# STATE OF THE ANTI- AND PRO-OXIDANT SYSTEMS DURING HEALING OF EXPERIMENTAL SEPTIC AND INFECTED WOUNDS

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The important biological role of oxygen has been established and the free-radical theory of its toxic action formulated [1, 4, 5, 11]. By itself the oxygen molecule is not a danger for the cell, but active intermediates and their derivatives, namely the superoxide anion-radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), the hydroxyl radical (OH), and singlet oxygen ($O_2$) [1, 4], formed continuously by enzymic and nonenzymic pathways, and lipid peroxidation products formed after initiation by them, may be extremely dangerous. They have a cytostatic action, they are able to damage the cell membranes and DNA molecules, they lower the concentration of sulfhydryl groups [1, 17], of fat-soluble hormones and vitamins, with subsequent potentiation of destructive processes [4, 17, 18] which lie at the basis of certain pathological states, including radiation sickness [1] and burns [4], atherosclerosis, and ischemic heart disease [8]. To protect cells against the harmful action of oxygen intermediates, the body has a protective mechanism, which is based on a balanced system of individual components and optimal spatial coordination of reactions, due to the arrangement of antioxidant enzymes in the immediate vicinity of regions of intermediate accumulation, and also a surplus of power relative to the level of oxygenation and intensity of lipid peroxidation (LPO) under normal conditions [2]. The key role in the antiradical defense of cells is ascribed to superoxide dismutase (SOD), which catalyzes the disproportioning reaction.

$$O_2^-:O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2,$$

as a result of which the concentration of $O_2^-$, participating in initiation of lipid peroxidation, in the cell is maintained at a low level ($10^{-11}$ M). It is claimed that $O_2^-$, interacting with $H_2O_2$ in accordance with Fenton's mechanism (with $Fe_2^{++}$ complexes as the catalyst), forms an extremely reactive hydroxyl radical, which can interact directly with acyl groups of unsaturated fatty acids of lipids. Irreversible injuries to living cells by free radicals and LPO products are one stage in the development of inflammation [16].

The aim of the present investigation was to study SOD activity and the concentration of hydroperoxides in the form of complexes, in the course of aseptic and infected wounds, assuming that this would give a more complete picture of the role of the cellular protective system and of the intensity of the course of destructive processes in them.

## EXPERIMENTAL METHODS

Two series of experiments were carried out on 230 male Wistar rats weighing 180-210 g. The experimental model consisted of aseptic and infected superficial wounds with an area of 400 mm$^2$. The model of an aseptic wound was formed by the method described previously [7]. To obtain a model of an infected wound, the edges and floor of the wound were additionally traumatized with toothed forceps and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus ($1.5 \cdot 10^9$ microbial cells in 1 ml of physiological saline) was introduced into the wound surface (inside a ring). The rings were removed after 3 days. Daily from the 1st through the 10th day and also on the 12th and 15th days after the operation, the protein con-

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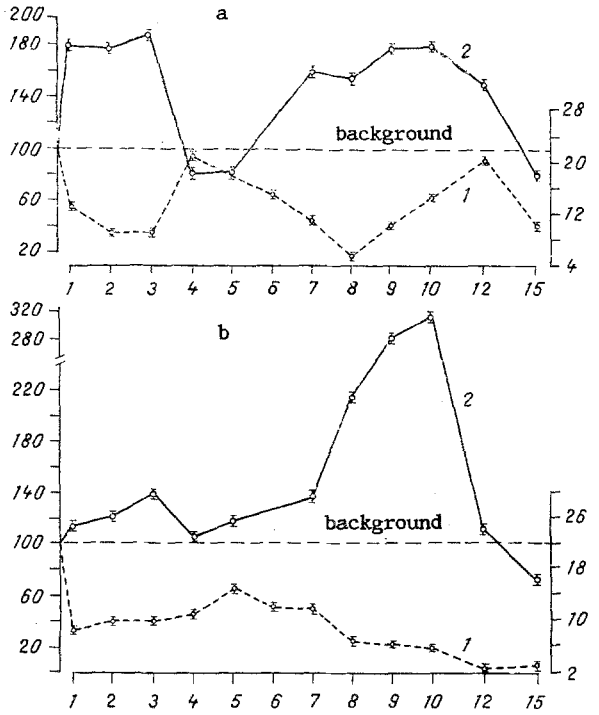


Fig. 1. Changes in SOD activity (1) and hydroperoxide concentrations (2) in granulation tissue of aseptic (a) and infected (b) wounds in rats. Abscissa) time after operation (in days); ordinate) on left – concentration of hydroperoxides (in nmoles/mg protein, in %); on right – SOD activity (activity units).

centration [20], SOD activity [14], and concentrations of hydroperoxides [12] were determined simultaneously in serum obtained by the standard method and in the supernatant of granulation tissue obtained from the same animals of both series and also from 10 intact animals (background). As the background value of SOD activity and hydroperoxide concentrations for the tissue, values of these parameters in tissue in the region of the wound, taken 5 min after infliction, were used. At each time point 8 to 10 rats were used. The results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that during healing of aseptic and infected wounds three distinct periods were observed in the time course of the changes in specific SOD activity and hydroperoxide concentrations in the granulation tissue.

The first period, one of an increase in the hydroperoxide concentration in tissues of the aseptic wound, corresponded to the time of greatest intensity of the inflammatory phase of the course of wound healing (Fig. 1a). The body responds to the action of different traumatic agents with a marked phagocytic reaction [6], which is accompanied by a "burst" of respiratory activity, leading to increased oxygen consumption by the cells and also to generation of $O_2^-$, OH, and $H_2O_2$, which have highly reactive bactericidal properties. These latter, combined with halide ions and myeloperoxidase, form a powerful system killing microorganisms [15], and which is probably responsible for the reduction of growth of microbial colonies in the wound during this period. Intensive production of $O_2^-$ causes oxidative damage to the lipid layer of the cell membranes, probably leading to hydroperoxide accumulation in the wound tissues at the same period (Fig. 1a). Hydroperoxides, together wtih active $O_2^-$ intermediates, act destructively on cells surrounding the tissues, inactivating the sulfhydryl groups of enzymes [17], disturbing vascular permeability, and leading to the development of edema [11, 16]. According to data in the literature [15, 16], intensive $O_2^-$ formation activates chemotaxis of the leukocytes, and this evidently leads to an increase in their number in the wound and to marked neutrophilic infiltration of its edges and floor. Aseptic inflam-

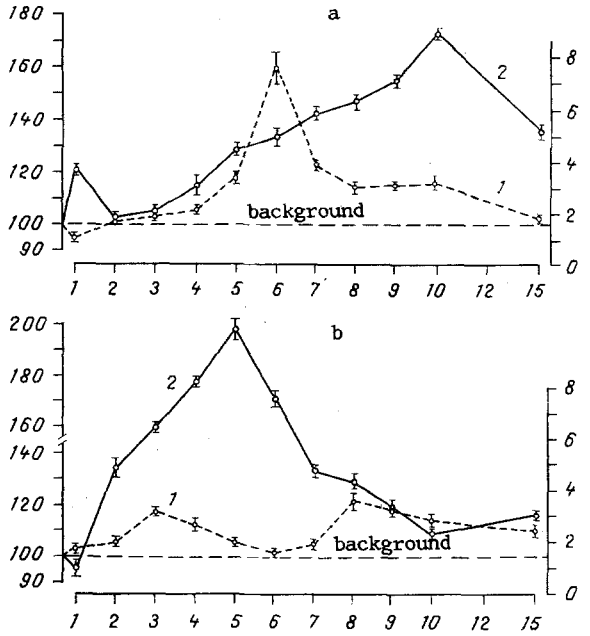


Fig. 2. Changes in SOD activity (1) and hydroperoxide concentration (2) in blood serum of rats with aseptic (a) and infected (b) wounds. Legend as to Fig. 1.

mation evidently develops as a result of these processes. Active forms of oxygen formed in excess in the wound lower specific SOD activity in the tissues [13, 18] and diffuse into the blood, possibly causing a decrease in the specific activity of SOD in the blood serum at this time (Fig. 2a). The active participation of hydroperoxides and superoxide radicals in the inflammatory process and the protective action of SOD also are confirmed by the results of other investigations [19], in which inflammatory phenomena subsided and neutrophilic infiltration in the pathological focus diminished under the influence of orgotein, a pharmacological preparation of SOD.

The second period, one of a fall in the hydroperoxide concentration in the tissues of the aseptic wounds on the 4th-5th days (Fig. 1a), is due to their absorption into the blood stream as a result of the increasing capillarization of the granulation tissue of the wound, dismutation of $O_2^-$, and a neutralization of hydroperoxides by SOD, the increase in the specific activity of which may be connected with the arrival of new blood cells (erythrocytes, lymphocytes, granulocytes), generating SOD exogenously [3], and to the release of the enzyme from phagocytosed erythrocytes and destroyed leukocytes. This evidently leads to weakening of the macrophagal reaction and of leukocytic infiltration of the tissue, to reduction of the edema, and to weakening of inflammation. The increase in the specific SOD activity in the serum toward the 6th day is evidently due to a response of the body to the arrival of hydroperoxides from the region of injury, and to increased synthesis of SOD (Fig. 1a, Fig. 2a) and to its release from the focus.

The third period (6th-15th days) begins with the conversion of metabolism to the plastic type and to supplying energy to the cells during the proliferative period. This causes active production of $O_2^-$ and hydroperoxides [3], which are "quenched" by the antioxidant system, which in turn, lowers the specific SOD activity in the tissues (Fig. 1a) [16, 18]. By the 10th day the hydroperoxide concentration in the serum is increased (Fig. 2a), probably due to lowering of specific SOD activity in the serum [13] and to reduced supply of SOD to the blood from the tissues in connection with the beginning of obliteration of blood vessels in the wound and of scar tissue formation [10]. By the 12th day normalization of specific SOD activity is observed in the tissues, possibly on account of maturation of granulation tissue and differentiation of fibroblasts [3], and to utilization of hydroperoxides by SOD and their retention at a certain level (Fig. 1a).

In the infected wounds, on the other hand, in the initial period (1st-3rd days), on account of the severe damage and destruction of muscle tissue during the operation, hemorrhage, and microbial invasion the intensive formation of a large flow of hydroperoxides after enzy-

mic dismutation of $O_2^-$ in the tissue and blood cells, synthesis of several enzymes and the lipid layer of the cell membranes were disturbed, and changes were observed in the concentrations of metallic ions, ceruloplasmin, transferrin, etc. [1]. This induced a protective response of "quenching" of hydroperoxides in the tissues (Fig. 1b) and a steady decline in specific SOD activity in them. The body may perhaps respond to the severity of the processes taking place by an increase in specific SOD activity in the serum, capable of restraining initiation and of quenching oxygen intermediates (Fig. 2b) in the serum and tissues.

The second period (4th-5th days) was characterized by activation of suppurative inflammation in the wound and by accumulation in the tissues of acid products and hydroperoxides, formed during disintegration of tissue cells and migrating leukocytes on entering the blood, causing an increase in the specific SOD activity in the tissues (Fig. 1b) and an exceptionally high $H^+$ concentration. This leads to the development of acidosis (a fall in the pH of the blood) [9] which, in turn, may perhaps provoke abnormally high initiation of $O_2^-$ and hydroperoxides [17] in the blood serum, with exhaustion of the antioxidant enzyme system (Fig. 2c).

The third period, characterized by an increase in the hydroperoxide concentration in suppurative wounds, corresponds to the 6th-10th days. During this period there was gradual weakening of the continuing suppurative inflammatory process in the tissues, accompanied by self-cleansing, and signs of proliferation began to appear, leading to an increase in the tissue concentration of hydroperoxides and to intensification of their destructive and cytostatic action on the cells [1, 18], to a sharp decline in specific SOD activity in the tissues (Fig. 1c), and to its subsequent decline [13]. This may perhaps be one of the conditions leading to delay of healing of infected wounds. During this same period tissues are evidently protected by their own tissue enzymes (glutathione peroxidase and catalase) [1], and by ceruloplasmin, transferrin, and so on, as a result of which the tissue hydroperoxide concentration is reduced, the hydroperoxide level falls gradually, and specific SOD activity in the serum falls below the background values by the 15th day.

During healing of aseptic and infected wounds the hydroperoxide concentration thus depends on the stages of wound healing, whereas the change in specific SOD activity in the tissues depends on the character and severity of the course of this process. On the basis of these results the use of antioxidants during the first days after an operation and in the period of proliferation of suppurative wounds can be recommended. In addition, the parameters listed above can be used with advantage as a test to determine the character of the course of wound healing and to assess the effectiveness of therapeutic measures.

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