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Acceleration of the Development of Benzopyrene-Induced Skin Cancer in Mice by Microwave Radiation

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Summary. Development and growth of skin cancer may be affected by various physical and chemical factors present in human environment. Of these factors electromagnetic radiation of radio- and microwave spectra are among the most common. In the present study Balb/c mice were exposed to chemical carcinogen, 3,4-benzopyrene, painted on the skin every 2nd day for a total of 6 months, and simultaneously irradiated with athermal (5 mW/cm^2) or subthermal (15 mW/cm^2) doses of 2,450 MHz microwaves. The other group of animals was preirradiated with microwaves at 10 mW/cm^2 power level for 1, 2, or 3 months and then treated with benzopyrene, as above. Control mice were exposed for 6 months to benzopyrene, resulting in the development of baso- or spinocellular skin carcinoma within approximately 9 months, and sham-irradiated with microwaves. The growth of the tumour was assessed according to a self-designed 7-range macroscopic scale, supported by microscopical examinations of skin sections.

All protocols of microwave irradiations resulted in a significant acceleration of the development of benzopyrene-induced skin cancer and in shortening of life span of the tumour-bearing hosts. This effect seemed to be dose-dependent since subthermal doses (15 mV/cm^2) and longer (3 months) expositions to microwaves were more efficient as compared to athermal doses (5 mW/cm^2) and shorter preirradiations. In addition, low-level, long-lasting exposure to microwaves led to a marked suppression of delayed hypersensitivity of mice treated with benzopyrene, as assessed by their reactivity to dinitrofluorbenzene (DNFB). It is suggested that the observed co-carcinogenic effect of microwave radiation may, at least in part, result from the inhibitory action of microwaves on cellular immune reactions of exposed animals.

Key words: Chemical carcinogenesis – Skin cancer – Microwaves

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Introduction

Several chemical substances present in the human environment are known as potent carcinogens capable of inducing skin cancer. These substances include aromatic hydrocarbons derived from coal tar, of which benzopyrene is one of the most active.

The other group of environmental "cancer-risk factors" comprises various physical and chemical agents, which per se do not induce neoplastic transformation of cells, but may accelerate this process, when already initiated by a given carcinogen, e. g., benzopyrene. These agents, called co-carcinogens [6, 14, 19, 20], seem to be more numerous in the environment than are classical carcinogenic factors.

Among the most common physical pollutants of human environment are various forms of electromagnetic energy, including radiations of microwave, radiowave, infrared, UV and ionizing spectra. In recent years experimental studies have focused on the biological effects of radio- and microwaves due to the rapidly growing occupational and environmental exposition to these frequencies [3, 10, 17].

Microwaves comprise a rather small fraction of electromagnetic spectrum, which in terms of a wave length ranges from 300 to 300,000 MHz. Energy of a quantum of this radiation is several thousand times too small to cause ionization of molecules, and thus microwaves are classified among the non-ionizing portion of electromagnetic radiation. Despite numerous studies on the biological effects and health hazards of microwaves (for review see [2] and [12]), no unequivocal data exist concerning their possible co-carcinogenic activity in animals and men.

In view of this, we attempted to evaluate the effect of long-term low-level microwave radiation on the development of skin cancer in mice chronically exposed to 3,4-benzopyrene. For irradiations athermal or subthermal doses of 2,450 MHz microwaves, frequently encountered in the occupational environment, were used throughout the experiments. The obtained results clearly indicate that microwave irradiation may, at least in our experimental system, stimulate the development of chemically induced skin cancer.

Material and Methods

Animals

All experiments were carried out on 600 adult male Balb/c mice, obtained from the breeding laboratory of the Institute of Hygiene and Epidemiology, Warsaw, Poland. Mice were housed in plastic cages, 10 mice per cage, and were fed a standard diet and water ad libitum.

Experimental Skin Cancer System

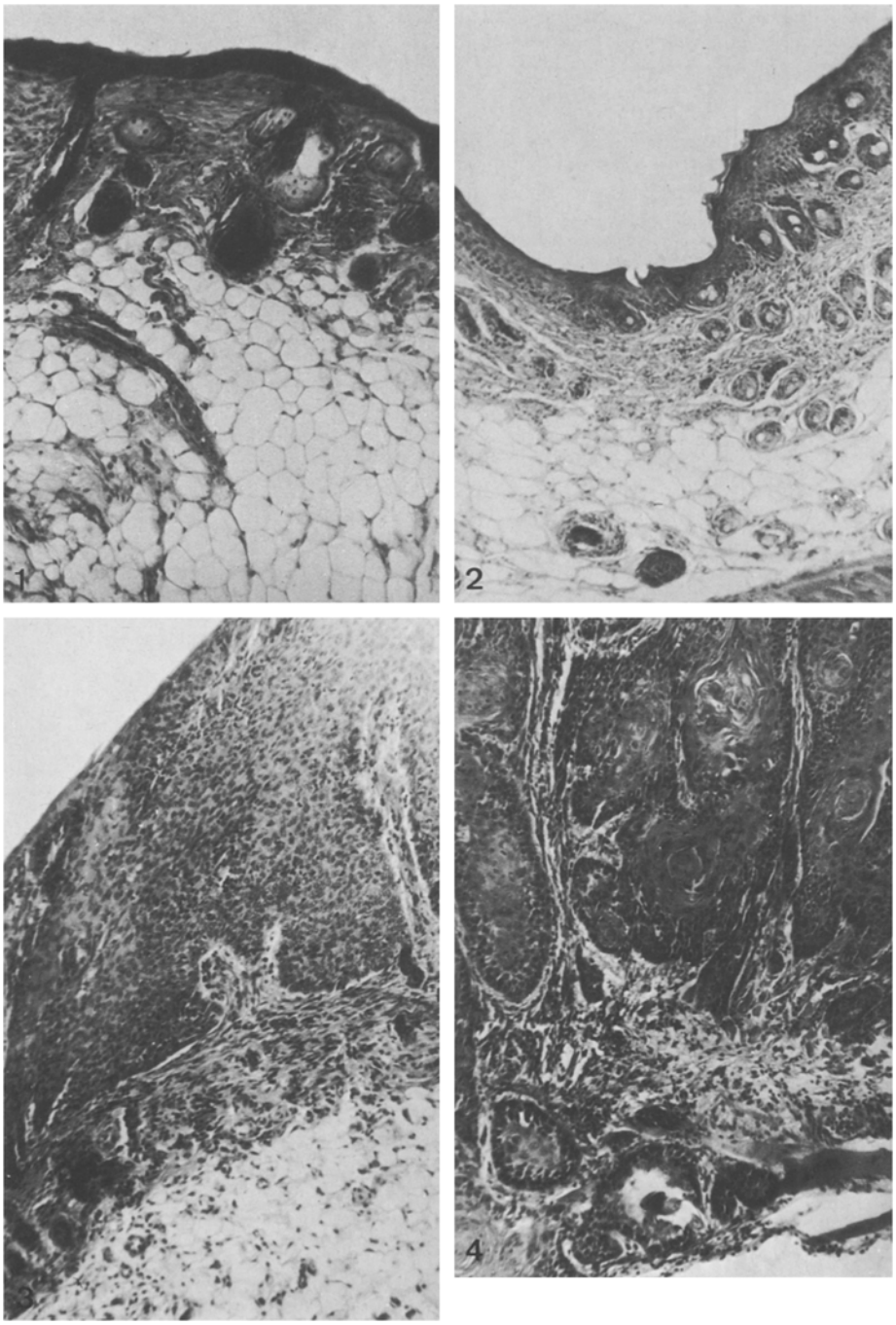
For induction of skin cancer in mice a 1% solution of 3,4-benzopyrene (Koch-Light Laboratories, Colnbrook, UK), in acetone and benzene (1:9) was used in the following manner: about 1 cm^2 of skin surface on the back of a mouse was shaved and 2 days later the first 0.01 ml of benzopyrene solution was dropped onto the shaved area and allowed to dry. The same dose of carcinogen was applied every 2 nd day for up to 6 months. This resulted in the development of skin cancer in 95% of animals after about 9 months. For the assessment of tumour induction and growth a 7-range scale of macroscopical skin changes was developed so that each pathological appearance on the surface of the exposed skin was closely related to microscopical images of appropriate skin sections, as summarized in Table 1. For

Table 1. Macroscopic and microscopic assessment of the development of skin cancer in mice chronically exposed to 3,4-benzopyrene

Weeks of exposition to benzopyrene	Macroscopic assessment		Histopathological assessment
	Stage	Skin change	
0– 4	0	None	Normal skin
4–10	1	Desiccation of skin surface	Thickening of epidermis (acanthosis and granulosis), increased amount of PAS-positive material and of collagen fibres in subepidermic area; intract basal membane
10–24	2	Desquamation and chemical epilation	Thickening of epidermis, atrophy of additional stuctures of skin, slight increase in PAS-positive material, marked thickening of collagen fibres in true skin; intact basal membrane
24–32	3	Intensive keratosis, inflammatory infiltration	Acanthosis, pappillomatosis, infiltration by lymphoid cells; focal increase of PAS-positive material, further thickening of collagen fibres in true skin; intact basal membrane
32–42	4	Small papillomas	Significant thickening of epidermis (acanthosis, papillomatosis, epithelial pearls). Characteristic features of spino- or basocellular carcinoma. Decreased amount of PAS-positive substance, irregular composition of collagen fibres; broken basal membrane
42–56	5	Cauliflower-like tumour	Considerable acanthosis and papillomatosis, numerous epithelial pearls. Weak PAS reactivity of true skin and of neoplastic cells. Hyalinosis and thickening of collagen fibres; disappearance of basal membrane
Over 56	6	Cancerous ulceration	Considerable acanthosis, invasion of cancerous cells to true skin, significant inflammatory infiltration composed of granulocytes and lymphocytes. Weak PAS reaction in the region of basal membrane which is absent. Hyalinosis and thickening of collagen fibres along with their partial loss

histological examinations skin sections were routinely processed and stained with hematoxylin and eosin (HE) (Fig. 1, 2).

In addition, the following histochemical examinations were perfomed in skin sections: (a) staining with Schiff reagent for PAS-positive material (Fig. 4); (b) collagen staining according to the method of



Figs. 1–4

Masson (Fig. 3) [7]; and (c) staining of reticular fibres according to the method of Gomori [7]. All histological images were referred to the appropriate stages of tumour development according to the macroscopical scale.

Microwave Irradiation

Microwave anechoic chamber was used for all irradiations; the animals were exposed in the far field of a standard-gain horn antenna, at 2,450 MHz. The radiation was produced by a Lucz 58 generator (USSR) of maximum power output 150 W, and fed to the antenna through a standard wave guide. Field distribution within the chamber was measured with a broadband isotropic radiation monitor (Narda Microwave Corp., Plainview, NY, USA). Temperature and humidity of the chamber were contolled during the expositions, and equaled to $22 \pm 1°C$ and $65 \pm 5\%$, respectively. For irradiations, mice restrained in a plastic cage, 10 mice per cage, were placed at the fixed area of the chamber where the power levels of the incident microwave energy equaled to 5, 10 or 15 mW/cm^2, for different irradiation regiments. Exposures were carried out for 2 h daily, 6 days per week, for a total of 1, 2, 3 or 6 months.

In additional experiments the specific absorption rate (SAR) of microwave energy in murine cadavers was tested by measuring the increase of body temperature in relation to field power density. By extrapolation it was established that under the conditions used SARs of 2, 4 and 6 mW/g were found for the respective power densities of 5, 10 and 15 mW/cm^2.

Experimental

All mice were divided into six experimental groups of 100 animals each. Mice in groups 1 and 2 were exposed to benzopyrene treatment and were simultaneously irradiated with 5 mW/cm^2 (group 1) or 15 mW/cm^2 (group 2) of microwave radiation for a total of 6 months. Groups 3–5 were composed of animals irradiated with 10 mW/cm^2 for 1 (group 3), 2 (group 4) or 3 months (group 5) prior to the beginning of benzopyrene treatment, as above.

Control animals were exposed to benzopyrene treatment for 6 months and sham-irradiated with microwaves, i. e. mice were placed in anechoic chamber for 2 h daily for the respective periods of time (1, 2, 3, or 6 months), but the generator was turned off throughout the whole session of "irradiation".

The process of the development of skin cancer was assessed in all groups of animals according to the 7-range macroscopical scale every 2 weeks, and the following indices were calculated: (a) mean cancer development time (MCDT), i. e. mean period of time in which all animals developed skin cancer to the step 4 on the scale; (b) cancer development time 50 (CDT_{50}), i. e. the period of time in which 50 % of mice developed a 4th-step skin cancer; (c) mean survival time 50 (MST_{50}), i.e. the period of time in which 50 % of the animals in the group were killed by the tumour.

Fig. 1. Histopathological examination of mouse skin exposed for 0–4 weeks to 3,4-benzopyrene. Normal mouse skin: thin epidermis, numerous additional structures of the skin, no inflammatory infiltrations present. The picture refers to stage 0 on the macroscopical scale of the tumour development. HE × 100

Fig. 2. Histopathological examination of mouse skin exposed for 10–24 weeks to 3,4-benzopyrene. Thickening of epidermis, atrophy of additional skin structures, collagenization and discrete lymphoid infiltration of true skin. The picture refers to stage 2 the on macroscopical scale. HE × 100

Fig. 3. Histopathological examination of mouse skin exposed for 32–42 weeks to 3,4-benzopyrene. Massive acanthotic hypertrophy of epidermis and focal growth of basocellular carcinoma; significant collagenization of true skin. The picture refers to stage 4 on the macroscopical scale of tumour growth. Staining according to the method of Masson, × 100

Fig. 4. Histopathological examination of mouse skin exposed for over 56 weeks to 3,4-benzopyrene. Massive invasion of spinocellular carcinoma cells into subcutanous tissue. Keratosis of superficial layer of cells containing small amounts of PAS-positive material. This picture refers to stage 6 on the macroscopical scale of tumour development. Histochemical staining with Schiff's reagent for PAS reaction, × 100

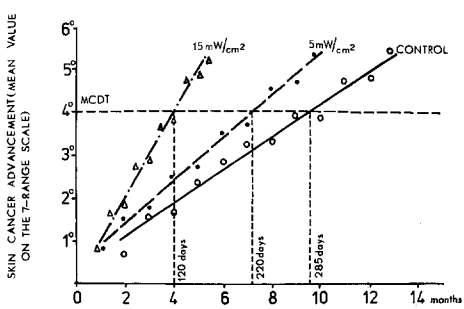
Statistical Analysis

The experimental points recorded every 2 weeks during 12 months were approximated to the straight line (the least square method) and the Pearson's correlation coefficient was calculated for each group. MCDT, CDT_{50} and MST_{50} were calculated from the above lines. Statistical significance of the differences ($P < 0.01$) between groups was tested by the χ^2 test (number of animals with developed tumours or survival rate in all groups at time of CDT_{50} or MST_{50}, respectively).

Results

Significant stimulation of the development and growth of benzopyrene-induced skin cancer was observed in mice irradiated with both 5 and 15 mW/cm^2 power density of microwaves, as compared with contol, sham-irradiated animals. This was reflected by a marked shortening of both MCDT and CDT_{50} in groups of irradiated mice, as shown in Figs. 5 and 6. Stimulatory effect of microwave radiation on the development of skin tumors was more pronounced after exposure to 15 mW/cm^2, and the difference was statistically significant ($P < 0.05$).

Acceleration of tumour growth in mice following irradiation with microwaves resulted in a shortened life span of animals exposed to both 5 and 15 mW/cm^2, as compared with control mice. Again, this phenomenon appeared to be dose-



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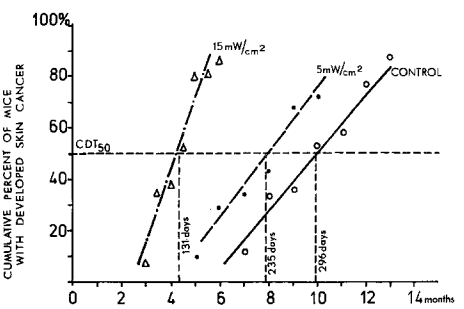

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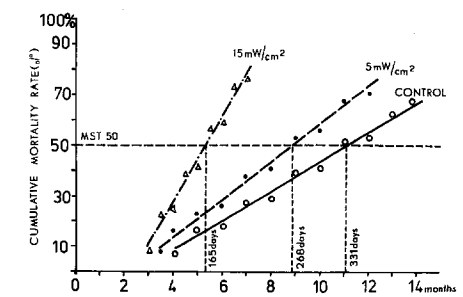

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Fig. 5. Mean cancer development time (MCDT) in mice painted with 3,4-benzopyrene and exposed to 2,450 MHz microwaves at field power densities of 5 or 15 mW/cm^2. Advancement of skin cancer was evaluated using a 7-range scale with the 4° (small papillomas) being the first step of fully development cancer. Control animals were exposed to 3,4-benzopyrene only

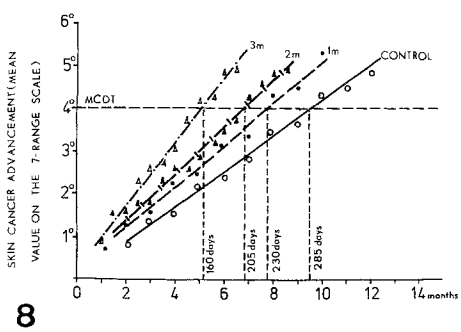
Fig. 6. Cancer development time (CDT_{50}) in 50% of mice painted with 3,4-benzopyrene and exposed to 2,450 MHz microwaves at field power densities of 5 or 15 mW/cm^2. Number of animals with fully developed skin cancer (4° – 6° on the 7-range scale) was counted every month in each group

Fig. 7. Mean surrival time (MST_{50}) of 50% of mice painted with 3,4-benzopyrene and exposed to 2,450 MHz microwaves at field power densities of 5 or 15 mW/cm^2

dependent, as irradiation with lower density of microwave energy led to the death of 50 % of the animals within 268 days, whereas exposure to higher dose resulted in the death of the same fraction of mice within only 165 days (Fig. 7). In the control group MST_{50} lasted 331 days, all the differences being stastistically significant ($P < 0.01$).

Exposure of mice to 10 mW/cm^2 power density of 2,450 MHz microwaves for 1,2 or 3 months prior to the beginning of the treatment with benzopyrene resulted in a significantly shortened period of development of skin cancer in these animals, as shown in Fig. 8–10. This stimulatory effect of microwave irradiation on the tumour development was most pronounced in mice irradiated for 3 months prior to the beginning of benzopyrene administration.

This was reflected by the smallest values of MCDT and CDT_{50} obtained in this group of animals (160 and 171 days, respectively), in comparison with the groups of mice preirradiated with microwaves for either 2 months (MCDT = 205 days and CDT_{50} = 210 days) or 1 month (MCDT = 230 days and CDT_{50} = 253 days). As the power density level was identical for all three protocols of irradiation, it seems possible that accumulation of microwave energy may account for the observed higher efficiency of longer-lasting periods of pre-exposure. In contrast, control, sham-irradiated animals yielded significantly higher values of MCDT and CDT_{50} in comparison with three experimental groups (Figs. 8, 9).



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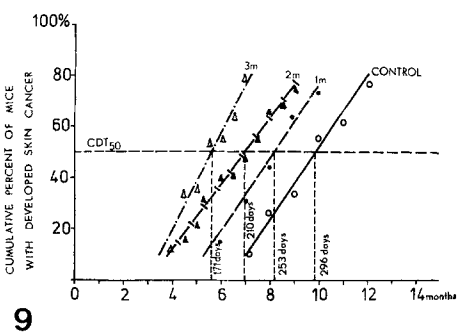

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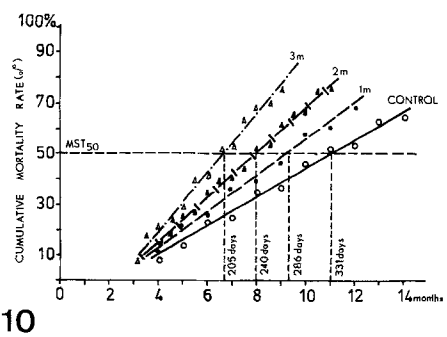

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Fig. 8. Mean cancer development time (MCDT) in mice preirradiated with 2,450 MHz microwaves during 1, 2 or 3 months and later painted with 3,4-benzopyrene. Advancement of skin cancer was evaluated using a 7-range scale with the 4° (small papillomas) being the first step of fully developed cancer. Control animals were exposed to 3,4-benzopyrene only

Fig. 9. Cancer development time (CDT_{50}) in 50 % of mice preirradiated with 2,450 MHz microwaves during 1,2 or 3 months and later painted with 3,4-benzopyrene. Number of animals with fully developed skin cancer (4° – 6° on the 7-range scale) was counted every month in each group

Fig. 10. Mean survival time (MST_{50}) of 50 % of mice peirradiated with 2,450 MHz microwaves during 1,2 or 3 months and later, painted with 3,4-benzopyrene

As in the case of stimultaneous exposure to benzopyrene and microwaves, previous irradiation for 1,2 or 3 months also decreased the life span of mice developing benzopyrene-induced skin tumour. This effect was also dose-dependent, as irradiation for longer periods of time resulted in more pronounced shortening of the life span, than did shorter expositions (Fig. 10). All differences were statistically significant ($P < 0.01$).

Discussion

Using the 7-range scale we were able to show that chronic irradiation with 5–15 mW/cm^2 power density of 2,450 MHz microwaves significantly accelerated the development of skin cancer induced with 3,4-benzopyrene. The acceleration was observed both when irradiation was applied simultaneously with benzopyrene and when exposure to microwaves preceded the administration of the carcinogen. Moreover, this effect seemed to be dose-dependent, as exposure to 15 mW/cm^2 led to more pronounced stimulation of tumour growth than did exposure to 5 mW/cm^2, and irradiation with 10 mW/cm^2 for 3 months was more effective in comparison with irradiations at the same power density for 1 or 2 months. It is worth mentioning here that in previous experiments carried out in our laboratories we never observed detectable skin changes in healthy mice exposed only to microwave fields for a long period.

The possible stimulatory effect of microwave radiation on the development of cancer in animals has been suggested in few laboratory studies [4, 8, 9, 15], but the obtained results are still to be supported by further experiments. In our previous study we have found that low-dose microwave irradiation may stimulate growth of spontaneous mammary carcinoma in C_3H/HeA mice [19]. Similar results were obtained earlier by Prausnitz and Süsskind [13], who observed higher incidence of chronic leukemias in mice exposed to long-term microwave irradiation. However, no clear-cut conclusions can be drawn from these studies as to the above effects. In view of the present results it is likely that at least two possible mechanisms may be responsible for the observed stimulation of tumour development in mice by microwave radiation. One possibility is that electromagnetic energy, which is transformed to heat inside tissues, may directly affect the structure and/or metabolism of actively proliferating cells in the epidermis. It was shown in numerous in vitro and in vivo studies that irradiation with microwaves may lead to various functional and structural alterations at the cellular level, including mutations and chromosomal aberrations [1, 5, 9, –11]. However, no direct carcinogenic effect of microwaves was found in these studies. On the other hand, most, if not all, of the observed cellular effects of microwave radiation could be related to substantial elevation of temperature of the exposed cells and tissues, what might render them susceptible to carcinogenic activity of various external physical or chemical agents. In our present study we used power densitiies of microwave energy, which do not result in detectable increase of body temperature. Thus, it is tempting to suggest the possible modulation by microwave radiation of the immune reactivity of the benzopyrene-treated animals, responsible for their antineoplastic resistance.

In a series of experiments it was shown, that acute high-level microwave irradiation of mice may lead to profound but reversible inhibition of cellular immune reactions, including natural antitumour resistance [15, 16]. Other authors also indicated that irradiations with various doses of microwaves led to modification of the cellular immune mechanisms in the exposed animals [8], but these results are contradictory. Few data exist, however, on the unequivocal immunosuppressive effect of athermal microwave irradiations in mice and rabbits [12]. In the present study we employed a test of cellular immune reactivity, which showed that long -term low-dose 2,450 MHz irradiation may suppress the mechanisms of delayed hypersensitivity in mice treated with benzopyrene. This was reflected by a marked inhibition of the benzopyrene-stimulated skin reactivity to dinitrofluorbenzene (DNFB), a specific inducer of cellular immune events responsible for delayed type hypersensitivity in mice, when benzopyrene treatment was preceded by or concomitant with microwave irradiation (results not shown). This inhibitory action of microwaves was qualitatively similar after irradiation with both 5 and 15 mW/cm^2 power levels, but the latter dose appeared to be significantly more effective.

Finally, a stress component of long-lasting irradiation of mice in a strange environment of the anechoic chamber may also be responsible for the above immunosuppressive effect of microwaves. This suggestion stems from the recent data on the profound alteration of the immune reactions in animals exposed to various stressors, such as heat, overcrowding or intensive physical exercises [2, 12]. The present study, however, does not provide us with results which can justify the more direct conclusions as to the possible role of stress in the co-carcinogenic effect of microwave radiation in mice treated with benzopyrene.

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