

## Prevention of Aging. Communication I.

### Individual Enzymatic Variations of the Antioxidant System and a Way to Correct this System by Means of Electrochemically Activated Systems

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**Abstract**—The effect of electrochemically activated systems (EGAS) on the enzyme activity of the antioxidant system (catalase, peroxidase, and superoxide dismutase) appears to be a normalizing effect that increases suppressed enzyme activity and decreases enhanced enzyme activity. Baseline enzyme activity is characterized by significant individual variations in both animals and humans. The effect of EGAS may be explained by the training effect of electroactivated systems due to the excess of electrons, with the EGAS redox potential being negative.

Reactive oxygen species (ROS) are the normal body components involved in many physiological processes that are controlled by special enzymatic systems. However, the free forms of ROS exert a significant damaging effect immediately or during the formation of long-lasting chain reactions. Physiological aging causes variably directed changes in ROS generation and the inactivation system: a decrease in ROS generation and defense activity of the body is accompanied by an increased body concentration of secondary damaging active macromolecules. The general pattern of the changes in the antioxidant system during aging is consistent with the concept of a global metabolic impairment in an senescent organism. Because the antioxidant system prevents the destruction of macromolecules, which are considered to be the main cause of aging, the increased activity of the antioxidant system, irrespective of any effects, is associated with an increased life span and less pronounced aging of macromolecules.

There is a way to pharmacologically stimulate the body's antioxidant defenses. Different pharmacological agents may have a pronounced geroprotective effect accompanied by anticarcinogenic, radioprotective, and biostimulating activity. Clearly, the effect on the redox potential (RP) of water and macromolecules is an important mechanism of the effect produced by these agents. New methods have been developed to obtain water-based substances without harm to the body and with the necessary RP indices, i.e., the electrochemically activated systems (EGAS) [1-8].

By means of the EGAS method, potable water can be adjusted to standard RP and pH indices and enriched with OH<sup>-</sup> or H<sup>+</sup> groups with electrochemical treatment.

During this treatment, the water simultaneously acquires several important properties: it becomes disinfected, dechlorinated, and detoxified due to the oxidation and reduction of toxic compounds and the precipitation of the salts of heavy metals.

As far as we know, there are three main factors of EGAS: (1) the formation of stable chemical compounds (acids in anolyte and alkalis in catholyte) that change the pH of liquids, which was initially the primary consideration of researchers; (2) the formation of unstable (metastable) superactive compounds (highly oxidized forms of ions or molecules and free radicals), which causes the physicochemical and training effect on the body's defense systems and changes in the RP; and (3) formation of metastable structural water anomalies after exposure to a high-intensity electrostatic field of electrodes (up to 8000000 V/cm), which results in no effect on the pH and RP but significantly changes the biophysical properties of the dissolved molecules, the substance activation conditions during enzymatic reactions, reactions at the film-cell membrane interface, etc.

Currently, there is only one patent for using EGAS in clinical practice [9] to treat purulent wounds, burns, inflammation of mucous membranes, and other pathological processes in skin and mucosa.

Studies showed pronounced antibacterial properties of anolytes, which are more effective than many modern antibiotics, and the effectiveness of the inhibition of microorganisms such as *Pseudomonas aeruginosa*, *Candida*, and other antibiotic-resistant microflora.

Catholyte facilitated regeneration, healing, and functional recovery; eliminated tissue acidosis; and

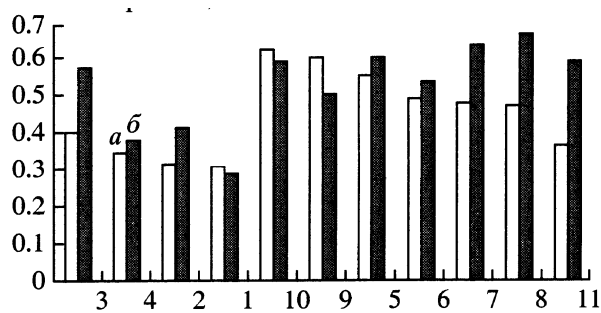
Decreasing peroxide, *E*

Fig. 1. The *in vivo* effect of the electrochemically activated systems (ECAS) on the catalase activity in rabbit erythrocytes and *in vitro* response to the effect of the ECAS on erythrocyte lysates. *a*, norm; *b*, ECAS; abscissa, № of rabbit. The catalase activity is given in extinction (*E*) decrease units that corresponds to fermented substrate. Rabbits nos. 1-4 are intact. An increase in enzyme activity corresponds to an increase in (*E*) extinction.

SOD activity (decreasing E)

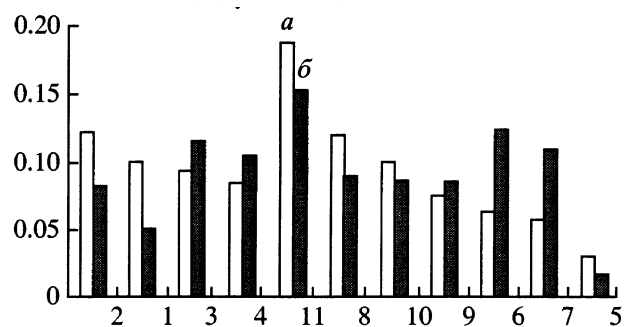


Fig. 2. The *in vivo* effect of the ECAS on superoxide dismutase (SOD) activity in rabbit erythrocytes and *in vitro* response to the effect of the ECAS on erythrocyte lysates. The SOD activity is given in extinction (*E*) decrease in the presence of SOD that corresponds to the amount of substrate (Nitro Blue Tetrazolinum) protected from superoxide. Rabbits nos. 1-4 are intact. An increase in enzyme activity corresponds to an increase in extinction (*E*). Other designations as in Fig. 1.

normalized many body parameters impaired during pathological process.

We studied individual enzyme activities and the effects of the ECAS on the activity of three main enzymes of the antioxidant system.

#### METHODS

The activities of catalase, peroxidase, and superoxide dismutase (SOD) were studied in peripheral blood erythrocytes in 11 rabbits and 10 healthy volunteers. Catalase was assayed by the reaction of peroxide with ammonium molybdate [10]; peroxidase activity, with Indigo Carmine [11]; and SOD activity, by the inhibited reduction of Nitro Blue Tetrazolinum (NBT) by super-

oxide ions generated in the reaction of  $\text{NAD} \cdot \text{H}$  with phenazine methosulfate [12].

The ECAS was obtained by passing a sodium chloride solution (1-2 g/l) through an Izumrud unit with two reaction blocks, which allowed us to obtain the ECAS with a redox potential ranging from +200 to -70 mV. A pH-meter with platinum electrodes was used to experimentally determine and monitor the RP according to the standard RP sampling regimen.

The ECAS (300 ml) with an initial RP activity of -70 mV (200 mg/l NaCl) was given to rabbits with water for two days. Then, we examined the level of blood activity of three main enzymes (peroxidase, catalase, and SOD) and the response of these enzymes to 10-min *in vitro* incubation with the standard ECAS solution (10% ECAS with 1g/l NaCl and RP = -65 mV). The experiment included seven rabbits; four rabbits were used as the control. The *in vitro* effect of the ECAS on the enzyme activity of human erythrocyte hemolysates was studied in the same way.

#### RESULTS AND DISCUSSION

The effect of the ECAS on the enzyme activity of the antioxidant system may be described as a normalizing effect that raises the decreased enzyme activity and decreases the enhanced enzyme activity. The marked individual variations of the baseline enzyme activity are observed in both animals and humans. This is evidence of the differences in the organism's reactivity. The effect of the ECAS is likely to result from the ROS generation due to the excess of electrons with the RP being negative.

Data showing the effect of the ECAS on the catalase, SOD, and peroxidase activity in erythrocytes of rabbits given the ECAS with drinking water for two days are shown in Figs. 1-3.

Unlike the other two enzymes, the peroxidase activity slightly changed under the effect of the ECAS. It averaged 69.8 s, with a standard deviation of 4.6 s (range, 65 to 76 s) in the control group. The reaction time decreased in the control group, which indicated the peroxidase activation up to 52.9 s (standard deviation of 18.0 s), with a greater enzyme activity variation ranging from 30 to 75 s. Most likely, such variation revealed the individual differences in the enzyme activation under the effect of the ECAS.

An analysis of the effect of the ECAS on erythrocyte lysates revealed the enzyme activation tendency in both groups. However, due to great variations, the means were nonsignificant (activation for 5.9 and 6.2 s with a standard deviation of 11.5 and 8.9 s in the control and test groups, respectively). The analysis of the effect of the ECAS on each animal reveals no clear differences. Thus, with both high and low enzyme activity, the *in vitro* effect of the ECAS appeared as either activation or inhibition of the response.

The catalase activity in the control group averaged 0.335 arb. units (expressed in decreasing extinction units proportional to the amount of fermented substrate, i.e., hydrogen peroxide) with a standard deviation of 0.041 arb. units (range, 0.305 to 0.393 arb. units). In the control group, the substrate consumption increased during the reaction time (catalase activation was observed) up to 0.503 arb. units (standard deviation of 0.089 arb. units) with a greater enzyme activity range of 0.353 to 0.613 arb. units. Most likely, such a range is evidence of the individual differences in enzyme activation under the effect of the ECAS.

The analysis of the effect of the ECAS on erythrocyte lysates revealed the enzyme activation tendency in both groups (by 21.1 and 19.1% in the control and test groups, respectively). However, due to great variations, the means were nonsignificant (standard deviations in groups were 21.6 and 28.1%, respectively).

Analysis of the effect of the ECAS on each animal shows that, if the animals are given the ECAS with water, the catalase activity in almost all animals from the test group is higher than that of the test rabbits. With mean and low enzyme activity, a stimulating effect of the ECAS on the erythrocyte lysates *in vitro* is observed. However, the enzyme superactivity (rabbits nos. 10 and 9) *in vivo* results in a reverse effect of the ECAS on the lysates: the catalase activity decreases after 10 min of incubation with the ECAS.

The SOD activity in the control group averaged 0.098 arb. units (expressed in the decreasing extinction units proportional to the NBT that is protected from the peroxide generated in the reaction of  $\text{NAD} \cdot \text{H}$  with phenazine methosulfate) with a standard deviation of 0.016 arb. units (range, 0.265 to 0.302 arb. units). The test group showed a slight decrease in the mean SOD activity down to 0.088 arb. units (standard deviation of 0.053 arb. units) with a greater enzyme activity range of 0.198 to 0.358 arb. units. Most likely, such a range is evidence of the individual differences in enzyme activation under the effect of the ECAS as in the case of two enzymes.

The analysis of the effect of the ECAS on erythrocyte lysates revealed a downward trend of the SOD activity in the control group (7.6%) with a significant standard deviation (38.2%), which makes comparison of the means inappropriate, whereas, in the test group, the SOD activation (17.2%) was observed with a significant standard deviation (58.8%).

Analysis of the effect of the ECAS on each animal shows that the increased SOD activity is accompanied by a reverse response to the ECAS in the *in vitro* test in both control and test groups, whereas decreased SOD activity causes activation as a response to the effect of the ECAS on the isolated rabbit erythrocytes. The exception was rabbit № 5, with very low SOD values, whose response to the ECAS appeared as a further decrease in the SOD activity.

In humans, the effect of the ECAS on the activity of all three enzymes after a 5-min incubation of 5% ECAS

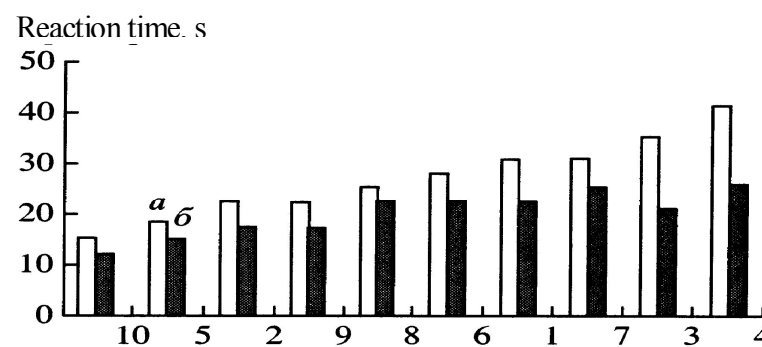


Fig. 3. The *in vivo* effect of the ECAS on peroxidase activity in rabbit erythrocytes and *in vitro* response to the effect of the ECAS on erythrocyte lysates. a, norm; b, ECAS; ordinate, reaction time; abscissa, № of rabbit.

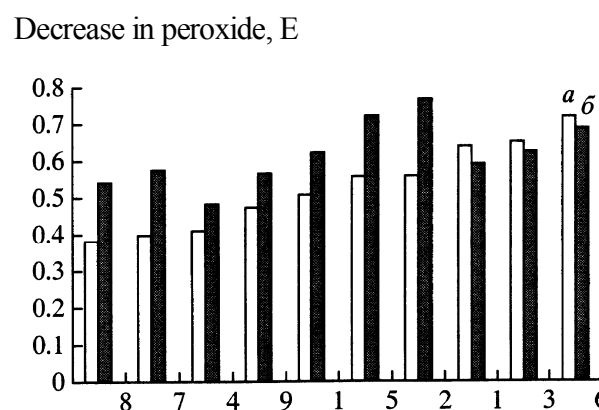


Fig. 4. The *in vitro* effect of the ECAS on catalase activity in human erythrocytes. a, norm; b, ECAS; ordinate, enzyme activity given in extinction units as in Fig. 1; abscissa, № of test (healthy donor).

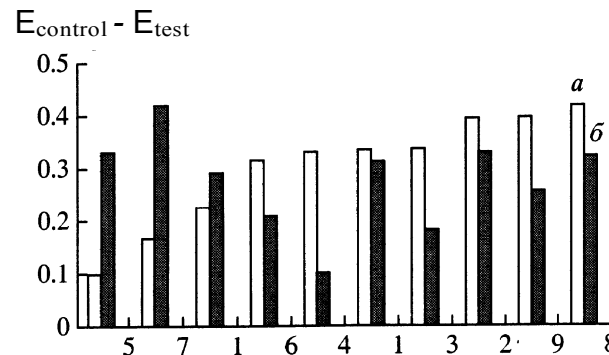


Fig. 5. The *in vitro* effect of the ECAS on the SOD activity in human erythrocytes. a, norm; b, ECAS; ordinate, enzyme activity given in extinction units ( $E_{\text{control}} - E_{\text{test}}$ ); abscissa, № of test (healthy donor).

solution ( $RP = -45 \text{ mV}$ ) with peripheral blood erythrocyte hemolysates was principally the same as in rabbit erythrocytes (Figs. 4 and 5).

Significant variations of baseline activity were observed in all three enzymes. As for peroxidase, the *in vitro* effect of the ECAS on erythrocyte hemolysates

of healthy donors under given conditions caused a decrease in peroxidase activity in all cases. As for catalase and SOD, as with rabbit erythrocytes, the low enzyme activity caused a decrease and vice versa. Thus, the ECAS produced a normalizing effect on the activity of the antioxidant system.

#### CONCLUSION

The enzyme activity of the antioxidant system showed significant individual variations in both rabbits and humans. The effect of the ECAS on the catalase, peroxidase, and superoxide dismutase activity appeared to be a normalizing effect, which raised the decreased enzyme activity and reduced the enhanced activity. The effect of the ECAS may be explained by the training effect of electrochemically activated systems due to the excess of electrons, with the ECAS redox potential being negative.

#### REFERENCES

1. *Vtoroe respublikanskoe soveshchanie po elektroaktivirovannym sredam* (Second Republican Meeting on Electroactivated Media), Kazan, 1987.
2. *Tez. Vseros. konf. po metodam i sredstvam sterilizatsii i dezinfektsii v meditsine* (Proc. All-Russia Conf. on Sterilization and Disinfection Techniques and Agents in Medicine), Moscow, 1992.
3. *Tez. Vtoroi Vseros. konf. po primeneniyu elektroaktivirovannykh metodov v meditsine* (Proc. Second All-Russia Conf. on Application of Electroactivation Techniques in Medicine), Konakovo, 1989.
4. *Vtoroe respublikanskoe soveshchanie po elektroaktivirovannym sredam* (Second Republican Meeting on Electroactivated Media), Kazan, 1987.
5. Dontsov, V.I., Krut'ko, V.N., and Podkolzin, A.A., *Starenie: mekhanizmy i puti preodoleniya* (Aging: Mechanisms and Ways of Prevention), Moscow, 1997.
6. Podkolzin, A.A. and Dontsov, V.I., *Faktory maloi intensivnosti v bioaktivatsii i immunokorreksii* (Low Intensity Factors in Bioactivation and Immunocorrection), Moscow, 1995.
7. Podkolzin, A.A. and Dontsov, V.I., *Starenie, dolgoletie i bioaktivatsiya* (Aging, Longevity, and Bioactivation), Moscow, 1996.
8. Khasanov, R.Sh., Application of Electrochemically Activated Systems (EGAS) in Complex Treatment of Purulent Wounds and Burns, *Cand. Sci. (Med.) Dissertation*, Moscow, 1986.
9. Podkolzin, A.A. and Khasanov, R.Sh., Patent Application 365284.13 (121800), 1983.
10. Korolyuk, M.A., Ivanova, L.I., Maiorova, I.G., and Tokareva, V.E., Method for Determination of Catalase Activity, *Lab. Delo*, 1988, № 1, P. 16.
11. Asatiani, V.S., *Fermentnye metody analiza* (Methods of Enzymatic Analysis), Moscow: Nauka, 1969.
12. Chevri, S., Chaba, I., and Sekei, I., The Role of Superoxide Dismutase in Cellular Oxidation Processes and the Method for Its Determination in Biological Materials, *Lab. Delo*, 1981, № 11, P. 678.