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# EFFECTS OF PULSED LOW-FREQUENCY MAGNETIC FIELD ON ACTIVITY OF REDOX ENZYMES IN THE ALBINO RAT LIVER (HISTOCHEMICAL INVESTIGATION)

[inhibition of succinate dehydrogenase in hepatic tissue of rats exposed acutely to 900 Oe fields, or chronically to 300 Oe fields, pulses 130 μsec duration, 10 pps between pulses, at 7 KHz pulse modulation

[Article by L.N. Yashina (Khar'kov), Institute of Industrial Hygiene and Occupational Pathology; Vliyaniye Impul'snogo Magnitnogo Polya Nizkoy Chastoty na Aktivnost' Ikislitel'no-vosstanovitel'nykh Fermentov v Pecheni Belykh Krys (Gistokhimicheskoye Issledovaniye), in Russian, submitted 14 September 1970, pp 53-56]

Among the enormous range of electromagnetic oscillations that man encounters in the course of industrial activity, pulsed magnetic fields (PMF) have not been investigated to date, although they are being increasingly used in several branches of industry. As for constant [static, continuous] magnetic fields (CMF), it was demonstrated experimentally that they have a direct effect on enzyme activity (Cook and Smith), processes of tissular respiration (Reno and Nutini), and alter the activity of a number of oxidative enzymes (M.A. Shishlo and L.L. Shimkevich).

In view of these investigations dealing with the effects of CMF on various aspects of biological oxidation, as well as of the significant role of energy metabolism in regulating the most diverse physiological processes, we deemed it purposeful to investigate the activity of some redox enzymes under the influence of PMF.

Experiments were conducted on 60 male rats weighing 150-170 grams, exposed to PMF with the following parameters: intensity of the magnetic field in the inductor constituted 72 kiloamperes/meter [ka/m](900 oersted); 24 kiloamperes/meter (300 oersted); pulse frequency -- 7 kilohertz, pulse duration -- 130 microseconds, interval between pulses -- 10 seconds. Two series of experiments were conducted. In the first series, experimental rats were exposed to 72 ka/m (900 oersted) in 15 sessions, each lasting 3 hours. In the second series, they were exposed to PMF with an intensity of 24 ka/m (300 oersted) for 1, 3, and 6 months, with 1.5-hour exposure daily. At set times, intact control animals were sacrificed concurrently with experimental ones. Some of the animals were sacrificed 1 and 2 months after termination of PMF exposure.

Histochemical techniques, which preserved mitochondrial structure and permitted observation of in situ reactions, were used on cryostat sections of hepatic tissue to investigate the activity of some redox enzymes located in the external membrane layer of the mitochondria (succinate dehydrogenase, malic, isocitric and glutamic acid dehydrogenases). Nitro-blue tetrazolium salt (nitro ST) was used as an electron and proton acceptor from specific dehydrogenases. Degree of enzymatic activity was assessed from the quantity of diformazan -- a blue chromophore formed as a result of running the nitro-ST reaction -- in the cells. For osmotic protection of mitochondria, polyvinylpyrrolidone was added to the incubation medium. In addition to unfixed hepatic tissue, some sections were first fixed in acetone cooled to 2° to remove lipid inclusions. Histochemical reactions were run using original prescriptions indicated in the textbook on theoretical and applied histochemistry by E. Pierce. In addition, RNA of hepatic tissue

was estimated according to Brachet, with RNAase and TCA (trichloroacetic acid) control, as well as proteins according to Danielli, SH groups according to Barnett and Seligman. Some of the liver sections were stained with hematoxylin-eosin for a morphological control.

In the cytoplasm of hepatic cells of control rats, it was established that there is distinctly marked activity of succinate dehydrogenase, isocitrate, malic, and glutamate dehydrogenases, which is documented by the large quantity of diformazan granules (Figure 1, a, and Figure 2, a). The end product of the reaction, diformazan, is deposited, as we know, in the hepatocyte cytoplasm, in the form of very fine blue granules, round in shape, in the mitochondria. Occasionally, large granules are encountered. We were impressed by the higher activity of enzymes in hepatocytes located on the periphery of the hepatic lobule (see Figure 2, a). Perhaps this fact is related to the distinctions of circulation in peripheral and central regions of hepatic lobes, since it was established that the peripheral cells are the first to be covered ["washed"] with blood entering the lobule and, consequently, receive a better supply of oxygen, as a result of which tissular respiration in them undergoes the Krebs cycle at a higher level than in the cells of the central regions of the lobule, with lower supply of oxygen (Novikoff).

Multiple exposure of the animals to PMF, in the first series of experiments, with an intensity of 900 oersted (for 10-15 sessions) elicited a significant decrease in succinate, isocitrate, and malate dehydrogenases, as documented by the decreased quantity of diformazan granules. A decrease in intensity of glutamate dehydrogenase reaction was also noted (Figure 1, b). We know that this enzyme, which participates in synthesis of different amino acids, as well as in the $NH_4^+$ binding reaction with keto acids by means of

which the liver performs its detoxifying function, is very specific for the liver and its level in blood serum rises only when hepatic cells are damaged.

In order to determine whether the inhibition of enzyme activity, which we observed, progresses as exposure to PMF extends, we continued it for 6 months (field intensity of 300 oersted, 1.5-hour exposure daily). Under these conditions, after 1, 3, and 6 months of exposure, and 1 and 2 months after stopping it, we estimated activity of succinate dehydrogenase, which is one of the "key enzymes" in the citric acid cycle of Krebs and plays an important part in synthesis of energy-rich macromolecular compounds. After 1 month of exposure to 300 oersted PMF, the rat liver failed to demonstrate differences in distribution of succinate dehydrogenase, as compared to control animals. The most distinct decrease in activity of this enzyme, as well as change in distribution of diformazan were noted in the hepatocyte cytoplasm after 3 months of exposure to PMF (see Figure 2, b), which could be considered an indication of poorer tissular respiration through the Krebs cycle. The regional differences with regard to succinate dehydrogenase activity persisted: enzyme activity was higher in the hepatic cells of paripheral regions than in the central parts of the lobule. However, by the end of the 3rd month of PMF exposure, the size of the lobular region with high enzyme activity was much smaller than in control animals. By the 3rd month of exposure, the hepatic tissue presented marked dystrophic changes, ending with necrobiosis in a number of cases, and total destruction of some parts of the hepatic parenchyma. The cells subject to necrobiosis presented signs of coagulation of cytoplasmic proteins, manifested by more intensive reaction for protein and protein SH groups, more intensive pyrroninophilia that was not removed

by RNAase and TCA. Such intensification of sorption properties of cytoplasm should be evaluated as the result of changes similar to denaturation in the structure of cytoplasmic proteins, described by D.N. Nasonov and his school in paranecrotic phenomena. In some hepatic cells, against a background of diffuse staining of cytoplasm, diformazan was localized in the form of swollen, enlarged, large polymorphous granules. Such changes in distribution of diformazan could be interpreted, in the light of the data in the literature (V.V. Portugalov; N.T. Raykhlin), as an index of the physical and functional state of mitochondria, in particular, swelling thereof, increased permeability of mitochondrial membranes upon further damage, with exit of enzymes into the principal substance of cytoplasm (hyaloplasm). The decrease in succinate dehydrogenase activity which we observed in some hepatic cells persisted toward the 6th month of exposure to PMF and activity was not restored, even 2 months after exposure.

The results of these investigations are indicative of inhibition of redox enzyme activity in hepatic tissue under the influence of PMF. The damage to mitochondrial membranes led to virtually total diffusion of the reaction product, diformazan, into the cellular cytoplasm, with deposition of coarse polymorphous granules.

Conclusions

1. Using histochemical techniques, we demonstrated inhibition of activity of succinate dehydrogenase, isocitrate, malate, and glutamate dehydrogenases in hepatic tissue under the influence of low-frequency PMF.

2. It was established that the activity of the enzymes studied was not the same in hepatocytes of different regions of hepatic lobules, and that this was apparently related to the difference in their functional activity.

3. The chronic effect of PMF, with an intensity of 300 oersted, leads to damage of mitochondrial membranes that are so important for the principal functional elements of the cell, the organelles.

4. The intensity of change in enzyme activity is related to the intensity of the field and duration of exposure. Some hepatic cells demonstrate inhibition of succinate dehydrogenase activity even 2 months after exposure to PMF.

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## Figure Captions

Figure 1. Decreased activity of glutamate dehydrogenase activity in the rat liver after 15 days of exposure to PMF, with an intensity of 900 oersted. Hess, Scarpelli, Pierce reaction. Magnification: 63×. a) control, b) experiment

Figure 2. Decreased succinate dehydrogenase activity in the rat liver after 3 months of exposure to PMF, with an intensity of 300 oersted. Reaction of Najlas et al. Magnification: 63×. a) control, b) experiment