EFFECTS OF 50 Hz CIRCULARLY POLARIZED MAGNETIC FIELDS ON SPONTANEOUS MAMMARY TUMORS IN RATS.

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ABSTRACT

Several epidemiological studies have suggested that residential or occupational exposure to power frequency magnetic fields (MF) might increase the risk of cancer. The objective of this study is to elucidate the possible carcinogenic effects of MF exposure using female Sprague-Dawley (Crj:CD) rats. A total of 360 rats was randomly divided into 6 groups of 60 rats each. Two groups were served as a negative control (vehicle : sesame oil only) or a positive control (single oral administration of 7,12-dimethylbenz(a)anthracene; DMBA, 90 mg/kg body weight at 50-52 days of age). Other four groups were simultaneously exposed to 0 (sham-exposed), 7, 70 or 350 μT(rms), continuous circularly polarized 50 Hz MF, 22 hrs/day, 7 days/week for 30 weeks from 8 weeks of age. Experiment was conducted under SPF condition and in a blinded manner. Ten animals in each group were served as satellite animals and their several hormonal concentrations in sera, such as melatonin and prolactin, collected at the midnight were measured. In addition, complete histopathological examination were performed in other 50 animals per each group. In the positive control group, the first mammary nodule was palpated at the 7th weeks of experiment in 5 out of 59 animals. Afterward, the incidence of palpable mammary nodules increased steadily and reached at 76% and 98 % of live animals at 14 weeks and the end of experiment, respectively. One out of 60 animals and 2 animals out of 60 animals had a nodule at the end of experiment in sham- and 350 μT-exposed group, respectively. Palpable mammary nodules were not detected in the negative control, 7- and 70 μT-exposed groups throughout the experiment. The incidences of palpable mammary nodules in the MF-exposed groups were not significantly different from those in the sham-exposed and negative control groups. Histopathologocally, most of palpable nodules were mammary tumors. The incidences of animals with mammary tumors per animals survived at the end of experiment were 4.1 and 100 % in the negative and positive control groups, and 0.0, 6.0, 8.0 and 6.0 % in the sham-, 7, 70 and 350 μT-exposed groups, respectively. These incidences in three MF-exposed groups were not significantly different from those in both the sham-exposed and negative control groups. Based on these results, it was not supported that continuous circularly polarized 50 Hz magnetic fields at up to 350 μT affect the incidence of spontaneous mammary tumors in female SD rats under the present experimental conditions.

INTRODUCTION

Several epidemiological studies have suggested an association between the increased risk of cancer and power frequency magnetic fields (MF) (Wertheimer and Leeper, 1979 : Savitz, et al., 1988 : Feychting and Ahlbom, 1993, 1994).

Several experimental studies *in vivo* have been conducted to examine the effects of MF on the incidence of mammary gland tumors. Beniashvili *et al.* (1991) injected *N*-methyl-*N*-nitrosourea (MNU; 50 mg/kg body weight) to female rats intravenously, then exposed to MF, and reported the significantly increased incidence and shorter latent time of mammary tumors in animals exposed to 50 Hz, 20 μT MF 180 min./day for the lifetime of rats than those in cage control animals. Löscher and his colleagues have conducted a series of mammary gland tumor initiation/promotion studies. They initiated female Sprague-Dawley (SD) rats, which is well known that the incidence of spontaneous mammary tumors is very high (Noble and Cutts, 1959), with 7,12-dimethylbenz[a]anthracene (DMBA) by four times' fractionated intragastric administration (5 mg of DMBA per injection) and exposed for 13 weeks, 24 hrs/day, to 50 Hz, up to 100μT horizontal MF. In these studies, they reported the increased mammary tumor incidence in a dose-dependent manner ((Löscher *et al.*, 1993 ; Mevissen *et al.*, 1996a, b) and the growth and the progression of mammary gland tumors due to MF exposure (Baum *et al.*, 1995).

Carcinogenicity study of MF is a few, but each report showed no effect of MF exposure on carcinogenicity during the lifetime of mice or rats (Yasui *et al.*, 1997 ; Mandeville *et al.*, 1997 ; NTP 1998).

The objective of this study is to elucidate the possible carcinogenic effects of MF exposure without chemical carcinogen administration using female Sprague-Dawley (SD ; Crj:CD) rats prior to 50 Hz horizontally or circularly polarized MF initiation/promotion studies.

MATERIALS AND METHODS

EXPOSURE SYSTEM : Identical four MF exposure rooms with barrier system were used in this study. The exposure facility has Merritt-type coils consisting of two orthogonal sets of 4-square bobbins, one set for vertical magnetic field and another for horizontal MF (Shigemitsu et al., 1993). These coils could generate up to about 700 μTrms circularly, or 500 μTrms linearly polarized horizontal or vertical continuous 50 Hz MF. The uniformity of MF flux density inside the coils where animals were kept was less than 3 % to an average field intensity inside the each coil of 2.2 m^3 (1.3m × 1.3m × 1.3m). MF was turned on gradually to avoid the transients. Four wooden shelves were installed in this uniform field space, and the animal cages were set on these shelves. The animal cages on the shelf did not influence the MF flux density applied. The background MF was less than 0.02 μT and the geomagnetic field was about 42 μT. Each facility was located separately, about 11 m apart to reduce mutual stray fields from adjacent facility.

ANIMALS : 396 of SPF Crj:CD (Sprague-Dawley, SD) female rats (SPF) were purchased at 6 weeks of age from Atsugi Breeding Center (Charles River Japan, Ltd.). All animal experimental rooms were barrier system, where temperature was set at 21-25 ° C, relative humidity within 30-70%, air ventilation more than 10 times per hour, and lighting 12 hours per day (7:00-19:00). Each record

certified that these values were maintained within set ones. 360 out of 396 animals were selected to reduce the standard deviation of body weights after acclimatization, then randomly divided into 6 groups of 60 rats each by the stratified body-mass procedure. Ten rats in each group were served as the satellite animals for serological hormonal assay. Two groups were served as a negative control (sesame oil ; vehicle only) or a positive control group (single oral administration of 90 mg/kg body weight of 7,12-dimethylbenz(a)anthracene; DMBA at 50-52 days of age). Rats in these groups were fasted for 20 hours before gastric intubation and then orally administered 6 ml/kg body weight of the vehicle or DMBA in order to make a dosage uniform per the body weights in rats. Other four groups were simultaneously exposed to 0 (sham-exposed), 7, 70 or 350 μT(rms), continuous circularly polarized 50 Hz MF, 22 hrs/day, 7 days/week for 30 weeks from 8 weeks of age. Exposure period was determined because the incidence of spontaneous mammary tumors in female SD rats is little or rare if any during this period (Okada et al., 1981).

OBSERVATION AND EXAMINATION OF RATS DURING 30 WEEKS OF MF EXPOSURE : Clinical observation was performed every day. Body weight and food consumption were measured weekly. All of rats were palpated weekly for the detection of mammary gland nodules. The size of each mammary gland nodule was recorded when detected. These observation, measurement and mammary nodule palpation were conducted when the MF exposure was off for the concurrent animal care(10:00-12:00 am).

EXAMINATION AFTER AUTOPSY : At the end of the experiment, animals except for the satellite were anesthetized with ether and the blood samples were collected from the abdominal aorta. At autopsy macroscopic lesions were recorded, and brain, thymus, liver, kidneys, adrenals and spleen were weighed. Hematology and blood serum chemistry evaluation was performed at the termination of the experiment.

HISTOPATHOLOGY : For the microscopic examination, tissues except for the satellite animals were fixed in 10 % neutral buffered formalin, dehydrated, embedded in paraffin, thin-sectioned, and stained with hematoxylin and eosin. Mammary gland lesions were classified into the non-neoplasm, pre-neoplasm and neoplasm histopathologically. Other tissues and organs were also examined histopathologically.

HORMONAL ASSAY : Ten satellite animals per each group in the proestrus stage were sacrificed by decapitation without anesthesia at 4.5 to 5.0 hours into dark cycle (between 23:30 and 24:00) at the termination of the experiment under the dim red light condition (less than 0.2 lux). When the estrus cycle of animals was out of order, blood was collected on the fifth day from the termination of the experiment. Animals were then discarded after the macroscopic observation of mammary gland nodules. The sera were analyzed for melatonin, estrogen (estradiol), progesterone, and prolactin.

RESULTS

CLINICAL FINDINGS : No animal died in 4 MF-exposed groups during the experiment. One and 12 animals died in the negative and positive control groups, respectively. The latter was observed mainly in the late stage of the experiment. : The incidences of clinical findings in 4 MF-exposed groups were few and similar to those in the negative control group. In the positive control group, pallor appeared at 15

weeks and its incidences from 22 weeks were significantly higher than those in the negative control and MF-exposed groups.

BODY WEIGHT AND FOOD CONSUMPTION : Body weight gain in the MF-exposed and negative control groups were comparable throughout the experiment, but the mean body weights in the positive control group were significantly lower than those in other groups from one week to the termination of the experiment (data not shown). In some cases, significant differences of the food consumption were observed between the negative control and MF-exposed groups, but were not consistent and considered to be biologically relevant, while those in the positive control group were lower than that in the negative control group throughout the experiment and occasionally showed significant values (data not shown).

ORGAN WEIGHTS : Organ weights of liver, kidneys, adrenals and heart in the positive control group were significantly higher than those in the negative control group, while that of thymus was significantly lower. Weights of spleen in this group were about three-fold compared with those in other groups, but not significant because of its extremely high standard deviations (data not shown). Furthermore, the similar tendency was observed in the relative organ weight to the body weights.

HEMATOLOGY AND BLOOD SERUM CHEMISTRY : In the positive control group, significantly increased values were observed in MCV, MCH, WBC and the percent of neutrophils, monocytes in WBC, while significantly decreased values were found in RBC, Hb, Ht, MCHC and the percent of lymphocytes (Table 1). In the blood serum chemistry examination, several parameters in the MF-exposed groups, such as the concentration of glucose increased and the values of creatinine and urea nitrogen decreased significantly when compared with those in the negative control group. These data among 4 MF-exposed groups, however, were not significantly different, so was considered to be due to the differences of micro-environment for animal keeping atmosphere.

MAMMARY NODULES AND HISTOPATHOLOGICAL FINDINGS : In the positive control group, the first nodule was palpated at the 7th weeks of experiment in 5 out of 59 animals. Afterward, the incidence of palpable mammary nodules increased steadily and reached at 76% and 98 % of live animals at 14 weeks and the end of experiment, respectively. One out of 60 animals and 2 animals out of 60 animals had a nodule at the end of experiment in the sham- and 350 μT-exposed group, respectively. Palpable mammary nodules were not detected in the negative control, 7- and 70 μT-exposed groups throughout the experiment (Fig. 1, 2). The incidences and the number of palpable mammary nodules in the MF-exposed groups were not significantly different from those in the sham-exposed and negative control groups. Histopathologocally, most of palpable nodules were mammary tumors (Table 2, 3, 4). The incidences of animals with mammary tumors per animals survived at the end of experiment were 4.1 and 100 % in the negative and positive control groups, and 0.0, 6.0, 8.0 and 6.0 % in the sham-, 7, 70 and 350 μT-exposed groups, respectively. Adenocarcinoma was the most frequent tumor (97% of live animals), following fibroadenoma (44%), adenoma (28%) and carcinosarcoma (15%) in the positive control group. In the negative control and MF-exposed groups except for the sham-exposed group (0.0%), 2 to 4 animals had a mammary tumor which was adenocarcinoma, adenoma or fibroadenoma (Photo 1-7). These incidences in the MF-exposed groups were not significantly different from those in both the sham-

exposed and negative control groups. Furthermore, the incidences of non-neoplastic or pre-neoplastic mammary gland lesions (hyperplasia) in the MF-exposed groups were not significantly different from those in both the sham-exposed and negative control groups. Several kinds of tumors, such as pituitary adenomas, skin squamous cell carcinoma, Zymbal's gland adenomas, were observed in each group, but the incidences were very low and not significantly different between groups. Other non-neoplastic lesions were also observed in the 4 MF-exposed groups and the negative control group, but the incidences were very low and not significantly different between groups except for the positive control group.

Hormonal assay : Melatonin concentration in sera in the positive control group was significantly decreased compared with that in the negative control group, however, those in the 4 MF-exposed groups were not significantly different from that in the negative cintrol group (Table 5). The concentrations of estrogen (estradiol), progesterone, and prolactin were not significantly different between 6 groups.

DISCUSSION

In this study, 4 groups of female SD rats were exposed to circularly polarized 50 Hz MF at up to 350 μ T for 30 weeks to elucidate the possible carcinogenic effects of MF exposure without chemical carcinogen administration. In addition, one group was administered 90 mg/kg BW of DMBA orally and served as a positive control group, another group was administered sesame oil and served as a negative control group. In the positive control animals, the administration of DMBA caused not only the significantly increased incidence of mammary gland tumors, but also hematological, blood serum chemical parameters and the melatonin concentration in sera. These changes seemed to occur due to the incidence of mammary gland tumors and the toxicity of DMBA administered. These parameters in animals exposed to 0 (sham), 7, 70 and 350 μT MF were not significantly different both between groups and compared with those in the negative control group.

Carcinogenicity study of MF is a few, but each report showed no effect of MF exposure on carcinogenicity using F344 rats and/or B6C3F1 mice during the lifetime of animals (Yasui *et al.*, 1997 ; Mandeville *et al.*, 1997 ; NTP 1998). In the present study, the incidences of mammary gland tumors were not affected by MF exposure, even if the exposure duration was 30 weeks.

Based on these results, it was not supported that continuous circularly polarized 50 Hz magnetic fields at up to 350 μT affect the incidence of spontaneous mammary tumors in female SD rats under the present experimental conditions.

ACKNOLEDGEMENTS

This work was funded by the the Agency of Natural Resources and Energy, the Ministry of International Trading and Industry (MITI), Japan. We acknowledge the members of the Peer Review Committee (Dr. M. Kato, Chairman, Professor emeritus, Hokkaido University ; Drs. S. Ueno, Professor, University of Tokyo ; T. Kusama, President, Oita University of Nursing and Health Sciences ; H. Otsu, Advisor for the Research, Institute for Environmental Sciences ; A. Maekawa, Director, Department of Pathology, Sasaki Institute ; H. Tanooka, Research advisor, CRIEPI).

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Table 1. Hematological Results of Animals at the end of Experiment (Mean±SD)

Group	Number of Animals	RBC (10^4/mm^3)	HGB (g/dL)	HCT (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	WBC (10^2/mm^3)
MF[a] 0 μTrms	50	752± 41.1	13.9±0.60	43.2±2.07	57.5± 2.08	18.5±0.62	32.2±0.70	83.9± 23.27
MF 7 μTrms	50	750± 35.5	14.0±0.58	43.3±1.97	57.8± 2.05	18.6±0.63	32.3±0.93	74.8± 22.99*
MF 70 μTrms	50	757± 35.1	14.0±0.68	43.4±2.18	57.3± 1.79	18.5±0.57	32.2±0.89	78.0± 20.09
MF 350 μTrms	50	751± 36.6	13.9±0.61	43.2±1.92	57.6± 2.21	18.6±0.64	32.3±0.83	75.4± 22.09*
Negative Control	49	742± 40.7	13.7±0.58	42.5±2.07	57.3± 1.75	18.5±0.56	32.3±0.61	86.9± 19.65
Positive Cpntrol[b]	39	577±174.8**	11.1±3.16**	35.9±8.39**	64.4±10.42**	19.4±1.97**	30.4±2.60**	450.8±1308.28**

PLT (10^4/mm^3)	Differential leukocyte counts (%)						
	Neutro		Lympho	Mono	Eosino	Baso	Others
	Band	Seg					
94.6±15.49	1.2±0.82	15.6± 5.46	80.2± 6.01	1.4±0.81	1.4±0.77	0.2±0.32	0.0± 0.00
94.4±17.01	1.0±0.98	19.6± 7.51**	76.3± 7.89**	1.3±0.71	1.6±1.04	0.2±0.31	0.0± 0.00
94.1±14.02	1.1±0.75	16.9± 6.59	79.1± 7.09	1.2±0.50	1.6±1.07	0.2±0.31	0.0± 0.00
93.2±11.93	0.8±0.68	17.7± 8.42	78.5± 8.47	1.3±0.74	1.4±1.08	0.3±0.39	0.0± 0.00
92.8±13.35	1.0±0.80	14.5± 6.07	81.9± 6.32	1.1±0.61	1.3±0.91	0.2±0.33	0.0± 0.00
94.5±48.13	1.6±1.01**	29.6±16.93**	63.7±19.19**	1.6±0.78**	0.7±0.66*	0.2±0.36	2.6±16.01[c]

a : 50 Hz Citcularly Polalized Magnetic Field Exposure
b : DMBA 90.0 mg/kg.BW
c : Large Lymphatic Leukocyte wity Atypia
* : $P < 0.05$ (vs negative control group)
** : $P < 0.01$ (vs negative control group)

Table 2. Histopathological Findings of Mammary Glands in Rats at the End of the Experiment.

Findings \ Group	MF[a] 0 μTrms (Sham)		MF 7 μTrms		MF 70 μTrms		MF 350 μTrms		Negative Control		Positive Control[b]	
No. of Rats	50		50		50		50		49		39	
	Number	(%)										
Nonneoplastic Lesions												
Hemorrhage	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.0)	7	(17.9) *
Necrosis	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	19	(48.7) **
Granuloma	0	(0.0)	1	(2.0)	0	(0.0)	3	(6.0)	3	(6.1)	1	(2.6)
Inflammatory cell Infiltration	0	(0.0)	0	(0.0)	1	(2.0)	1	(2.0)	0	(0.0)	26	(66.7) **
Galactocele	2	(4.0)	5	(10.0)	5	(10.0)	7	(14.0)	4	(8.2)	2	(5.1)
Preneoplastic Lesions												
Focal Hyperplasia	4	(8.0)	6	(12.0)	4	(8.0)	6	(12.0)	5	(10.2)	13	(33.3) **
Focal Hyperplasia with atypia	1	(2.0)	4	(8.0)	0	(0.0)	5	(10.0)	1	(2.0)	3	(7.7)
Neoplastic Lesions												
Adenoma	0	(0.0)	1	(2.0)	1	(2.0)	0	(0.0)	0	(0.0)	11	(28.2) **
Adenocarcinoma	0	(0.0)	0	(0.0)	1	(2.0)	2	(4.0)	2	(4.1)	38	(97.4) **
Fibroadenoma	0	(0.0)	2	(4.0)	2	(4.0)	1	(2.0)	0	(0.0)	17	(43.6) **
Fibrosarcoma	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.6)
Carcinosarcoma	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	6	(15.4) **

a : 50 Hz Circularly Polarized Magnetic Field.
b : DMBA 90.0 mg/kg Body Weight.

* : P<0.05 (vs Negative Control).
** : P<0.01 (vs Negative COntrol).

Table 3. The Incidence (%) of Mammary Gland Lesions in Rats at the End of the Experiment

Findings \ Group	MF[a] 0 μTrms (Sham)		MF 7 μTrms		MF 70 μTrms		MF 350 μTrms		Negative Control		Positive Control[b]	
No. of Rats	50		50		50		50		49		39	
	Number	(%)										
Incidence of Nonneoplastic Lesions	2	(4.0)	5	(10.0)	5	(10.0)	7	(14.0)	4	(8.2)	29	(74.4) **
Incidence of Preneoplastic Lesions	5	(10.0)	7	(14.0)	4	(8.0)	8	(16.0)	5	(10.2)	14	(35.9) **
Incidence of Neoplastic Lesions	0	(0.0)	3	(6.0)	4	(8.0)	3	(6.0)	2	(4.1)	39	(100.0) **
Incidence of Rats with Mammary Gland Lesions	6	(12.0)	9	(18.0)	8	(16.0)	10	(20.0)	8	(16.3)	39	(100.0) **

a : 50 Hz Circularly Polarized Magnetic Field.
b : DMBA 90.0 mg/kg Body Weight.

* : P<0.05 (vs Negative Control).
** : P<0.01 (vs Negative COntrol).

Table 4. Preneoplastic and Neoplastic Lesions of Rat Mammary Glands at the End of Experiment.

Group	MF[a] 0 μTrms (Sham)	MF 7 μTrms	MF 70 μTrms	MF 350 μTrms	Negative Control	Positive Control[b]
Findings No. of Rats	50	50	50	50	49	39
Preneoplastic Lesions (Total)						
Focal Hyperplasia	4	11	8	15	13	14
Focal Hyperplasia with Atypia	1	8	0	8	2	4
Total	5	19	8	23	15	18
Number of Lesions per Rat						
Focal Hyperplasia	0.08 ± 0.274 c	0.22 ± 0.737	0.16 ± 0.618	0.30 ± 1.111	0.27 ± 1.036	0.36 ± 0.537 *
Focal Hyperplasia with Aty	0.02 ± 0.141	0.16 ± 0.584	0.00 ± 0.000	0.16 ± 0.548	0.04 ± 0.286	0.10 ± 0.384
Total	0.10 ± 0.303	0.38 ± 1.193	0.16 ± 0.618	0.46 ± 1.358	0.31 ± 1.278	0.46 ± 0.682 **
Neoplastic Lesions (Total)						
Adenoma	0	1	1	0	0	13
Adenocarcinoma	0	0	1	4	2	197
Fibroadenoma	0	2	2	1	0	40
Fibrosarcoma	0	0	0	0	0	1
Carcinosarcoma	0	0	0	0	0	10
Total	0	3	4	5	2	261
Number of Tumors per Rat						
Adenoma	0.00 ± 0.000	0.02 ± 0.141	0.02 ± 0.141	0.00 ± 0.000	0.00 ± 0.000	0.33 ± 0.621 **
Adenocarcinoma	0.00 ± 0.000	0.00 ± 0.000	0.02 ± 0.141	0.08 ± 0.444	0.04 ± 0.200	5.05 ± 3.371 **
Fibroadenoma	0.00 ± 0.000	0.04 ± 0.198	0.04 ± 0.198	0.02 ± 0.141	0.00 ± 0.000	1.03 ± 1.769 **
Fibrosarcoma	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.03 ± 0.160
Carcinosarcoma	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.26 ± 0.850 **
Total	0.00 ± 0.000	0.06 ± 0.240	0.08 ± 0.274	0.10 ± 0.463	0.04 ± 0.200	6.69 ± 3.122 **
Number of Tumors per Tumor-Bearing Rat						
Adenoma	None	1.00	1.00	None	None	1.18 ± 0.603
Adenocarcinoma	None	None	1.00	2.00 ± 1.414	1.00	5.18 ± 3.311
Fibroadenoma	None	1.00	1.00	1.00	None	2.35 ± 2.029
Fibrosarcoma	None	None	None	None	None	1.00
Carcinosarcoma	None	None	None	None	None	1.67 ± 1.633
Total	None	1.00	1.00	1.67 ± 1.155	1.00	6.69 ± 3.122
Metastasis (Total)	0	0	0	0	0	5

a : 50 Hz Circularly Polarized Magnetic Field.
b : DMBA 90.0 mg/kg. Body Weight
c : Mean±SD.
* :P<0.05 (vs Negative Control).
**:P<0.01 (vs Negative Control).

Table 5. Endocrinolpgical results in sattelite animals.

Group	Number of animals	Melatonin (pg/mL)	Estradiol (pg/mL)	Progesterone (ng/mL)	Prolactin (ng/mL)
MF[a] 0 μTrms	10	35.2 ± 12.43	25.2 ± 20.80	12.2 ± 12.41	75.0 ± 145.28
MF 7 μTrms	10	49.4 ± 13.49	58.8 ± 92.55	13.8 ± 10.09	35.4 ± 14.73
MF 70 μTrms	10	47.3 ± 20.75	57.2 ± 86.40	12.3 ± 6.72[c]	319.3 ± 886.55
MF 350 μTrms	10	35.5 ± 12.96	23.1 ± 16.15	17.8 ± 19.29	25.7 ± 8.10
Negative Control	10	54.8 ± 22.08	19.3 ± 9.39	12.2 ± 11.32	42.8 ± 56.95
Positive Control	8	29.0 ± 22.53 *	22.6 ± 22.77	13.7 ± 14.11	106.9 ± 170.36

a : 50 Hz Circularly Porilized Magnetic Field.
b : DMBA 90.0 mg/kg Body Weight.
c : n=9
* : P<0.05 (vs Negative Control).

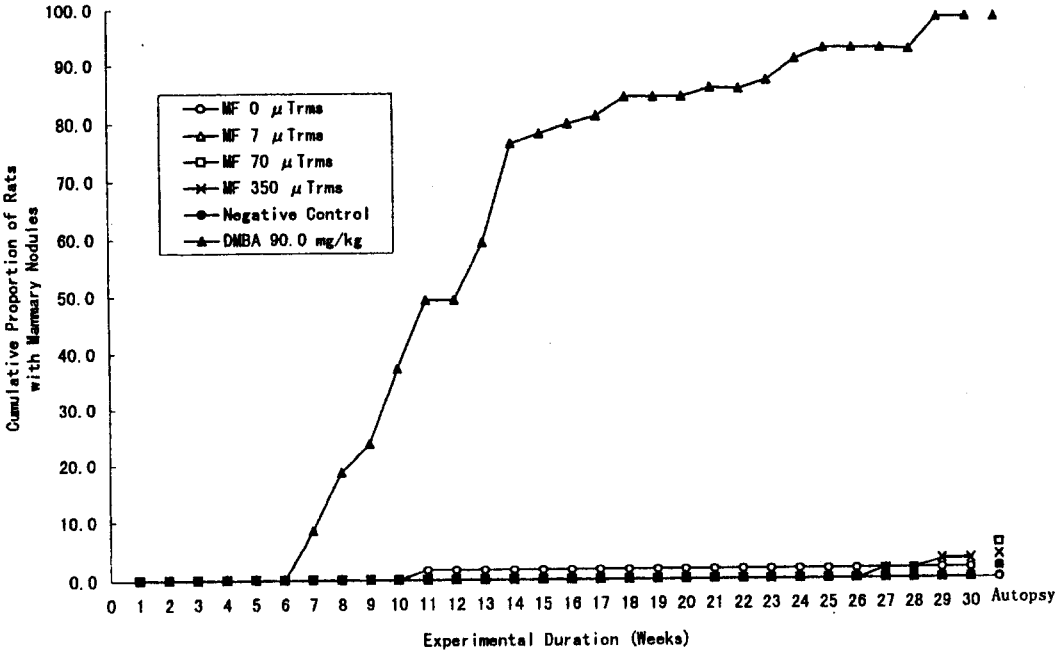

Fig. 1. Cumulative Proportion of Rats with Palpable Mammary Gland Nodules in Carcinogenicity Study.

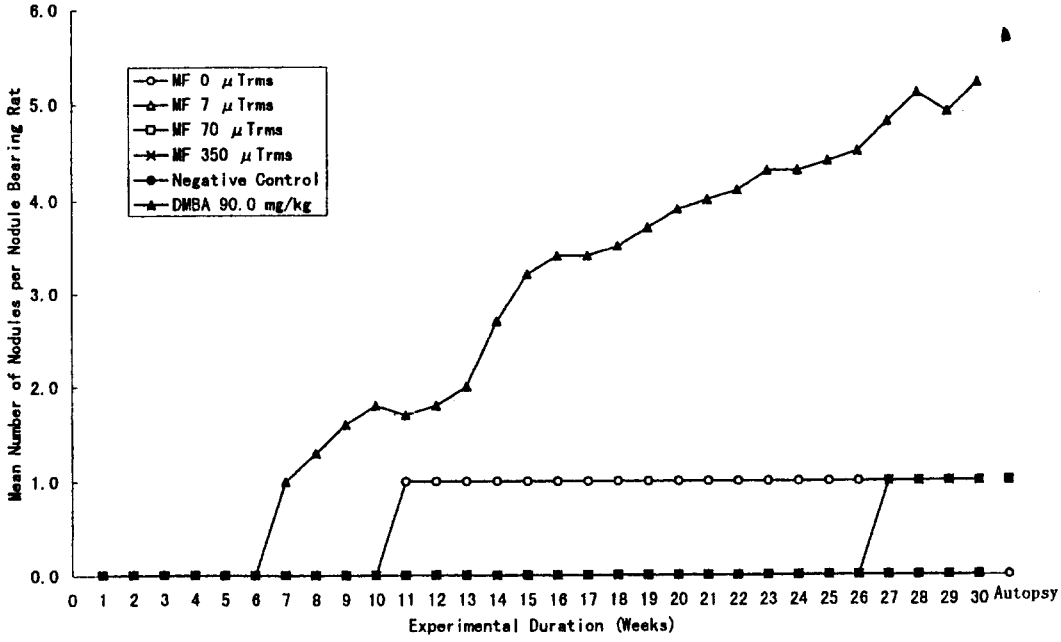

Fig. 2. Mean Number of Mammary Gland Nodules per Nodule-Bearing Rat in Carcinogenicity Study


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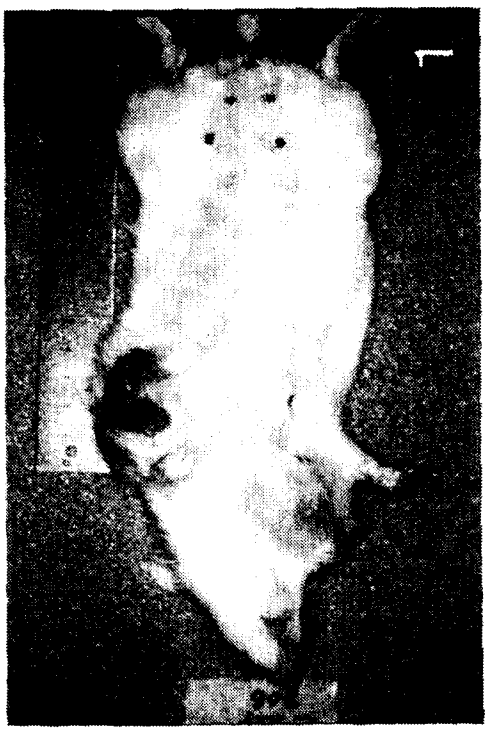
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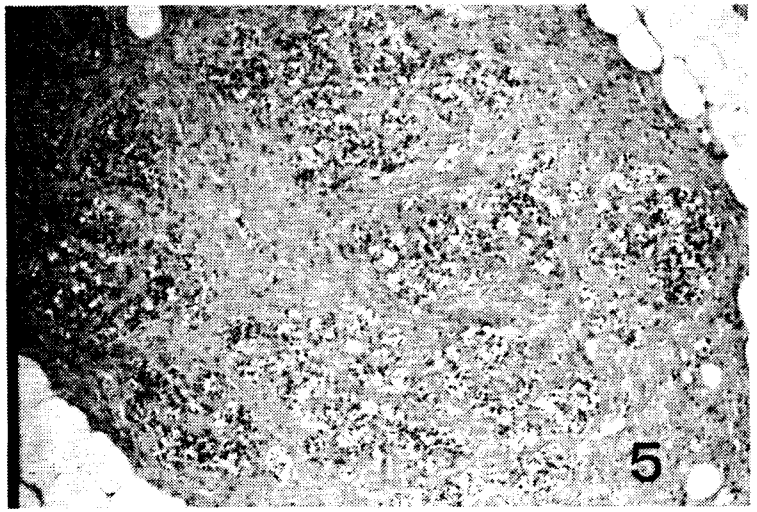

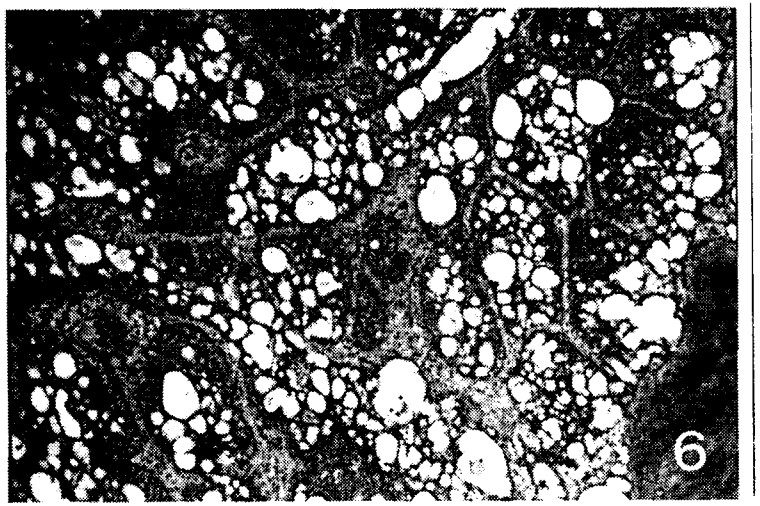

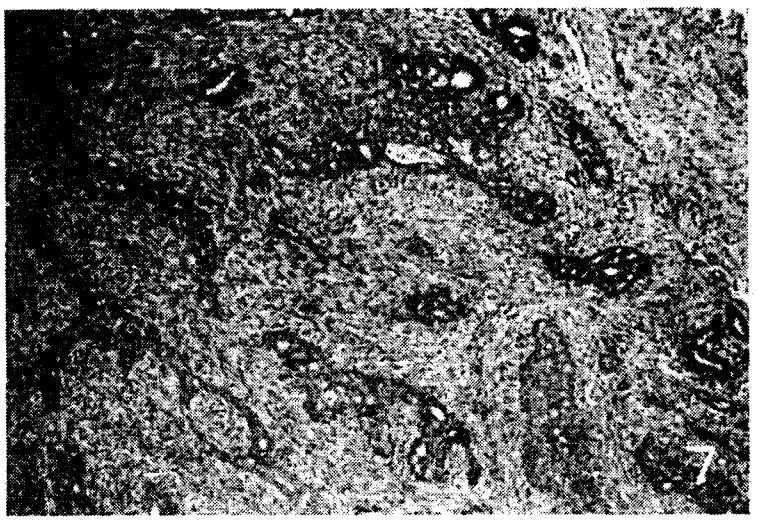

Photo 1. External appearance of tumor-bearing rat.
Photo 2. Internal appearance of tumor-bearing rat.
Photo 3. Normal mammary gland in negative control. HE stain, ×100.
Photo 4. Adenocarcinoma (tubular type), HE stain, ×100.

Photo 5. Fibroadenoma, HE stain, ×100.
Photo 6. Adenocarcinoma (cribriform type), HE stain, ×100.
Photo 7. Carcinosarcoma, HE stain, ×100.