THE INFLUENCE OF MAGNETIC FIELDS ON THE PRIMARY HEALING OF INCISIONAL WOUNDS IN RATS

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ABSTRACT

Our studies were conducted to examine the influence of extremely low frequency magnetic fields (ELF-MF) on skin wound healing in male adults rats. We used 40 Hz and 10 mT sinusoidal fields. We evaluated the rate of wound healing by determining the tissue hydroxyproline concentration and scar imaging in electron microscope. The systemic body response to ELF-MF was detected by analysis of blood morphological and biochemical parameters, such as: RBC, WBC, hemoglobin, hematocrit, reticulocytes, electrolytes, urea, and total protein concentration. ELF-MF induced the increase of hydroxyproline level in scar tissue and intensified the maturation of collagen seen in the electron microscope. The increase of reticulocyte number in blood confirmed that the healing process in experimental animals was supported by the activation of the oxygen supply and utilization processes, as a result of erythropoietic intensification, without simultaneously upsetting cellular energetic processes. We did not obtain changes in bio-

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chemical parameters in blood, such as: electrolytes, urea, and total protein concentration, so we concluded that ELF-MF evoked no negative systemic response.

Key Words: Magnetic fields; Primary healing; Incisional wounds in rats

INTRODUCTION

One of the basic problems of contemporary surgery is the impairment of wound healing. This complicated biological process can be defined as a homeostatic answer by the organism to damaging trauma. Considered from the point of phylogenetic development, it helps the species to survive.\[27\] At present, recognition of the biological basics of healing determines underpins modern surgery. This knowledge has been evolved over the last 100 years; but recently surgeons and other researchers have revealed some exact mechanisms of this process and partly influence and control its course.\[2,9\]

In spite of medical progress, chronic wounds are a serious problem not just for physicians, but also present social, psychic, and often financial problems for patients.

There are many ways to characterize wounds. Regarding the time of healing, we can divide them into acute and chronic.\[1,3,5,36\]

- Acute wounds—surgical and posttraumatic, heals quickly (days, weeks) without complications as a rule.
- Chronic wounds—complications of acute wounds, or as idiopathic symptoms of other diseases (venous ulcers, bedsores, etc.).

There is no problem with acute wound treatment since they usually heal quickly, or are covered with flap transplants. Chronic wounds heal over a long time, or do not heal at all. A long process of recovering from illness or an unfavorable course of healing contributes to crippling and sometimes to the patient’s death.\[19,35\]

This has been the stimulus for physicians and researchers to look for methods of treatment supporting classical therapeutic methods. One such useful method turned out to be therapy by extremely low frequency magnetic fields (ELF-MF). These fields are characterized by frequencies to 100 Hz, field strength 1–20 mT, and electric field intensity from 150 to 500 V/m.\[10,11,28\]

The following biophysical and biological effects are bases for the use of magnetic fields in the treatment of chronic wounds.\[28,29,37\]

1. Usage of fields of therapeutic parameters exerts antiphlogistic, antiseptic, and analgesic activities, which have positive influences on wound healing and the patient’s frame of mind.
2. ELF-MF helps to increase oxygen diffusion and hemoglobin oxygenation, which enhances oxygen utilization processes and tissue oxygenation.
3. External magnetic fields intensify anaerobic respiration, breaking the lipid peroxydation process chains, which leads to disintegration of cellular membranes and respiratory enzymes.
4. Collagen determines the mechanical properties of scars and is the foundation of their structure. It has piezoelectric generator properties, reacting to external field changes. ELF-MF promotes collagen synthesis, probably by suppression of cAMP level as a second messenger.

Pulsed magnetic fields also accelerate:

1. Angiogenesis and development of collateral circulation in damaged tissue;
2. Fibrocyte to fibroblast transformation;
3. Steering the growth of collagen bundles, according to lines of the magnetic field;
4. Epithelialization processes.

Present clinical data confirm the high efficacy of magnetotherapy in the treatment of poorly healing chronic wounds.\(^{14,17,31,32}\) They have almost no support in experimental investigations. There are few works in the world literature describing ELF-MF influence on experimental wound healing.\(^{15,16,23,24}\) The profile of these works bases the estimation of the ELF-MF influence on acute, surgical wounds, with a later shift to chronic wound pathophysiology, because the biochemical and morphological mechanism leading to final effect is identical in both processes.

Pulsed magnetic field has antiphlogistic, antiswelling, and antiseptic activity, which supports natural processes of cleansing wounds.\(^{17,34}\) It intensifies tissue respiration processes, utilization of oxygen, promotes vasodilatation, angiogenesis, and in consequence, tissue regeneration.\(^{13,21,28}\)

We assumed that low frequency pulsed magnetic field might stimulate the healing process by influencing local homeostatic mechanisms. We observed effects of ELF-MF activities on primary wound healing by picturing the scar in the electron microscope and by measuring the quantity of tissue hydroxyproline. The systemic activity of magnetic field was estimated by measuring morphological elements of blood, some biochemical compounds, and the activity of malic dehydrogenase (E.C.1.1.3.7)—the key enzyme of Krebs Cycle—in liver homogenates.

**MATERIALS AND METHODS**

**Animals**

Thirty male white rats of the Sprague-Dawley (Centralna Zwierzętnia Doświadczalna Śląskiej Akademii Medycznej) strain were used for this study. The average mass was 271.4 ± 25.3 g. Each rat was housed in a separate cage to compare the increase of body mass. Access to food and water was unlimited. Animals were kept at 20–22°C under a standard regimen with 12:12 hr light–dark cycle (darkness 19:00–7:00 hr). Animals were divided into two groups of 15 rats each:

- Group BP—the experimental group, received a normal incisional skin wound and was exposed to ELF-MF. This group was divided into three subgroups (five animals each). Animals from each subgroup were killed respectively—first on the 6th, second on the 10th, and third on the 14th day of exposure.
• Group KP—control, received a normal incisional dorsal wound as well, but was not exposed on magnetic field. This group was also divided into three subgroups as in group BP.

Procedure

At the beginning of the experiment after 24 hr of starving, each animal was anesthetized with 2.5% Thiopental, applied intraperitoneally (40 mg/kg). Each rat was shaved on its dorsal surface and disinfected with Braunoderm. Then the animals received an incisional dorsal wound, beginning between scapulas, 4 cm long, embracing the panniculus carnosum. All wounds were sutured with Amisil M 4-0. According to Paul et al. such wounds are an optimum (standard in presentation) model of wounds for biochemical and morphological assay of healing in experimental animals.

All animals of group BP were exposed daily to magnetic field, at the same time (12.00 hr), with following parameters: 10 mT, 30 min, 40 Hz, and sinusoidal impulse. Exposure was performed systemically, animals were placed in a special cage filling all space of the coil. The electric coils were covered with wood panels, which effectively eliminated a thermal effect inside the coil. Temperatures in the room and in the operant chamber were monitored by separate probes. The mean difference between the room ambient temperature and the operant chamber temperature was 0.5°C. The cage was constructed to conduct experiments with animals in the ELF-MF generated by the coil. The cage and the coil have the same parameters: cylindrical shape, 30 cm in length, and 40 cm in diameter. The first exposure was performed about 2 hr after operation. The first subgroup was exposed six times, the second was exposed 10 times, and the third was exposed 14 times.

Animals were killed also on the 6th, 10th, and 14th day. They were put to death at noon after 24 hr of starving. Before killing, all animals were weighed and anesthetized. They were shaved carefully on the back, and skin flaps with scars were removed with a 2 mm margin of healthy skin. Part of each scar was fixed with a 2.5% glutaraldehyde solution and embedded in epon. They were cut as semithin ultrathin sections by an Ultramicrotome OUM 3 (Reichert). Sections were visualized in an electron microscope (JEM 100CX, Jeol) with an enlargement of 8.3 × 1000. The remaining fragment of each scar was frozen at −10°C, and used next for an hydroxyproline assay according to Prockop and Udenfriend. Next we opened the chests to obtain blood from the heart ventricles. Blood analyses (with reticulocytosis), for total protein, urea, sodium, and potassium were performed. After opening the abdominal cavity, we removed the liver for a malic dehydrogenase activity assay.

Instrumentation

To generate extremely low frequency magnetic field, a cylindrical coil was used connected with a computer driver (Ambit®). Surgery was performed with Aesculap® tools, blade No. 22 (Jai Surgical). For disinfection, we applied Braunoderm®.
Table 1. Number of RBCs [T/l]

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<tr>
<th>Group</th>
<th>6th Day</th>
<th>10th Day</th>
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<tr>
<td>Group BP</td>
<td>6.55 ± 0.3</td>
<td>5.78 ± 0.99</td>
<td>6.66 ± 0.41</td>
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<tr>
<td>Group KP</td>
<td>6.18 ± 0.26</td>
<td>5.77 ± 0.6</td>
<td>6.16 ± 0.11</td>
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<tr>
<td>BP/KP ( p(\alpha) )</td>
<td>0.027*</td>
<td>0.083</td>
<td>0.021*</td>
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\(^*p(\alpha)<0.05.\)

(B Braun). Blood analysis was performed with Cell Dyn 1600 (Abbot), Medica Easy Lyte Plus and Corning 480.

Data Analysis

All results were expressed as mean±SD. Statistical significance of difference between control and exposed rats was determined using factor analysis of deviations with the Mann–Whitney \( U \)-test, adjusted for a limited sample size (\( n = 5 \)). A value of \( p < 0.05 \) was regarded as significant. For computer analysis, we used STATGRAPHICS\(^\text{®}\) program (Version 7 for DOS).

RESULTS

Erythrocytes (RBC)

The number of RBCs significantly increased further in group BP (6%, \( p < 0.05 \)) on the 6th day of research. On the 14th day of research, RBCs increased further in group BP (7.5%, \( p < 0.05 \)) (Table 1).

Reticulocytes

The number of reticulocytes was significantly higher (67.5%, \( p < 0.05 \)) in group BP on the 6th day of research. On the 10th day of investigation, the number of reticulocytes increased significantly in group BP (36%, \( p < 0.05 \)). Between the 10th and 14th day of the experiment, the number increased in group BP (24%, \( p < 0.05 \)). Their numbers were higher in group BP on the 14th day (51%, \( p < 0.05 \)) (Table 2).

Table 2. Number of Reticulocytes [l/l]

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<th>6th Day</th>
<th>10th Day</th>
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<tr>
<td>Group BP</td>
<td>0.0418 ± 0.0061</td>
<td>0.0354 ± 0.0071</td>
<td>0.0466 ± 0.0086</td>
</tr>
<tr>
<td>Group KP</td>
<td>0.0178 ± 0.0011</td>
<td>0.0226 ± 0.0048</td>
<td>0.0228 ± 0.0052</td>
</tr>
<tr>
<td>BP/KP ( p(\alpha) )</td>
<td>0.049*</td>
<td>0.011*</td>
<td>0.012*</td>
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</table>

\(^*p(\alpha)<0.05.\)
Table 3. Concentration of Hemoglobin [mmol/l]

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<th>6th Day</th>
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<th>14th Day</th>
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<tr>
<td>Group BP</td>
<td>87.86 ± 2.41</td>
<td>85.25 ± 4.46</td>
<td>86.74 ± 6.39</td>
</tr>
<tr>
<td>Group KP</td>
<td>83.27 ± 3.53</td>
<td>83.76 ± 2.35</td>
<td>81.03 ± 2.48</td>
</tr>
<tr>
<td>BP/KP p(α)</td>
<td>0.021*</td>
<td>0.083</td>
<td>0.025*</td>
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*p(α) < 0.05.

Hemoglobin

The concentration of hemoglobin was significantly higher in group BP on the 6th and 14th days of research (5.5% and 7%, p < 0.05, respectively) (Table 3).

Hematocrit

On the 6th day of the experiment, the hematocrit level was significantly higher in group BP (7%, p < 0.05) (Table 4).

Leucocytes (WBC)

The number of WBCs was significantly higher in group KP on the 10th day of investigation (42%, p < 0.05) and significantly decreased between the 6th and 10th days (60%, p < 0.05) and between the 6th and 14th days (50%, p < 0.05) in BP (Table 5).

Sodium

There were no significant differences in sodium concentration between groups.

Potassium

Between the 10th and 14th days, potassium concentration significantly decreased in group BP (14%, p < 0.05).

Table 4. Hematocrit Level [l/l]

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<th>6th Day</th>
<th>10th Day</th>
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<tr>
<td>Group BP</td>
<td>0.352 ± 0.018</td>
<td>0.355 ± 0.037</td>
<td>0.354 ± 0.033</td>
</tr>
<tr>
<td>Group KP</td>
<td>0.327 ± 0.018</td>
<td>0.344 ± 0.02</td>
<td>0.332 ± 0.015</td>
</tr>
<tr>
<td>BP/KP p(α)</td>
<td>0.021*</td>
<td>0.354</td>
<td>0.173</td>
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*p(α) < 0.05.
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Table 5. Number of WBCs [G/l]

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<th>6th Day</th>
<th>10th Day</th>
<th>14th Day</th>
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<tbody>
<tr>
<td>Group BP</td>
<td>8.6 ± 1.8</td>
<td>3.4 ± 0.6</td>
<td>6.8 ± 2.3</td>
</tr>
<tr>
<td>Group KP</td>
<td>8.8 ± 1.2</td>
<td>5.8 ± 1.6</td>
<td>7.1 ± 3.5</td>
</tr>
<tr>
<td>BP/KP p(α)</td>
<td>0.752</td>
<td>0.036*</td>
<td>0.916</td>
</tr>
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</table>

*p(α) < 0.05.

Total Protein

There were no significant differences in total protein concentration between groups.

Urea

There were no significant differences in urea concentration between groups.

Hydroxyproline

Concentration of hydroxyproline was measured in the scars.

On day 6, its concentration was significantly higher in group BP (40%, p < 0.05). Between the 6th and 10th days, hydroxyproline increased significantly in group BP (25%, p < 0.05) and in group KP (37.5%, p < 0.05). On the 10th day, hydroxyproline significantly increased in group BP (29%, p < 0.05). Between the 10th and 14th days, its level significantly increased in group BP (13%, p < 0.05). On the 14th day of investigation, hydroxyproline was higher in group BP (27.5%, p < 0.05). Between the 6th and 14th days, hydroxyproline increased significantly in group BP (35%, p < 0.05) and group KP (46.5%, p < 0.05) (Table 6).

Malic Dehydrogenase (MDH)

Activity of MDH significantly decreased in group KP between the 6th and 14th days (25.5%, p < 0.05).

Table 6. Concentration of Hydroxyproline [mg/g of Dry Tissue]

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<th>6th Day</th>
<th>10th Day</th>
<th>14th Day</th>
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</thead>
<tbody>
<tr>
<td>Group BP</td>
<td>27.12 ± 3.36</td>
<td>36.23 ± 3.37</td>
<td>41.49 ± 3.78</td>
</tr>
<tr>
<td>Group KP</td>
<td>16.11 ± 3.22</td>
<td>25.77 ± 3.43</td>
<td>30.04 ± 4.36</td>
</tr>
<tr>
<td>BP/KP</td>
<td>0.012*</td>
<td>0.011*</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

*p(α) < 0.05.
Body Mass

We compared body mass between the 1st and the last day for every rat, assuming the difference as a ratio 1.00 with no difference.

We noticed no statistical difference in the change of body mass in experimental animals during the investigation.

Ultrastructure of Scars in Electron Microscopy

In the BP group after 6 days from the beginning of research, we saw in central part of the section the appearance of collagen fibers (Figure 1). They are characterized by transverse stripes, created by breaks between tropocollagen molecules, passing each other at about 40 nm. Light and dark stripes allow us to identify and distinguish collagen in the scar. There is no such characteristic striping in pictures of group KP on the 6th day (Figure 2). One can see only necrotic and clotted blood fragments with no regular features of a growing scar.

After 10 days from the beginning of the investigation, the collagen structure started to become distinct (Figure 3). The collagen created a regular network with parallel fibers, clearly separated from the deeper layers of skin and from the layers of the new epidermis. In the scars of group KP, the new appearing collagen fibers were only just beginning to appear on the 10th day of experiment (Figure 4). Their structure was clearly less well defined in comparison with group BP, with no regular bundling of fibers.

Figure 1. Ultrastructure of group BP 6 scar in scanning electron micrography. All magnifications = 8300 x.
Figure 2. Ultrastructure of group KP 6 scar in scanning electron micrography.
Figure 4. Ultrastructure of group KP 10 scar in scanning electron micrography.

The ultrastructural picture of scars in group BP showed a mature formed net of collagen on the 14th day (Figure 5). The fibers were regular, with a dense, unbroken structure. The collagen of the exposed animals seemed to be very similar to mature collagen of normal skin, not revealing the features of uncontrolled growth. In animal

Figure 5. Ultrastructure of group BP 14 scar in scanning electron micrography.
of group KP, there were no such regularities of collagen structure (Figure 6). The fibers were not so regularly arranged with clearly visible gaps in their continuity. We perceived bifurcation in tropocollagen structure, proving its weaker structure. There was no distinct separation among collagen fibers, and there were many necrotic remains.

DISCUSSION

We found in the world literature many clinical studies describing the use of ELF-MF in wound treatment. However there is no wide experimental exploration of this problem. Patino et al. used 50 Hz, 20 mT sinusoidal magnetic field in treatment of experimental burn wounds in rats. The first control group of animals had wound dressings imbibed with Nitrofurazone, the second with a solution of NaCl. The surface of the wound was measured with a special planimeter on the 7th, 14th, and 21st day of experiment. The researchers proved statistically the positive effect of therapy with magnetic field in relation to the control group. The foundations of an effective activity of magnetotherapy on burn wounds were proved. [24]

A further investigation was executed by Ottani et al. They detected the influence of ELF-MF (50 Hz, 8 mT) on rats with a dorsal incisional wound. The rate of healing was assessed in the electron microscope by detecting the maturity of capillary vessels and quantity of collagen in new forming scar. [23]

The influence of extremely low frequency magnetic fields and low-energy laser on burn wounds was also investigated by Biniszkiewicz. [4] He proved that a wound exposed to a magnetic field and laser was cleansed quicker and was better perfused. The maximum effect was obtained using both methods together.
On the 6th and 14th day, we observed in our investigations a significantly higher quantity of erythrocytes and concentration of hemoglobin in the blood of experimental animals. The stimulating erythropoietic activity of low frequency magnetic fields has already been demonstrated in earlier works by Margonato et al. [21] and Sieron et al. [30]. The activation of this process, appearing as an increase of erythrocyte numbers and elevated hemoglobin concentration in the blood of experimental animals, augments the oxygen supply to tissue, which prevents anaerobic transformation and formation of free oxygen radicals [3,38].

The process of oxygen supply, intensified by ELF-MF, is also amplified by faster neangiogenesis and changes in rheological features of blood. There is evidence in the world literature [8,13,28] showing changes in plasma stickiness, shape of erythrocytes, a change in ionic permeability, and the reactance of their cellular membranes.

From the beginning of the experiment, there was a significantly higher number of reticulocytes in the experimental group, which stayed constant throughout the time of the investigation. As the result of the influence of ELF-MF, such increased erythropoiesis gives the possibility for better utilization of oxygen, beginning from the intensification of the diffusion process in the lungs, to its transport to and utilization in tissues. As far as the morphological parameters of blood are concerned, we noticed that in spite of the considerable stimulation of erythropoiesis, there was no clumping of blood cells in group BP, which was proved by the constant level of the hematocrit and blood morphology in the experimental animals. Such a state is possible due to the influence of magnetic fields on electric potential charges on RBC cellular membranes and the enlarged protective activity of ELF-MF against free radicals, which damage these membranes. Comparing the experimental groups, we did not see a significant change over the entire period of research in the number of leukocytes in blood. There were also no significant changes of this number between particular days of investigation, and their level stayed constantly within normal limits. We expect such relations, considering our proposed model of wound healing.

Types I and III collagen build the scar. Their concentration in the healing tissues is the basis of the physical and chemical resistance of the scar. Disturbance of collagen synthesis can lead to setbacks or handicaps in healing. [33] The concentration of hydroxyproline in healing tissues is a reliable indicator of collagen deposition, which might be considered as a function of wound healing (over time). At each time period of our research, we noted significant differences in the hydroxyproline concentration between groups of the experiment. On the 6th day, this difference was the highest, slightly decreased on the 10th and 14th day. The hydroxyproline concentration in the healing tissue reflects the level of newly synthesized collagen, and attains its summit physiologically between the 14th and 21st day of healing. After this time, the quantity of the collagen in the scar did not increase, but still consolidated the wound structurally due to collagenase present in the healing wound. [35] This enzyme easily melts the collagen and in this way an equilibrium exists, making possible the reconstruction of scar and modeling to surrounding tissues. In this period, the collagen changes only the mechanical properties of scars, attaining after 6 weeks 50% of the natural strength of healthy tissue. [27]

Magnetic fields, in therapeutic parameters, have a stimulating influence on collagen synthesis. We can propose that the reason for this is a strong fibroblast activation
in the connective tissue surrounding the wound, thus increasing the synthesis of collagen and its excretion to form the scar. The activation of fibroblasts takes place by a decrease in adenyl cyclase activity and then the subsequent decrease of cAMP concentration. The next step in the increase of collagen synthesis is the higher activity of proline 4-hydroxylase, under the influence of an iron ion, which determines the activity of its prosthetic group.\[6,12,18,28]\ The collagen also plays a very important role as a structural element of blood vessels growing to the wound. The increased synthesis of collagen leads to their quicker maturation and increased oxygen supply to healing tissue as well.

The total collagen concentration in the healing tissue correlated with the pictures of scars in the electron microscope. Already after 6 days of magnetic field exposure, the appearance of distinct weaving of the net of collagen was visible in the newly forming skin layer, while in control animals the collagen net was completely absent. The collagen net in rats from the KP group appeared only on the 10th and 14th day of the experiment. On these days, the electron microscope pictures showed a weak net of collagen; intermittent, not showing the strong structural monolith of a new-forming scar. The collagen of the scar was visible in group BP as a network of equal tropocollagen fibers, uninterrupted with a condensed structure. On the 14th day of research, this structure appeared as a well organized and equal collagen net, similar to the collagen of healthy skin.\[5\] Magnetic fields thus have a beneficial activity on collagen synthesis, as seen in the microscope pictures. There is also a problem with uncontrolled scar growth and keloid formation. Given this possibility, our microscope investigations should have been extended to the measurement of the thickness of the collagen fibers. However, the lack of access to a suitable microscope limited this possibility.

After complicated and long operation procedures, surgeons observe losses of body mass in the postsurgical period.\[35\] However, in our experiment, we did not note any decrease of body mass in the experimental animals. We explain it by noting that all animals used in the research were actively growing and developing during the whole experiment. Such wounds and the experimental conditions were not able to change the metabolism of the experimental animals, so we considered the wounds as local surgical disorders. This fact we tried to confirm by detecting changes in the urea and the total protein concentration in blood serum. We noticed no differences on the 6th, 10th, and 14th day of investigations. However the homeostatic control of these values, in all periods of the experiment, is extremely important for the correct supply of proteins and amino acids to the regenerating tissues. In our experiment, we did not note changes in the concentration of sodium and potassium in serum. The concentration of these two ions stayed constant in both experimental and control groups.

Extremely low frequency magnetic fields beneficially influence the regeneration process, not intensifying systemic catabolic changes of malic dehydrogenase activity in the livers of either experimental animals. MDH (E.C.1.1.3.7)—a key enzyme of the Krebs cycle can be treated as an indicator of cellular changes in the energetic process.\[22\] Although magnetic fields enhance the endogenous mechanism of tissue oxygen supply and utilization, they do not influence cellular energetic processes, which remain very stable.
CONCLUSIONS

1. The systemic activity of extremely low frequency magnetic fields beneficially influences the collagen synthesis in experimental wounds in male rats and leads to better stabilization and mechanical strength of scar.

2. Collagen fibers, as observed in the electron microscope, present higher density and more regular position of tropocollagen in the scars of rats exposed to ELF-MF. This correlates with a higher concentration of collagen in the treated scar, reflecting its better maturity.

3. The healing process in experimental animals is supported by activation of the oxygen supply and utilization processes as a result of erythropoietic intensification, without simultaneously upsetting cellular (mitochondrial) energetic processes.

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