LITERATURE CITED

1. Yu. Ya. Perov and Yu. V. Postnov, Byull. Éksp. Biol. Med., No. 6, 755 (1976).
2. R. I. Sokolova, A. A. Nekrasova, and N. T. Legon'kaya, Arkh Patol., No. 6, 29 (1977).
3. R. I Sokolova and A. M. Vikhert, Kardiologiya, No. 7, 56 (1981).
4. B. N. Tsibel and L. S. Khodasevich, Kardiologiya, No. 5, 102 (1980).
5. B. N. Tsibel and E. V. Nefedova, Byull. Éksp. Biol. Med., No. 3, 358 (1985).
6. E. Anggard, S.-O, Bohman, S. E. Grifi'in, et al., Acta Physiol. Scand., **84**, 231 (1972).
7. W .H. Beirwaltes, S. Schryver, P. S. Olson, et al., Am. J. Physiol., **239**, 602 (1980).
8. J. G. Gerbert, R. D. Olson, and A. S. Wies, Kidney Int., **19**, 816 (1981).
9. M. A. Knepper, R. A. Danielson, G. M. Saidel, et al., Kidney Int., **12**, 313 (1977).
10. I. P. Kokko, Kidney Int., **19**, 791 (1981),
11. K. V. Lemley and W. Kriz, Kidney Int., **31**, 538 (1987).
12. M. D. Lifchitz, Kidney Int., **19**, 781 (1981).
13. B. E. Muirhead and J. A. Pitcock, The Renal Papilla and Hypertension, New York (1980), pp. 35-61.
14. M. Rathaus, E. Rodjarny, B. Weiss, et al., Clin., Sci., **60**, 405 (1981).
15. L. Tobian, M. Ischii, and M. Duke, J. Lab. Clin. Med., **75**, 309 (1969).

CHANGES IN PHAGOCYTIC ACTIVITY IN COMPLICATED EXPERIMENTAL WOUNDS

V. V. Zakharov, A. V. Nikolaev, Ch. R. Ragimov, L. A. Mamedov, A. P. Khokhlov, A. B. Shekhter, N. D. Egorova, and V. A. Sitarov

UDC 617-001.41-002.3-092:616.155.3-008.13

KEY WORDS: suppurative inflammation, coefficient of disturbance of the antioxidative system, ascorbic acid.

An increase in the number of postoperative septic complications, which cannot always be prevented, and difficulties in their treatment are among the urgent problems of modern surgery [2, 7]. The mechanism of formation of a suppurative inflammatory process in postoperative wounds is mainly dependent on a disturbance of phagocytosis [6]. Phagocytic cells, on contact with foreign bodies or microorganisms, actively take up oxygen and generate superoxide anion-radicals ($O_2^{\cdot-}$), from which are formed hydroxyl radicals ($^{\cdot}OH$). which possess high bactericidal and also destructive actions, initiating lipid peroxidation (LPO) [10, 13, 14]. Protection against excessive intensification of LPO is effected both by the antioxidative system of the tissues and by phagocytic cells, which secrete antioxidants, especially ascorbic acid, into the intercellular space [17]. The latter substance can eliminate free radicals in accordance with the following scheme: ascorbic acid + $2O_2^{\cdot-}$ + $3H_2$ → dehydroascorbate + $2H_2O_2$, converting toxic oxygen radicals into less toxic. Ascorbic acid also protects glutathione and protein SH-groups against oxidation and is thus an additional factor in antioxidative protection [15, 19].

Hence the interest of the study of the role of ascorbic acid and LPO products on phagocytic activity in the formation of the septic focus.

I. M. Sechenov First Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 324-327, March, 1991. Original article submitted January 25, 1990.

0007-4888/91/0003-0410$12.50

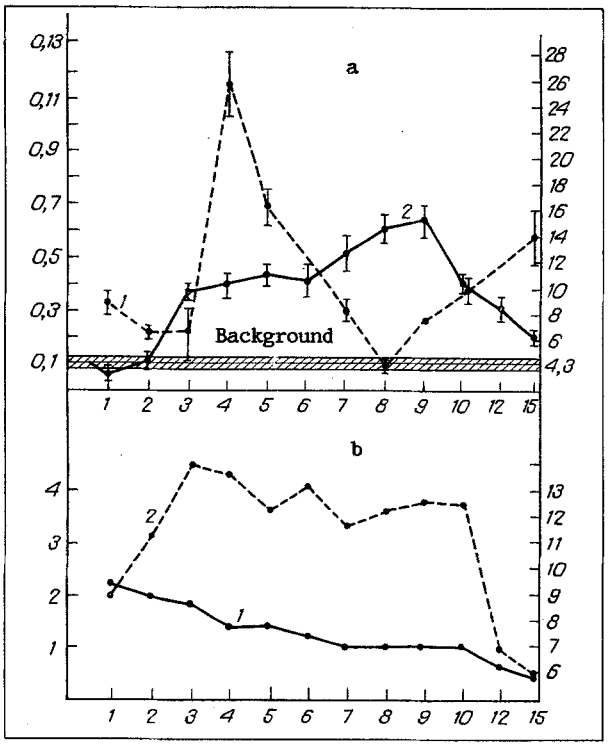

Fig. 1. Time course of ascorbic acid level and changes in values of K_{dist} (a), neutrophilic infiltration, and integral parameter of macrophagal reaction (b) in granulation tissue from rats with wounds not complicated by infection. Here and in Fig. 2: abscissa, time after operation (in days); ordinate: a) K_{dist}, relative units (1), on right – ascorbic acid concentration, mg% (2); b) neutrophilic infiltration, points (1), on right – integral parameter of macrophagal reaction, points (2).

EXPERIMENTAL METHOD

Experiments were carried out on 220 mature male Wistar rats weighing 200 g. Extensive superficial wounds, either uncomplicated or complicated by infection, served as the experimental model [3]. The total ascorbic acid concentration (ascorbic acid and dehydroascorbate) [18], superoxide dismutase (SOD) activity [9], and the concentration of hydroperoxides [8] were determined simultaneously and daily from the first through the 10th, 12th, and 15th days after the operation, in blood serum obtained by the standard method under hexobarbital anesthesia [4], and in the supernatant of granulation tissue from the same animals of both series and also from ten intact animals (background). The coefficient of disturbance of the antioxidative reserve (K_{dist}), which we first suggested, was calculated in relative units by the equation:

$$K_{dist} = \frac{\text{relative SOD activity (in percent)}}{\text{level of hydroperoxides (in percent)}}.$$

Tissue samples from the wound were taken at the same times for morphological investigation: sections were stained with hematoxylin and eosin, by Van Gieson's method, with toluidine blue, and by Brachet's reaction. A different inflammatory and reparative feature, including neutrophilic infiltration, and an integral parameter of the macrophagal reaction, consisting of a set of features (total number of macrophages, phagocytic activity of epithelioid cells and large macrophages, multinuclear cells, resorption of necrotic tissues and fat cells – on a 5-point system), were evaluated. At each time point 8-10 rats were used, after they were decapitated. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During healing of the wounds not complicated by infection, values of K_{dist} and the ascorbic acid concentration fluctuated and were opposite in phase, so that 4 periods could be conventionally distinguished: I) 2 days after the operation, II) 3-5 days, III) 6-8 days, and IV) 9-15 days (Fig. 1a).

In period I after wounding phagocytic cells, mainly neutrophils and to a lesser degree, macrophages, with considerable respiratory activity, were activated. This led to initiation of LPO by the biomembranes of the microorganisms and surrounding tissues. The damaging action was evidently neutralized mainly by the intrinsic lipid antioxidants of the cells (SOD). The investigation showed that no ascorbic acid was released during this period.

Conversely, a significant decrease in the ascorbic acid concentration to 3.44 ± 0.50 mg%, $p < 0.05$, was observed (compared with background values of ascorbic acid of 4.31 ± 0.49 mg%).

Period II began with enhancement of the macrophagal reaction and neutrophilic infiltration in the tissues of the wound bed on the 3rd day (Fig. 1b) and it was accompanied by an increase in values of the antioxidant potential (K_{dist} = 1.174 ± 0.12 relative units), largely due to an increase in ascorbic acid. This restores optimal relations between pro- and antioxidants in the tissues, thereby protecting the biomembranes of the undamaged tissues. Favorable conditions were thus created for active phagocytosis and, at the same time, for normal repair of damaged structures.

Period III was characterized by an increase in the concentrations of LPO products and a consequent reduction in relative SOD activity, leading to lowering of the values of K_{dist} in the tissues of the wound bed (Fig. 1a), whereas "quenching" of intermediates was effected by ascorbic acid. The observed increase in the total ascorbic acid concentration was evidently due to accumulation of its oxidized form, mainly dehydroascorbate. Ascorbic acid helps to preserve the antioxidant potential of healthy tissue cells.

In period IV, when scar tissue was formed in the wound and function of the neutrophils and macrophages became less important than in the previous periods (Fig. 1b), the antioxidant system of the wound tissues stabilized, as a result of which a considerable quantity of ascorbic acid was utilized for collagen synthesis and maturation. As a coenzyme of the enzymes catalyzing oxidation of lysine and proline, ascorbate plays an important role in the formation of the intercellular matrix [15].

Four periods also can be distinguished in the course of wound healing complicated by infection, but they were shifted relative to those described above. For instance, on the first day after the operation K_{dist} was not significantly increased as when wound healing was complicated by infection (Fig. 2a). The stronger response of the body and tissues to the severity of the operative trauma, and to microbial invasion, evidently was responsible for the intensity of neutrophilic infiltration and increased activity of the macrophagal reaction in the tissues than in the case of an uncomplicated course (Fig. 2b). This reaction was accompanied by a significant increase in the ascorbic acid concentration (6.11 ± 0.80 mg%, $p < 0.001$); in the case of healing of an uncomplicated wound 3.44 ± 0.50 mg%).

A small quantity of ascorbic acid is known to be present in the form of a complex with metals of transitional valency, and possesses high prooxidative activity, so that it is used in model systems to initiate LPO [1]. A similar picture was observed in the present case. The very small increase in ascorbic acid (not significant, see Fig. 2a), against the background of an increase in concentrations of metals (Fe^{2+}, Cu^{2+}) [5], has a prooxidative action, thereby curtailing the compensatory reactions of the body aimed at removing free radicals in the tissues.

The final formation of sepsis in the postoperative wounds was observed on the basis of morphological features on the 5th day after the operation (growth of neutrophilic infiltration and macrophagal activity in the complicated wounds was sharply increased compared with uncomplicated; Fig. 2b). Activity of macrophages and neutrophils was accompanied by a considerable increase in the ascorbic acid concentration and SOD activity, leading to limitation of spread of the pathological process. This suggests that the antioxidative system of the tissues can still withstand activity of the proantioxidative system also. Starting as early as on the 6th day after the operation, when signs of proliferation began to appear in the tissues of the wound bed against a background of continuing septic inflammation, a decrease was observed in the values of K_{dist} evidently on account of increased production of oxygen intermediates and initiation of LPO. Their accumulation led to "exhaustion" of the antioxidative system of the tissues and to a decrease in the ascorbic acid concentration (to 11.63 ± 0.72 mg%, $p < 0.001$); at the same time the intensity of the macrophagal reaction weakened (Fig. 2b), prolonging the inflammatory process. After the 8th day a significant increase in the ascorbic acid concentration was observed to 22.18 ± 1.17 mg% ($p < 0.001$), evidently due to the oxidized form of ascorbic acid, for the level of antioxidants in the tissues became further exhausted, as confirmed by the fall in the values of K_{dist} and a tendency for its values to become even more depressed in the future (Fig. 2a), on account of accumulation of LPO products in the tissues. This led to a disturbance of phagocytosis, a fact which does not contradict the concept of suppression of macrophagal

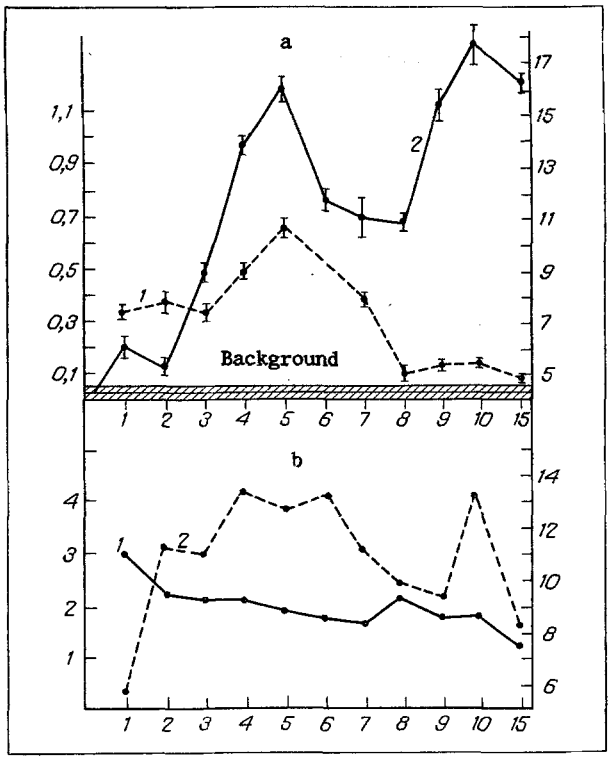

Fig. 2. Time course of ascorbic acid concentration and changes in values of K_{dist} (a), neutrophilic infiltration, and integral parameter of macrophagal reaction (b) in granulation tissue of rats with wound complicated by infection.

activity in the presence of an excess of the oxidized form of ascorbic acid and of peroxidation products [1, 12, 18]. Under these circumstances oxidation of proline into hydroxyproline is disturbed and scar tissue formation retarded, thereby prolonging the course of wound healing.

It can thus be postulated that on the 1st day after the operation, in the case of wound healing complicated by infection, marked activation of LPO takes place not only due to increased traumatization of the tissues, but also due to the additional prooxidative effect of ascorbic acid, associated with metals. This leads to enhancement of destructive processes in the tissues. During resolution of the suppurative process in the wound LPO products accumulate, and against the background of disturbance of ascorbic acid metabolism, this leads to exhaustion of antioxidative protection of the tissue cells, to weakening of activity of phagocytosis, to inhibition of collagen biosynthesis and, consequently, to delay of scar formation.

LITERATURE CITED

1. L. K. Bakhova and T. K. Fadeeva, Vopr. Med. Khim., No. 1, 22 (1988).
2. M. I. Kuzin and B. M. Kostyuchenok, Wounds and Wound Infection [in Russian], Moscow (1981).
3. L. A. Mamedov, N. Yu. Kosaganova, and G. T. Rikhireva, Vopr. Med. Khim., No. 6, 82 (1986).
4. L. A. Mamedov, A. V. Nikolaev, and V. V. Zakharov, Patol. Fiziol., No. 6, 61 (1987).
5. A. I. Strukov and V. V. Serov, Pathological Anatomy [in Russian], Moscow (1985).
6. I. V. Struchkov, K. N. Prozorovskaya, and L. M. Nedvetskaya, Immunology in the Prevention and Treatment of Suppurative Surgical Diseases [in Russian], Moscow (1978).
7. I. V. Struchkov, All-Union Congress on Surgeons [in Russian], Moscow (1981), pp. 7-8.
8. T. Asakawa and J. Maisushitas, Lipids, **5**, 137 (1980).
9. K. Asuda, M. Takahashi, and N. Nagate, Agric. Biol. Chem., **38**, 471 (1974).
10. B. M. Babior, R. S. Kipnes, and J. T. Curnutte, J. Clin. Invest., **52**, 741 (1974).
11. D. A. Bass, J. W. Parce, and L. R. Dechatelet, J. Immunol., **130**, 1910 (1983).
12. A. Bendich, L.J. Machlin, and O. Scandura, Free Radicals Biol. Med., **2**, 430 (1986).

13. J. W. Callahan and J. A. Lowden, Lysosomes and Lysosomal Storage Diseases, New York (1981), pp. 48-83.
14. S. J. Klebanoff and C. B. Hamon, J. Reticuloend. Soc., **12**, 170 (1972).
15. S. Lewin, Vitamin C: Its Molecular Biology and Medical Potential, London (1976).
16. E. Peacock and R. van Winkle, Wound Repair, Philadelphia (1976), p. 699.
17. P. Roberts, H. Hemila, and M. Wikström, J. Med. Biol., **62**, 88 (1984).
18. J. H. Roe and C. A. Kuether, J. Biol. Chem., **147**, 399 (1943).
19. K. Schmidt and U. Moser, Int. J. Vitamin. Nutr. Res., **27**, Suppl., 363 (1985).

THE EPITHELIAL TUBULES OF THE THYMUS ARE THYMOSIN-DEPENDENT STRUCTURES

N. A. Voloshin, V. A. Malyzhev, and A. G. Yakhnitsa

UDC 612.438

KEY WORDS: thymus, epithelial tubules, thymosin.

Considerable progress in the study of the thymus has led to the unanimous conclusion that it is an organ with dual function: lymphopoietic and hormonal [5, 6, 7]. More than 14 different substances with biological activity have been isolated from the thymus, and some of them have been synthesized artificially [1, 9, 12, 15]. Using monoclonal antibodies to thymosin and thymulin, the sources of synthesis of the thymus hormones have been localized, namely the epithelial cells of the cortex and medulla [13, 15]. Methods of determination of hormones in the blood have been developed [9]. However, the question of how thymus hormones enter the circulation has not yet been settled, and the role of the corpuscles and epithelial tubules of the thymus in this process has not been identified.

The possibility that the epithelial tubules may be involved in the secretory process is indicated by histochemical and histoautoradiographic investigations [2, 10], which have demonstrated the ability of the epithelial cells lining the walls of the tubules to synthesize proteins and glycoproteins has been confirmed. Epithelial tubules are detectable constantly in the early postnatal period of development [2, 10, 11]. The character of the morphological changes arising in the epithelial tubules of the thymus following injection of exogenous thymus hormones has not been established, and the investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

The test object was the thymus of Wistar rats aged 1 month. The experimental animals were given an injection of thymosin-5, obtained by the usual method [12] on the 1st, 2nd, and 3rd days after birth in the interscapular region, in a single dose of 50 μg in 0.05 ml of solution. The control animals received 0.05 ml of physiological saline at the same times. The intact, control, and experimental animals were decapitated on the 1st, 3rd, 7th, 14th, and 30th days after birth. The thymus was quickly removed and fixed in Bouin's or Carnoy's fluid. Sections 5-7 μ thick were stained with hematoxylin and eosin, alcian blue, and Schiff's reagent. Between 30 and 50 serial longitudinal and transverse sections were cut from each block, and each was examined visually for the presence of epithelial tubules.

Department of Human Anatomy, Zaporozhe Medical Institute. All-Union Research Center for Radiation Medicine, Academy of Medical Sciences of the USSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. S. Efimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 327-330, March, 1991. Original article submitted May 3, 1990.

 0007-4888/91/0003-0414$12.50