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## Histological characteristics of cutaneous and thyroid mast cell populations in male rats exposed to power-frequency electromagnetic fields

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### Abstract

**Purpose:** The objective of this study was to determine whether mast cells (MC) in skin and thyroid gland, cutaneous nerve fibers and eosinophils are sensitive to the influence of electromagnetic fields (EMF).

**Materials and methods:** The experiment was performed on two-month-old Wistar male rats, exposed to 50 Hz EMF (100–300  $\mu$ T, 54–160 V/m) for 4 h a day, seven days a week during one month. After sacrifice, samples of skin and thyroid were processed for toluidine blue staining or indirect immunohistochemistry. The M42 grid placed in the ocular of a light microscope and a special microscopic frame placed in the ocular of a fluorescence microscope were used for stereological analysis.

**Results:** The numerical and volume density of intact type A MC in the thyroid of the exposed group was significantly higher compared to the control. A number of MC and immunoreactive nerve fibers were observed in the skin and of histamine-immunoreactive MC in the thyroid of exposed animals. The differences in stereological data were not statistically significant by the Mann-Whitney test.

**Conclusions:** The results indicate certain alterations of cutaneous and thyroid MC in rats exposed to EMF. However, the possible outcome of changes in the MC population under EMF influence on morphophysiological properties of other structures in skin and thyroid requires further investigation.

**Keywords:** Cutaneous mast cells, thyroid mast cells, electromagnetic fields, stereology

### Introduction

A number of epidemiological investigations have raised the question of whether human subjects are sensitive to environmental electric and magnetic fields. The importance was particularly attached to the relation of skin symptoms and exposure of workers to visual display terminals (VDT). Results based on a self-administered questionnaire to office workers proposed that there might be an interaction between psychosocial factors and electric fields in the workplace, which increases the risk of skin symptoms among VDT workers (Eriksson et al. 1997). Investigation of facial skin complaints of office workers before and after the electrostatic field of VDT were reduced resulted in the conclusion that removing the field can probably help in reducing the skin symptoms (Skulberg et al. 2001). In patients with

self-reported 'sensitivity to electricity' subjected to 30-min periods of stress situations and simultaneous exposure to electromagnetic fields (EMF) from VDT, no alterations of mast cells (MC) in the skin were shown (Lonne-Rahm et al. 2000). According to the recent literature review data, it is concluded that this topic is not substantiated with sufficient data and requires further investigation (Levallois 2002).

One of the early studies of extremely low frequency electromagnetic field (ELF-EMF) influence on thyroid gland provided by Lafreniere and Persinger (1979) has shown that neither alterations in serum T3 and T4 concentrations, nor in the number of thyroidal follicles, were found in rats exposed to 0.5 Hz EMF perinatally and/or as adults. On the contrary, Udintsev et al. (1978) have found an increased level of circulating T4 in rats exposed to 50 Hz EMF of 20 mT for 18 h, but a decreased

concentration of circulating thyroid hormones after a single exposure to EMF. Investigating the influence of single exposure of rats to 20 mT ELF-EMF, Zagorskaya and Rodina (1990) found that the concentration of thyroid hormones remained lowered for two months after the exposure. On the other hand, Selmaoui et al. (1997) reported no significant differences in serum T3 and T4 levels between sham-exposed men and men exposed to continuous and intermittent 50 Hz magnetic field of 10  $\mu$ T for one night.

Previous investigations of the authors of this paper indicated changes in the dermis and epidermis in people exposed to video display terminals (VDT) with symptoms assigned to electrosensitivity (Johansson et al. 1994, 1996). Alterations in thyroid gland parenchymal and stromal elements have been observed as well, after various duration of exposure to power frequency EMF (Matavulj et al. 1996, 1999a, Rajkovic et al. 2003).

In the skin and thyroid gland MC are known to play an important role in the homeostatic regulatory mechanisms in both organs. MC in normal rat skin reside in the region of the dermis and hypodermis. The location of MC under the epidermis has particular significance, since these cells present a barrier to different external environmental stimuli and play a mediating role in the presence of infectious agents (Senol & Fireman 1997). Hence they are involved in the maintenance of homeostasis in the whole organism too (Galli 2000, Gurish & Austen 2001). In the thyroid gland, MC are located perifollicularly and perivascularly and are known to be involved in the regulation of thyroid function (Melander 1977). Serotonin released from MC granules stimulates release and synthesis of thyroid hormones by a direct action on the thyroid follicular cells (Melander & Sundler 1972), while histamine exerts an increase in thyroid blood flow and capillary permeability (Melander et al. 1975).

Therefore, the purpose of the present study was to evaluate the possible influence of ELF-EMF on skin and thyroid MC using the methods of classical histology and immunohistochemistry, primarily with histamine as a marker of MC degranulation. Skin nerve fibers and eosinophils were of additional interest in terms of their importance in interactions with MC.

## Materials and methods

### *Animals*

The experiment was performed on 24 white male rats of the Wistar strain. Animals were 1.5 months old when they arrived in the laboratory and they were kept for two weeks until the beginning of the

experiment. Animals were housed under laboratory conditions with  $20 \pm 2^\circ\text{C}$  and subjected to a controlled photoperiod (14 h light, 10 h dark). Access to tap water and pelleted food was omitted during exposure hours. Twelve animals were exposed to the influence of ELF-EMF for 4 h a day, seven days a week for one month. Twelve animals served as controls and were maintained in a similar environment, but without the presence of artificially produced ELF-EMF. The investigation was made with the permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

### *Exposure system and the EMF*

The exposure system, by which ELF-EMF was produced, was made of a single coil of solenoid type (Electronic Equipment Factory 'Novkabel', Novi Sad, Serbia and Montenegro) equipped with a cooling system and energized from 50 Hz, 220 V and 10 A via an autotransformer, which provided a 100 V output. Cages with animals were placed at the both sides of the coil at a 12 cm distance and were covered with a plastic lid. The EMF produced by the coil was in the horizontal direction relative to the geomagnetic field, it was inhomogeneous and of decaying intensity along the animal cages with 300  $\mu$ T and 160 V/m value on the side of the cage near the coil and 100  $\mu$ T and 54 V/m on the opposite side, while the value of the electric field at any other point in the room was less than 10 V/m. Although the exposure system was equipped with a cooling system, the temperature inside the cages with animals was measured during exposure hours with a digital thermometer (Testo 925, Testo GmbH, Lenzkirch, Germany). The average temperature of the control cages was measured to be  $20.0^\circ\text{C}$  and the exposed cages  $19.8^\circ\text{C}$ . The residential values of the magnetic (AC milligaussmeter, model 42B-1, Monitor Industries, Boulder, USA) and electric fields (HI-3607 ELF sensor, Holaday Industries, Eden Prairie, USA) were measured to be 0.2  $\mu$ T and 2.9 V/m, while the value of the geomagnetic field (Gauss/Tesla meter, model 4048, F.W. Bell, Orlando, USA) was 40  $\mu$ T.

### *Specimen preparation*

Immediately after the last hour at the last day of exposure, animals were sacrificed by diethyl ether (Lachema, Neratovice, Czech Republic) narcosis. The samples of skin from interscapular region were taken as well as the samples of thyroid gland with adjacent parts of trachea and surrounding connective tissue. All specimens were fixed at  $4^\circ\text{C}$  in a mixture of paraformaldehyde (4%; Merck, Darmstadt, Germany) and saturated picric acid (14%; Merck, Darmstadt, Germany). Thereafter,

the tissue samples were rinsed in 0.1 M Sörensen's buffer containing 10% sucrose (Merck, Darmstadt, Germany), 0.01%  $\text{NaN}_3$  (Merck, Darmstadt, Germany) and 0.02% Bacitracin (Sigma Chemicals Co., St. Louis, USA) and cut into  $14\mu\text{m}$  thick sections using a cryostat (Microm, Heidelberg, Germany). Sections were further processed for toluidine blue staining or indirect immunohistochemistry.

#### *Immunohistochemistry*

In order to demonstrate MC, eosinophils or nerve fibers in skin and MC in thyroid samples, the indirect immunofluorescence technique was used. Sections were kept at  $4^\circ\text{C}$  overnight in a humid atmosphere during incubation with the following primary antibodies: rabbit antibodies to histamine (1:2000; Euro-Diagnostica, Arnhem, Netherlands), serotonin (5-HT; 1:250; Verhofstad et al. 1983), eosinophil cationic protein (ECP; 1:500; produced by Claus M. Reimert; for details see Johansson et al. 2000) or mouse or rabbit antibodies to protein gene product 9.5 (PGP 9.5; 1:2000; UltraClone Limited, Isle of Wight, England). Monoclonal and polyclonal antibodies to PGP 9.5 were used to demonstrate nerve fibers in the skin due to the ability of these antibodies to detect small nerve fibers in peripheral tissues. PGP 9.5 staining enabled the histological and morphometrical analysis of nerve fibers in skin tissue samples including the delicate nerve endings in the epidermis.

Sections were further rinsed in phosphate-buffered saline (PBS; all components by Merck, Darmstadt, Germany), incubated for 30 min at  $37^\circ\text{C}$  in rhodamine (TRITC)-conjugated donkey anti-rabbit IgG (1:160; Jackson ImmunoResearch Laboratories, Inc., West Grove, USA), rinsed and mounted. For PGP and histamine double-staining, fluorescein (FITC)-conjugated donkey anti-mouse IgG (1:160; Jackson ImmunoResearch Laboratories, West Grove, USA) was also used. All antibodies were diluted in 0.3% Triton X-100 (Sigma Chemicals Co., St. Louis, USA). To test for any possible non-specific binding of primary antisera, PBS was applied to certain sections instead of each primary antibody. For observation and photography a Nikon Microphot-FXA fluorescence microscope (Tokyo, Japan) was used. All sections were blind-coded and analyzed by the same observer.

#### *Stereological analysis*

Toluidine blue stained skin and thyroid sections and sections stained according to immunohistochemistry protocol were used for quantitative estimation of MC, eosinophils and nerve fibers. Skin sections

were analyzed starting from the epidermal-dermal junction and thyroid sections from the middle of the lobe (facing trachea) to the periphery.

Analysis on toluidine blue sections was performed using a multipurpose stereological grid M42 placed in the ocular of a Reichert light microscope. For the stereological analysis of cutaneous MC one tissue sample taken from each of 12 control and 12 exposed animals was used and for the analysis of thyroid gland MC a thyroid sample taken from each of 10 control and 10 exposed animals. From a series of frozen sections made of each tissue sample, 2 sections of skin were randomly chosen and the MC were counted on 25 test fields per section (a total of 50 test fields) and 4 sections of thyroid were chosen and MC were counted on 25 test fields per section (a total of 100 test fields). Analysis of both skin and thyroid sections was made using ocular  $10\times$  and objective  $40\times$  magnification. The total MC numerical (Nvm) and volume (Vvm) density were determined. Additionally, the numerical and volume density of three morphological types of MC were determined according to Bani-Sacchi (1966): type A MC are compact with abundant cytoplasmic granules, type B MC are characterized by numerous cytoplasmic extensions that implement the initiation of degranulation, and type C MC are degranulated cells with extruded granules in the intercellular space.

Histamine- and serotonin-containing MC, ECP-labeled eosinophils and PGP-positive nerve fibers were estimated according to the principles of design-based stereology. The number of immunoreactive cells or nerve fibers per projected  $\text{mm}^2$  were counted using a special microscopic frame under the  $20\times$  objective on 2 test fields per each of 2 skin sections per one tissue sample taken from each of 12 control and 12 exposed animals and 4 test fields per each of 2 thyroid sections per one sample taken from each of 10 control and 10 exposed animals.

Estimations were made by the same observer on blind-coded sections. A non-parametric Mann-Whitney U test was used for statistical analysis to evaluate the differences between the control and the exposed group,  $p$  values less than 0.05 were considered significant.

## **Results**

### *Histological and stereological analysis of skin*

**Toluidine blue.** MC with decreased metachromasia and cell volume were noted in the papillary dermis of the exposed animals as well as released MC granules in the cutaneous connective tissue (Figure 1a & b). Only solitary, darker stained cells were found in control animals (Figure 1c). Degranulated MC in the exposed group were frequently seen in close

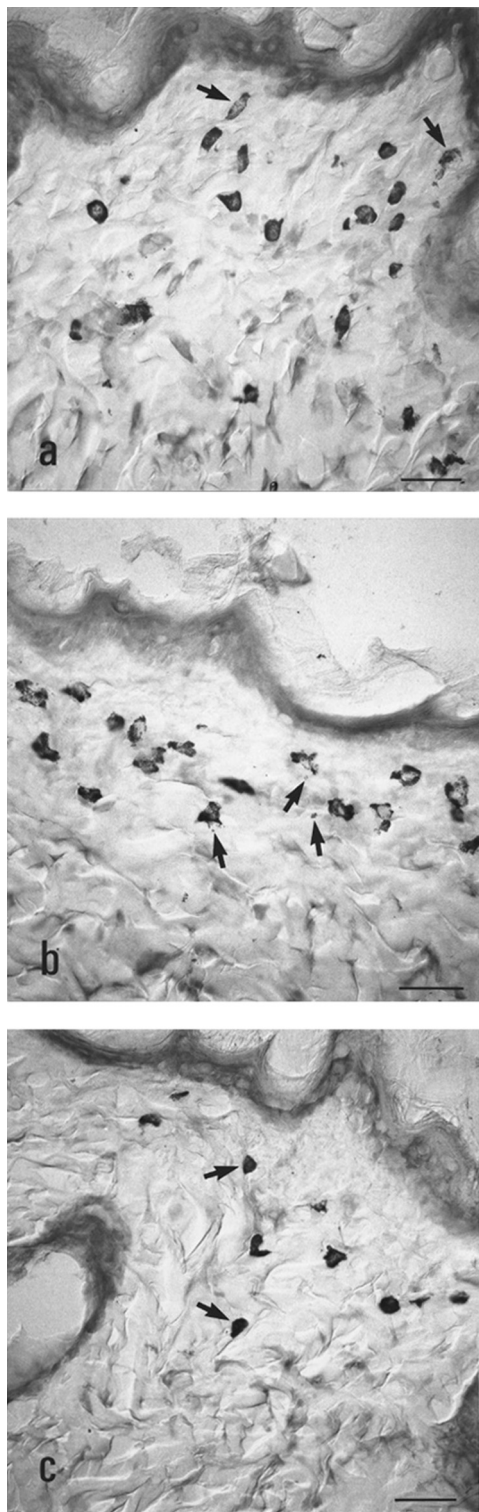


Figure 1. Mast cells (MC) in the upper dermis of toluidine blue-stained skin sections of animals exposed to ELF-EMF (a,b) and a control animal (c). (a,b) An increased number of MC is seen in the upper dermis. Demasked cell nuclei and degranulated cells are situated closely to the epidermis (arrows) in (a). A number of MC with decreased metachromasia and cytoplasmatic processes indicating their degranulation and released granules in the surrounding connective tissue (arrows) are found in (b). In the control, dark-stained MC in the *stratum papillare* (arrows) were observed (c). Bars indicate 50  $\mu$ m.

proximity to blood vessels. Stereological analysis showed that the differences in the calculated values of all stereological parameters between the control and the exposed group were not statistically significant at the  $p=0.05$  level according to the Mann-Whitney test (Table I).

**Histamine.** Solitary histamine-positive MC with decreased cell volume and weak fluorescence were observed in the papillary dermis of the exposed group. Occasionally, individual MC were located closely to the basal cell layer of the cutaneous epidermis. In 6 out of 12 of the exposed animals, large MC arising in lines towards the epidermis were found, but only in 2 animals from the 12 control group animals. Differences in the calculated values of the histamine-positive MC number between the control and the exposed group were not statistically significant at the  $p=0.05$  level according to the Mann-Whitney test (Table I).

**PGP 9.5.** In animals exposed to EMF, the distribution density of immunoreactive nerve fibers in the epidermis was higher, as compared to controls (Figures 2a,b, and 3a–c). However, these differences, as well as for the PGP-positive nerve fibers in the skin dermis, were not statistically significant at the  $p=0.05$  level according to the Mann-Whitney test (Table I).

**Histamine and PGP 9.5 double-staining.** Pale fluorescent histamine-positive MC closely apposed to nerve fibers and free MC granules in their vicinity were observed in the papillary dermis of the exposed group. On the sites of the migration process towards the epidermis, lineages of MC always accompanied nerve fibers.

**ECP.** Immunolabeled eosinophils in the dermis of exposed animals were found in groups or solitary, and diffusely scattered in the control group. According to the Mann-Whitney test, no statistically significant differences at the  $p=0.05$  level were found in the calculated number of ECP-positive cells between the groups (Table I).

#### *Histological and stereological analysis of thyroid gland*

**Toluidine blue.** MC in the exposed animals showed weak metachromatic staining and were found in the interfollicular connective tissue closely positioned to blood vessels. MC in control sections were frequently observed adjacent to follicular cells. Stereological analysis showed an increased numerical and volume density of A type MC in the exposed group as compared to the control, which was

Table I. The number of observations, median values with the lower and upper quartiles of all investigated stereological parameters in the skin of control and exposed animals and the p-level according to the Mann-Whitney U-test are presented. Vvm presents the volume density of mast cell and Nvm the numerical density of mast cells.

| Stereological parameter                               | N  | Control group |                |                | Exposed group |                |                | p-level |
|-------------------------------------------------------|----|---------------|----------------|----------------|---------------|----------------|----------------|---------|
|                                                       |    | Median        | Lower quartile | Upper quartile | Median        | Lower quartile | Upper quartile |         |
| Vvm (%)                                               | 12 | 0.50          | 0.43           | 0.74           | 0.52          | 0.48           | 0.85           | 0.40    |
| Nvm ( $\text{mm}^{-3}$ )                              | 12 | 13263         | 10642          | 14077          | 14944         | 12020          | 19170          | 0.09    |
| Vvm type A (%)                                        | 12 | 0.00          | 0.00           | 0.00           | 0.00          | 0.00           | 0.05           | 0.12    |
| Vvm type B (%)                                        | 12 | 0.17          | 0.14           | 0.28           | 0.09          | 0.09           | 0.17           | 0.08    |
| Vvm type C (%)                                        | 12 | 0.33          | 0.24           | 0.48           | 0.41          | 0.28           | 0.55           | 0.23    |
| Nvm type A ( $\text{mm}^{-3}$ )                       | 12 | 0             | 0              | 0              | 0             | 0              | 799            | 0.19    |
| Nvm type B ( $\text{mm}^{-3}$ )                       | 12 | 2814          | 2443           | 3882           | 2797          | 2449           | 4113           | 0.95    |
| Nvm type C ( $\text{mm}^{-3}$ )                       | 12 | 8535          | 6919           | 11110          | 10161         | 8784           | 15252          | 0.08    |
| Histamine + cells ( $\text{No}/\text{mm}^2$ )         | 12 | 103           | 50             | 173            | 94            | 42             | 134            | 0.54    |
| PGP + fibers in epidermis ( $\text{No}/\text{mm}^2$ ) | 12 | 970           | 747            | 1781           | 1326          | 795            | 1660           | 0.64    |
| PGP + fibers in dermis ( $\text{No}/\text{mm}^2$ )    | 12 | 288           | 203            | 329            | 221           | 148            | 331            | 0.62    |
| ECP + cells ( $\text{No}/\text{mm}^2$ )               | 12 | 106           | 84             | 161            | 165           | 64             | 213            | 0.43    |

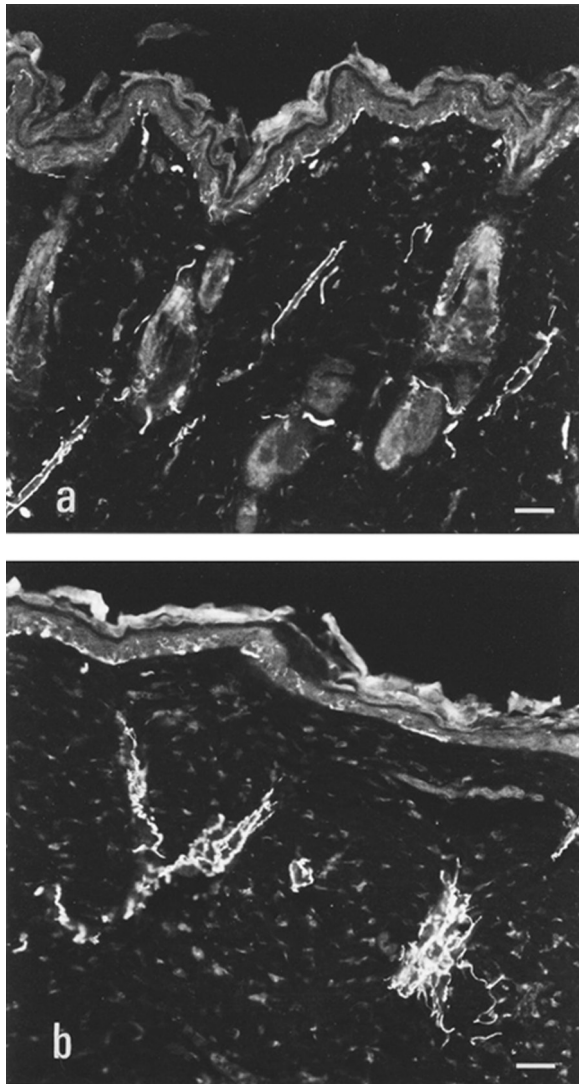


Figure 2. PGP-positive nerve fibers in the epidermis and dermis in skin sections from an animal exposed to ELF-EMF (a) and a control animal (b). Bars indicate 50  $\mu\text{m}$ .

statistically significant (both at  $p < 0.05$ ) (Table II). Differences between the control and the exposed group for other calculated stereological parameters were not statistically significant at the  $p = 0.05$  level (Table II).

**Histamine.** A number of degranulated histamine-containing MC, released granules in the thyroid interfollicular space and degranulated cells in close proximity to blood vessels were observed in the exposed animals (Figure 4a,b). However, according to the Mann-Whitney test, the differences in the calculated values of the number of histamine-positive cells between the groups were not statistically significant at the  $p = 0.05$  level (Table II).

**Serotonin.** MC showing bright serotonin fluorescence were noted in both experimental groups. Occasionally, MC with decreased fluorescence or reduced volume were found in the exposed group, indicating their degranulation. According to the Mann-Whitney test there were no statistically significant differences at the  $p = 0.05$  level in the calculated number of serotonin-positive cells between the control and the exposed group (Table II).

## Discussion

The results of the present experimental investigations indicate certain changes in the MC population of skin and of thyroid gland in male rats exposed one month to 50 Hz EMF. Skin alteration refers mainly to the finding of numerous MC in the upper dermis, of type A MC and of histamine-immunoreactive MC in the thyroid. Additionally, more immunoreactive nerve fibers were found in the cutaneous epidermis and upper dermis. Alterations of skin ECP and thyroid serotonin-positive MC are related to

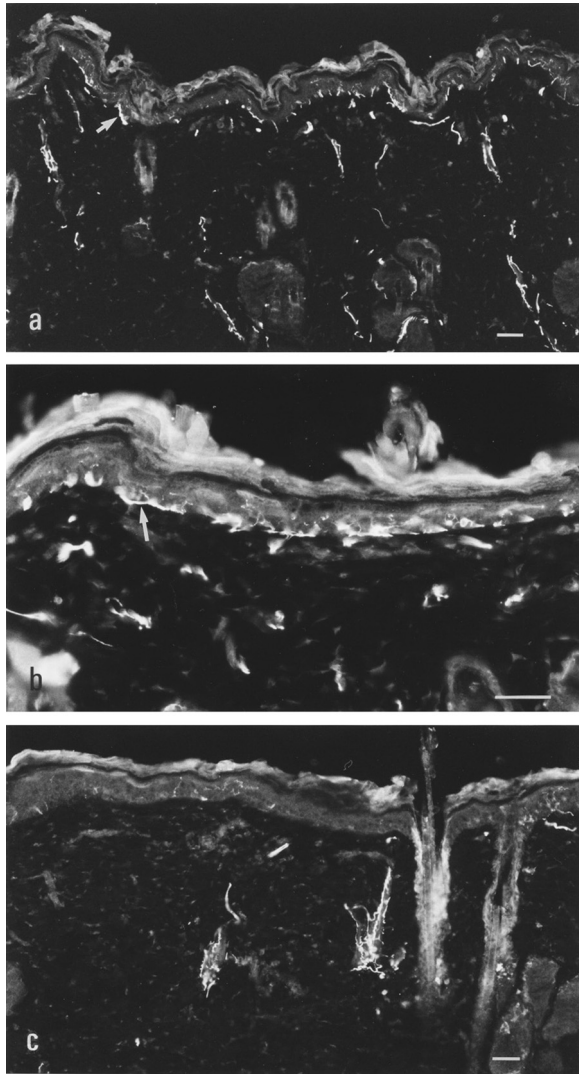


Figure 3. PGP-positive nerve fibers in the epidermis and dermis of skin sections of animals exposed to ELF-EMF (a,b) and a control animal (c). An increased number of nerve fibers in the epidermis and the appearance of a few thicker fibers under the epidermis (arrows) is seen in (a,b). Bars indicate 50  $\mu\text{m}$ .

rearrangement of these cells in respective organs. However, these histological findings were not substantiated by the stereological analysis, except for the numerical and volume density of type A MC in the thyroid gland, which was found to be significantly altered.

The results of histological analysis showed that the majority of toluidine blue-stained MC in the papillary dermis of the exposed animals were degranulated as indicated by decreased cell metachromasia, decreased cell volume and free granules extruded into the connective tissue surroundings. Stereological data are supportive to these findings as the numerical density of degranulated MC increased in the exposed group and the volume density of type B MC decreased compared to the controls. The value of the numerical density of degranulated MC considerably contributed to the value of the total MC numerical density in both experimental groups. This was more prominent in the exposed group indicating an increase of MC population in the skin of animals exposed to EMF. These observations are further strengthened by frequent findings of degranulated histamine-positive MC. Accumulation of larger groups of MC occasionally seen in upper dermis of the exposed group is probably due to MC migration from the deeper parts of the dermis towards the epidermis. The basis of this assumption could be found in the observation of histamine-positive MC arising from the deep dermis in straight lines towards the epidermis. It should be stressed that these cells had many cytoplasmatic processes, which precede degranulation or point to the initiation of this process. Therefore, it could be hypothesized that these cells enter a phase of advanced degranulation while reaching the upper dermis, which was particularly evident on toluidine blue-stained skin sections of the exposed animals. The response of cutaneous

Table II. The number of observations, median values with the lower and upper quartiles of all investigated stereological parameters in the thyroid gland of control and exposed animals and the p-level according to the Mann-Whitney U-test are presented. Vvm presents the volume density of mast cell and Nvm the numerical density of mast cells.

| Stereological parameter                       | N  | Control group |                |                | Exposed group |                |                | p-level |
|-----------------------------------------------|----|---------------|----------------|----------------|---------------|----------------|----------------|---------|
|                                               |    | Median        | Lower quartile | Upper quartile | Median        | Lower quartile | Upper quartile |         |
| Vvm (%)                                       | 10 | 0.29          | 0.24           | 0.33           | 0.35          | 0.31           | 0.38           | 0.15    |
| Nvm ( $\text{mm}^{-3}$ )                      | 10 | 6163          | 4128           | 7268           | 6590          | 6041           | 6933           | 0.40    |
| Vvm type A (%)                                | 10 | 0.10          | 0.05           | 0.14           | 0.190         | 0.14           | 0.21           | 0.015*  |
| Vvm type B (%)                                | 10 | 0.02          | 0.00           | 0.05           | 0.05          | 0.02           | 0.05           | 0.27    |
| Vvm type C (%)                                | 10 | 0.12          | 0.12           | 0.19           | 0.13          | 0.07           | 0.14           | 0.64    |
| Nvm type A ( $\text{mm}^{-3}$ )               | 10 | 2983          | 1483           | 3929           | 4441          | 3495           | 5232           | 0.03*   |
| Nvm type B ( $\text{mm}^{-3}$ )               | 10 | 231           | 0              | 446            | 447           | 315            | 559            | 0.25    |
| Nvm type C ( $\text{mm}^{-3}$ )               | 10 | 2645          | 2038           | 3007           | 2005          | 1389           | 2666           | 0.20    |
| Histamine + cells ( $\text{No}/\text{mm}^2$ ) | 10 | 132           | 90             | 170            | 217           | 170            | 240            | 0.06    |
| Serotonin + cells ( $\text{No}/\text{mm}^2$ ) | 10 | 348           | 259            | 442            | 340           | 254            | 379            | 0.54    |

Values labeled by \* indicate the difference between the groups at  $p < 0.05$  according to the Mann-Whitney test.

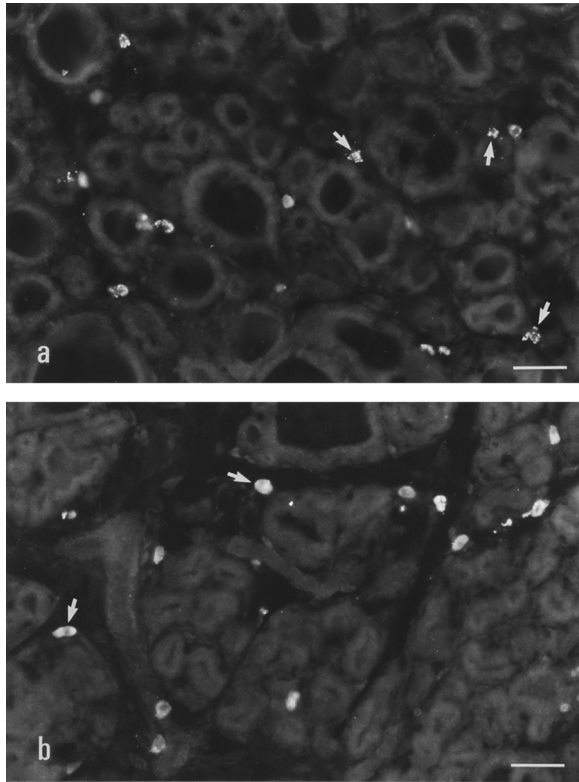


Figure 4. Histamine-positive mast cells in the thyroid gland of an animal exposed to ELF-EMF (a) and a control animal (b). A number of MC with cytoplasmic processes and degranulated cells (arrows) (a) and predominantly intact MC (arrows) (b) are observed. Bars indicate 50  $\mu$ m.

MC to the influence of EMF by histamine release has already been proposed (Gangi & Johansson 2000).

The EMF influence is believed to be a specific physiological stress situation and cells are thought to respond to EMF similarly to other such stressors (as hyperthermia or oxidative stress), and a stress response is even considered as a general mechanism of EMF interaction with cells (Lin et al. 1997). Our finding of immunoreactive nerve fiber-MC contacts in the skin implicates the possible role of neuropeptides in triggering MC degranulation in the presence of EMF, the latter acting as the stress factor.

Our findings of MC alterations in the skin of animals exposed to 50 Hz EMF are in agreement with data reported by Johansson et al. (2001). The investigation was carried out on healthy volunteers exposed to TV/PC screens for 2 or 4 h. After the provocation, an increased number of MC in skin biopsies was observed in the region of the papillary dermis. Cellular migration towards the epidermis was also noted with a loss of granular content by some cells and shrunken cytoplasm, pointing to possible degranulation, as commented on by the authors. Similarities in the pattern of MC response in this investigation and our present study imply a possible model of MC reaction to weak EMF

whether of static or alternating nature. However, only further investigation in this direction would reveal any solid base for this assumption.

Stereological analysis of thyroid gland toluidine blue-stained sections in the present study revealed a significant increase in type A MC number and volume of the exposed group. Early studies of TSH action on thyroid MC demonstrated the correlation between the level of circulating TSH and the number of MC in the gland (Melander et al. 1971). Therefore, the above-mentioned alterations of thyroid MC could have resulted from their sensitivity to TSH action. However, this should be taken cautiously, because there were no TSH measurements in our study. Literature data, however, provide seemingly opposite results of the EMF influence on TSH. Ushintsev et al. (1978) found increased levels of circulating TSH in rats exposed to 50 Hz EMF of 20 mT for 18 h. Conversely, Selmaoui et al. (1997) reported that either a continuous or intermittent 50 Hz magnetic field has no effect on TSH secretion in humans exposed to the field of 10  $\mu$ T intensity for one night.

In contrast to our present findings, the results of previous investigation in our laboratory showed a statistically significant decrease in numerical and volume densities of type A and of type B MC in rats exposed to 50 Hz EMF (50–500  $\mu$ T) from 24 h after birth, 7 h a day, 5 days a week during 4 months (Matavulj et al. 1999b). This could be attributed to different exposure duration in the two experiments and to different exposure dynamics.

In the present study, a number of histamine-positive MC was observed in exposed animals with an abundance of degranulated cells and extruded granules in the thyroid interfollicular space. In view of the possibility that released histamine was affecting thyroid microcirculation in exposed animals, one of our previous investigations is of interest. Namely, this previous investigation demonstrated the dilatation of blood capillaries in the thyroid gland of male rats exposed 7 h a day, 5 days a week to ELF-ELF (50 Hz and 50–500  $\mu$ T) during two months of postnatal life (Matavulj et al. 2000).

Literature data regarding EMF influence on MC suggest alterations of MC in different organs of different animal species. The sensitivity of MC in mice, rats and rabbits from dermal, intestinal and popliteal lymph node to power-frequency EMF was reported by Iurina et al. (1997) after exposure to EMF (50 Hz and 2 kA/m, 16 kA/m or 32 kA/m) for 4 h during 5 days. Also, the number of MC in rat brain was found to be affected by weak, extremely low-frequency EMF (7 Hz or 40 Hz and 50 nT or 500 nT) applied during 15 nights from midnight to 8 h (Cook et al. 2000). However, rat peritoneal MC showed no significant degranulation, measured by

histamine release, after treatment with 5 mT 60 Hz magnetic field during 30 min to 2 h in *in vitro* conditions (Price & Strattan 1998).

Results obtained in our investigation thus demonstrate certain alterations in morphology, distribution and number of cutaneous and thyroid MC in male rats exposed to power-frequency EMF during one month. The reaction of the MC in these two investigated organs showed differences, particularly at the level of expressed metachromasia and morphological appearance. However, the possible outcome of changes in the MC population on morphophysiological properties of other tissue structures/cells in the skin and thyroid gland under the influence of ELF-EMF, as well as of more high-frequency fields, has yet to be determined.

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