respect to the tumors palpated before necropsy, only those neoplasms that were subsequently histologically verified as mammary tumors are shown.

L/R2 was a complex in which many of the first tumors developed in sham controls, the delay in tumor appearance in L/R2 in sham controls may be involved in the differences between incidence curves (Fig. 1), although the higher incidence of tumor development in L/R1 of MF exposed rats (Fig. 3; Table 1) is certainly more important in this respect.

Tumor multiplicity, *i.e.*, mean number of tumors per tumor-bearing rat, is shown in Fig. 4. MF-exposed rats tended to develop more tumors than sham-exposed rats, the difference being significantly different ($P < 0.05$) for 16, 17, 19, and 20 weeks of exposure. At the time of necropsy, no significant difference in tumor multiplicity was seen.

With respect to the size of tumors as estimated by palpation, MF-exposed rats tended to have larger tumors until the 16th week of exposure (Fig. 5), but the difference to sham-exposed rats was only significant at 12 weeks ($P = 0.0427$). After the dissection of tumors, tumor volume tended to be higher in MF-exposed rats (Fig. 5), but the difference was not statistically significant.

Histopathology. The incidence of histologically verified DMBA-induced mammary tumors was 50.5% in sham-exposed and 64.7% in MF-exposed rats, the difference being statistically significant (Table 3). The predominant type of tumors was invasive adenocarcinomas, which were observed in 42.4% of sham-exposed and 52.5% of MF-exposed rats. In both groups, comparable incidences of benign lesions (adenomas or fibroadenomas) were determined. In terms of total numbers of grossly recorded mammary tumors, again the predominant type of neoplasm was adenocarcinoma in both groups of rats (Table 4). All of the types of mammary lesions occurred more frequently in MF-exposed rats (Table 4). Hyperplasia were only found in sham-

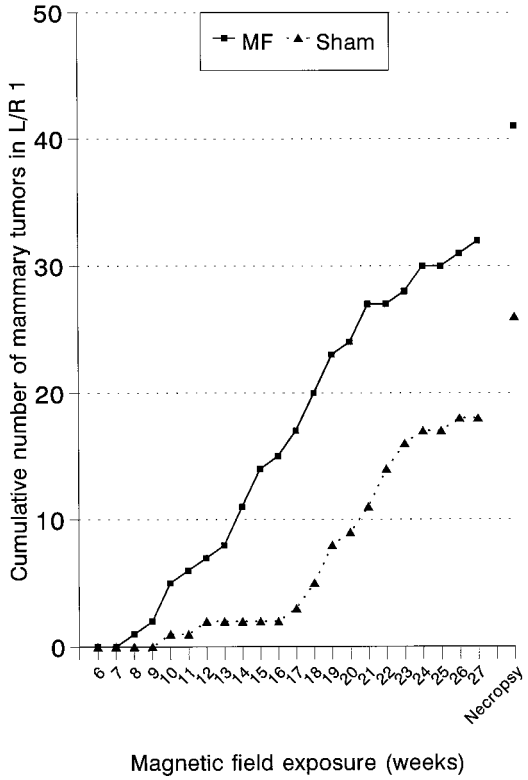

Fig. 3. The cumulative number of mammary tumors in the cranial thoracic mammary complexes (*L/R1*) as a function of duration of MF exposure. DMBA was administered at 10 mg/rat after 1 week of MF exposure. Data from weeks 6–27 are from palpation, whereas data shown for necropsy relate to the numbers of macroscopically visible (and histologically verified) mammary tumors at the time of necropsy (*i.e.*, after 27 weeks of exposure). With respect to the tumors palpated before necropsy, only those neoplasms that were subsequently histologically verified as mammary tumors are shown.

Table 2 *Latency-to-onset of the first palpable mammary tumor after the application of DMBA in sham- and MF-exposed rats*

Data are from mammary tumors that were subsequently verified histologically at the time of necropsy. Two types of calculations were done. Latency-to-first tumor in each rat (independently of the mammary complex in which the first tumor appeared) was used for calculation of the mean (±SD) tumor latency (shown as "overall" latency). In addition, the mammary complex in which the first tumor appeared in each rat was used to calculate tumor latency (±SD) separately for each of the six mammary complexes. The number of rats is given in brackets after each figure. The statistical significance between groups is shown by *P* values, indicating a significant difference between groups of rats in which the first tumor was palpated in L/R2. For group sizes <4, no statistical comparisons were done (nd, not determined). Because some rats developed more than one tumor at the same time, the sum of the number of rats with first tumor in one of the six mammary complexes is not identical to the number of rats shown for overall tumor latency.

	Latency-to-onset of first tumor (days)		
	Sham-exposed controls	MF-exposed rats	Intergroup difference *P*
Overall	131 ± 32 (n = 50)	125 ± 42 (n = 64)	0.375
First tumor in			
L/R1	117 ± 26 (n = 11)	111 ± 40 (n = 23)	0.6334
L/R2	137 ± 31 (n = 15)	106 ± 36 (n = 19)	0.0135
L/R3	129 ± 38 (n = 12)	144 ± 37 (n = 9)	0.3791
L/R4	182 ± 9.9 (n = 2)	147 ± 73 (n = 3)	nd
L/R5	131 ± 26 (n = 9)	149 ± 39 (n = 11)	0.2654
L/R6	124 ± 46 (n = 3)	161 ± 20 (n = 2)	nd

exposed rats; however, because we did not perform serial sections of the mammary glands, the present data on hyperplasia are certainly not reliable.

Other neoplastic and nonneoplastic lesions grossly recorded in the mammary glands of sham- and MF-exposed rats are shown in Table 5. There were no significant differences between the groups.

Intergroup differences were found when histologically verified mammary tumors were evaluated according to the location in the six mammary complexes (Table 6). Thus, significantly more (30 of 99) rats of the MF group had tumors in the cranial thoracic (L/R1) complex compared with sham controls (18 of 99 rats). Tumor multiplicity and tumor volume did not differ between groups in the six mammary complexes.

Other Findings. No differences between groups were seen in body weight gain or general behavior during the period of exposure. Average body weight (± SD) in MF- and sham-exposed groups was 161 ± 6.7 g and 160 ± 6.4 g, respectively, at the onset of exposure and 300 ± 30 g and 307 ± 36 g, respectively, after 27 weeks of exposure. Furthermore, weights of liver and spleen at the time of necropsy did not differ significantly. Liver weights in MF- and sham-exposed rats (mean ± SD) were 9.99 ± 1.45 g and 10.1 ± 1.75 g, respectively. Spleen weights in MF- and sham-exposed groups (mean ± SD) were 0.52 ± 0.12 and 0.56 ± 0.28 g, respectively.

DISCUSSION

The present study substantiates that, at least under the experimental conditions used in our laboratory, 50-Hz, 100-μT MF exposure significantly facilitates development and growth of mammary tumors in the DMBA rat model of breast cancer. Compared with sham controls, the incidence of histologically verified mammary gland tumors observed grossly in female SD rats after 27 weeks of MF exposure was significantly increased by 28%. Previous experiments of our group with 20 mg DMBA/rat (four weekly gavage doses of 5 mg) and 13

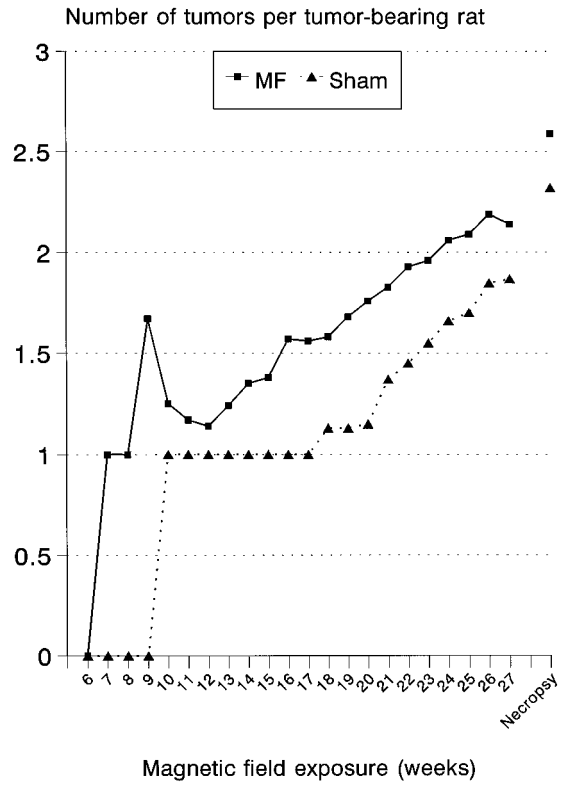

Fig. 4. The mean number of mammary tumors per rat with mammary tumors as a function of duration of MF exposure. DMBA was administered at 10 mg/rat after 1 week of MF exposure. Data from weeks 6–27 are from palpation, whereas data shown for necropsy relate to the numbers of macroscopically visible (and histologically verified) mammary tumors at the time of necropsy (*i.e.*, after 27 weeks of exposure). With respect to the tumors palpated before necropsy, only those neoplasms that were subsequently histologically verified as mammary tumors are shown. The individual number of tumors/rat ranged between 1 and 10. Statistical evaluation of data indicated a significant difference in tumor number/rat between groups for week 16, 17, 19, and 20 ($P < 0.05$).

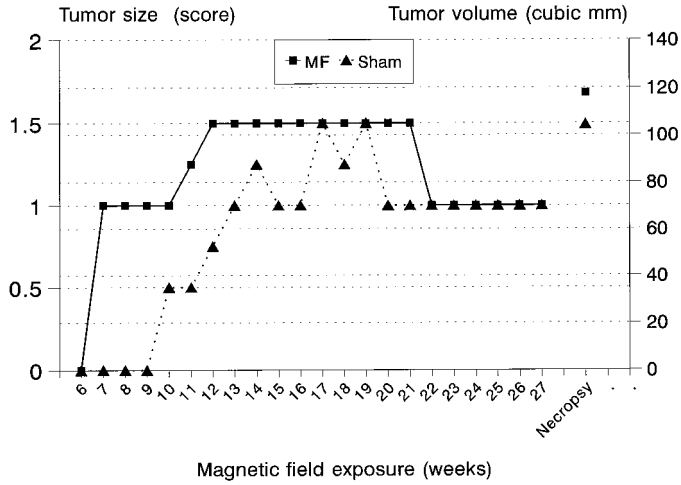

Fig. 5. The size of mammary tumors as a function of duration of MF exposure. DMBA was administered at 10 mg/rat after 1 week of MF exposure. During exposure, tumor size was estimated by palpation, using a scoring system. These data are shown as medians of all of the tumors palpated at the different weeks during exposure (the cumulative numbers of tumors in the MF-exposed and sham-exposed groups of rats are illustrated in Fig. 2). Only those palpable neoplasms that were subsequently histologically verified as mammary tumors are shown. Statistical evaluation of data indicated a significant difference in tumor size between groups only for week 12 ($P = 0.0427$). At the time of necropsy (*i.e.*, after 27 weeks of exposure), the volume of mammary tumors was determined for all of the macroscopically visible (and histologically verified) tumors and is shown as median of 166 tumors in MF-exposed and 116 tumors in sham-exposed groups. Interquartile ranges for tumor volume were 25.65–361.3 mm^3 in controls and 31.7–565.5 mm^3 in MF-exposed rats. Tumor volumes did not differ significantly between groups.

Table 3 *Incidences of grossly recorded mammary gland neoplasias induced by DMBA in sham- and MF-exposed rats*

	Sham-exposed controls ($n = 99$)	MF-exposed animals ($n = 99$)
No. of rats with		
Mammary tumors (total)	50	64[a]
Adenomas	21	24
Fibroadenomas	7	11
Adenocarcinomas	42	52
Hyperplasias	3	0

[a] Significantly different from control ($P = 0.044$).

weeks of MF exposure at 100 μT yielded significant differences to concurrent sham control of 50% (5) and 34% (8). Thus, the present protocol with one 10-mg DMBA application, MF exposure for 1 week before DMBA application, and prolongation of MF exposure to 27 weeks apparently did not increase the effect of MF exposure on breast cancer development and growth, but rather led to a similar effect as obtained with 20 mg DMBA but only 13 weeks of exposure, which indicates that the magnitude of the MF effect depends on both DMBA dose and duration of exposure. However, if the present experiment would have been terminated 13 weeks after DMBA (14 weeks after the initiation of MF exposure) as in our previous experiments, tumor incidence (based on palpation of subsequently verified mammary tumors) would have been 23.2% in MF-exposed compared with 8.1% in sham-exposed rats (Fig. 1), thus indicating that tumor incidence in MF-exposed rats was increased 3-fold ($P = 0.003$). Because tumor incidence in sham controls 13 weeks after application of DMBA with 20 mg of DMBA was substantially higher compared with tumor incidence 13 weeks after 10 mg of DMBA, this may indicate that the magnitude of the MF effect at the same duration of exposure depends on the basal (control) tumor incidence in this model, *i.e.*, the lower the control tumor incidence the higher the increase in tumor incidence by MF exposure. Indeed, when our data from the previous and present experiments with 100 μT MF exposure in the DMBA model are plotted as shown in Fig. 6, there appears to be an inverse relationship between control incidence and the magnitude of the MF effect on tumor incidence 13 weeks after DMBA application.

Additional observations in our previous studies with 100 μT were a significant increase in tumor volume and a higher frequency of malignant mammary tumors in MF-exposed rats, which indicated that MF exposure had affected the progression of DMBA-induced lesions (7), which is in line with observations of Beniashvili *et al.* (4) using nitrosomethylurea to produce mammary tumors in female rats. A tendency toward larger tumors in MF-exposed rats and a higher frequency of invasive adenocarcinoma was also observed in the present study. Likely explanations for these effects of MF exposure on tumor development and growth in the DMBA model are a marked MF-induced increase in ODC in mammary tissue, indicating enhanced proliferation of breast stem cells at risk for malignant transformation (16), and impaired immune surveillance in response to prolonged MF exposure (17). Furthermore, a reduction of melatonin's oncostatic action on mammary tumor growth by MF exposure could be involved (18).

The presence and proliferative state of TEBs at the time of DMBA administration are considered to play an important role in the genesis of carcinomas in the mammary tumor model (9). TEBs, which are composed of an actively proliferating epithelium, are the most actively growing terminal ductal structures in the rat mammary gland, which explains the high susceptibility of the TEB to neoplastic transformation in response to DMBA and other chemical carcinogens (9). Any factor at the time of DMBA treatment that enhances the proliferative state of the mammary epithelium seems important in determining the appearance of carcinomas. Because we recently found that MF exposure enhances ODC in the mammary gland (14) and that this effect is already present after 1–2 weeks of MF exposure at 100 μT (10), we began MF exposure in the present experiments 1 week before application of DMBA.

An interesting finding, not reported before, was that MF exposure affected the development of mammary tumors unequally across the six mammary complexes of the female rat. It has been previously described that not all of the mammary glands respond to the administration of DMBA in the same fashion; tumor incidence in thoracic mammary glands is higher than in the abdominal glands (9, 19–21). This was also found in the present sham-control experiment, in which more than 70% of all of the grossly recorded tumors were found in the three thoracic complexes. This different carcinogenic response is thought to be due to the asynchronous development of mammary glands in different topographic areas; thoracic glands lag behind in

Table 4 *Absolute numbers of grossly recorded mammary gland neoplasias induced by DMBA in sham- and MF-exposed rats*

	Sham-exposed controls ($n = 99$)	MF-exposed animals ($n = 99$)
Mammary tumors (total)	116	166
Adenomas	24	35
Fibroadenomas	8	13
Adenocarcinomas	84	118
Hyperplasias	8	0

Table 5 *Other histopathological findings in the mammary glands of sham- and MF-exposed rats*

	Sham-exposed controls ($n = 99$)		MF-exposed animals ($n = 99$)	
	Number	Incidence	Number	Incidence
Cystic dilatation	2	1/99	1	1/99
Epidermal cysts	20	18/99	14	13/99
Trichofolliculomas	3	3/99	8	7/99
Sebaceous adenomas	2	2/99	6	5/99

Table 6 *Grossly recorded histologically verified mammary tumors in the six mammary glands of sham- and MF-exposed rats*

	Sham-exposed controls ($n = 99$)				MF-exposed animals ($n = 99$)			
Mammary complex	Number	Incidence	Multiplicity (tumors/rat)	Volume (mm^3)	Number	Incidence	Multiplicity (tumors/rat)	Volume (mm^3)
L/R1	26	18/99	1.44 ± 0.7	158 (17–885)	41	30/99[a]	1.37 ± 0.7	212 (39–1393)
L/R2	25	19/99	1.32 ± 0.5	41.9 (17–147)	33	24/99	1.38 ± 0.8	62.8 (29–484)
L/R3	32	22/99	1.45 ± 0.9	105 (37–218)	42	28/99	1.5 ± 0.7	116 (48–314)
L/R4	7	7/99	1	73.3 (13–118)	9	9/99	1	6.3 (4.2–297)
L/R5	21	19/99	1.11 ± 0.3	238 (44–448)	33	24/99	1.38 ± 0.6	189 (73–490)
L/R6	5	5/99	1	572 (452–647)	8	8/99	1	91 (23–3118)

[a] Significantly different from control ($P < 0.05$).

development and retain a higher concentration of TEBs, *i.e.*, the site of origin of mammary carcinomas (9). We have recently found that the cranial thoracic mammary complexes (L/R1) are particularly sensitive to 50-Hz MF exposure at 100 μT in terms of ODC increase (10), which may explain the higher susceptibility of these complexes to cocarcinogenic or tumor-promoting effects of MF exposure seen in the present experiments. To prove whether the cranial thoracic glands also responded to MF exposure more markedly than other mammary complexes in the previous studies of our group with MF exposure in the DMBA model, we reexamined one of our previous studies (7) and found a similar enhanced susceptibility of L/R1 to increased tumor incidence in response to MF exposure as in the present study.[5] Furthermore, the finding of a significantly decreased tumor latency in MF-exposed rats with first tumor in the middle thoracic complexes (L/R2) as determined in the present study indicates that these thoracic mammary complexes exhibit an increased sensitivity to MF effects, too. These data thus strongly indicate that not only the basal (control) tumor incidence (see above) but also the site of origin of mammary carcinoma determine to which extent MF exposure increases mammary tumorigenesis in the DMBA model.

Ekström *et al.* (22) recently reported that intermittent 50-Hz MF exposure at flux densities of 250 or 500 μT with a 15-s-on/15-s-off schedule for 21 weeks did not significantly affect mammary tumor growth in response to intragastric application of 7 mg of DMBA/SD rat. However, the MF exposure scheme was different in the study of Ekström *et al.* (22) and was applied in a strict promotional scheme, *i.e.*, MF exposure was started 1 week after DMBA administration. Furthermore, the subline of SD outbred rats used by Ekström *et al.* (22) was much more sensitive to DMBA than our SD rats, pointing to genetic differences between the two outbred sublines of SD rats used. We have previously shown (23) a linear relationship between flux density and MF effect on tumor incidence in the DMBA model when the effects of flux densities between 1 and 100 μT were evaluated, which could indicate that further increase of flux density as used by Ekström *et al.* (22) would also increase the effect of MF on DMBA-induced mammary tumors. However, as recently demonstrated by us (24), the effect of MF in the DMBA model is lost at higher flux density, indicating a "window effect" of MF exposure in the low μT range.

In a recent draft technical report of the United States Department of Health and Human Services prepared for public review and comment (25), a series of studies on the effects of 50 or 60-Hz, 100 μT MF exposure in the DMBA model in SD rats were described. Different DMBA dosing protocols were used, *i.e.*, four times 5 mg/rat and 13 weeks exposure, four times 2 mg/rat and 13 weeks of exposure, and 1× 10 mg DMBA/rat and 26 weeks of exposure. In none of the experiments were significant MF effects observed. Unfortunately, although the studies were conducted in an attempt to replicate our previous MF studies in the DMBA model, there were various differences from our experiments, including another diet, shorter exposure per day (*e.g.*, 500 h less exposure in 13 weeks), the use of different rooms for sham and MF exposure, differences in the exposure systems, and the use of a subline of SD rats with markedly higher susceptibility to DMBA than our rats. Because of this higher sensitivity to DMBA, two of the three DMBA protocols used in the United States study resulted in almost 100% tumor incidence in sham controls, which prevented obtaining any additional effect by MF exposure (25). Thus, because of these various differences, these experiments cannot be considered as replicate studies of our experiments. It has previously been demonstrated that there are inherent differences between SD rats obtained in the United States and SD rats obtained in Europe in regard to their mammary neoplastic response to DMBA, as well as in their response to radiation (26). The use of different rat sublines and other experimental differences between studies on MF exposure in the DMBA model will eventually allow the evaluation of which environmental and genetic factors are critical for the effects of MF exposure in this model.

In conclusion, the present study demonstrates that, at least under the conditions of our laboratory conditions, MF exposure significantly facilitates mammary tumorigenesis using a standard DMBA protocol as previously used for the evaluation of dietary, hormonal, and envi-

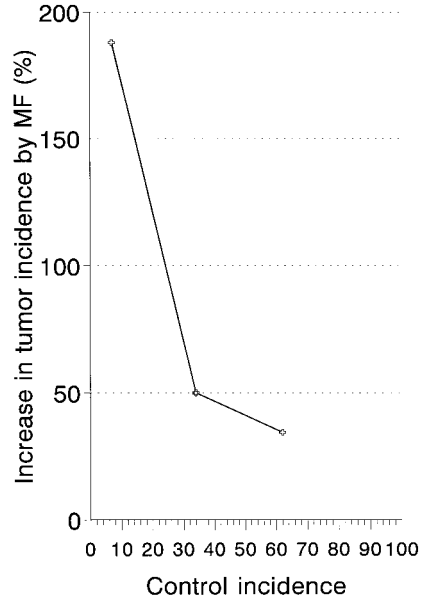

Fig. 6. The relationship between control incidence of mammary tumors and increase in tumor incidence by MF exposure. Data are from three separate experiments with 50-Hz MF exposure at 100 μT. In each experiment, a sham control group of 99 rats was exposed together with a MF group of 99 rats. The sham-control mammary tumor incidence is plotted against the MF-induced increase in mammary tumor incidence determined 13 weeks after the application of DMBA in the same experiment. Data are from the present study (from palpation 13 weeks after DMBA) and two previous studies with higher doses of DMBA (7, 8).

[5] Unpublished observations.

ronmental factors affecting the development and growth of mammary cancer (21, 27, 28) and points to a particular susceptibility of the cranial thoracic part of the mammary complex of the SD rat to MF effects on carcinogenic responses. The latter finding is in line with recent observations that this part of the mammary gland of juvenile SD rats exhibits a unique sensitivity to MF exposure in terms of increased proliferation (10), which we think can be explained by the melatonin hypothesis of the potential association between electric power and breast cancer (6), including the reported interaction between MF exposure and melatonin's functional effects at the cellular level (cf. Ref. 29). At present, it is not possible to predict from our animal studies any human risk of MF exposure, although several residential and occupational studies have indicated an association between EMF exposure and increased female breast cancer risks (30–35). More definite conclusions about an association between MF exposure and increased breast cancer risks have to await replication of our experimental data by other groups and the results of several ongoing prospective epidemiological studies. On the basis of the results of epidemiological studies of childhood leukemia with residential MF exposure and chronic lymphocytic leukemia with occupational MF exposure and following the working procedures and evaluation method of the IARC, a Working Group organized by the National Institute of Environmental Health Sciences (NIEHS) of the United States NIH recently concluded that 50/60-Hz MF are possibly carcinogenic to humans (35). The Working Group further concluded that there is inadequate evidence in experimental animals for the carcinogenicity of exposure to 50/60-Hz MF (35), necessitating more studies with adequate experimental design to address the question of whether animal studies confirm or refute the findings of epidemiological studies. The present experiments, which uncovered several experimental factors that are important in the carcinogenic effect of MF exposure in the DMBA model of breast cancer, further substantiate the electric power/breast cancer hypothesis of Stevens and colleagues (1–3).

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