Erysod, Erythrocyte Superoxide Dismutase Preparation: Effects on LPO Processes and Morphological Changes in the Viscera of Rats with Burns under Conditions of Delayed Antishock Infusion Therapy

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Biochemical and morphological changes in the kidneys, liver, heart, and lungs were studied in rats with deep burns of 20% body surface. Erysod (0.47 mg/kg/day) added to antishock therapy notably reduced the intensity of LPO processes in tissues both in cases when infusion therapy was started immediately (by 8-20% 12 h and by 5-24% 24 h after the injury) and when this therapy was 6 h delayed (by up about 36% after 12 h). This prevented injuries to visceral organs during the acute period of thermal injury and prevented the development of burn disease.

Key Words: *thermal injury; infusion therapy; superoxide dismutase; lipid peroxidation*

The skin with underlying tissues become a potent source of free radicals in thermal injuries; this leads to hyperproduction of active oxygen forms (AOF) and sharp intensification of LPO processes in the viscera during the first hour after the injury. Cytolysis and activation of LPO in ischemic areas adjacent to the necrotic zone in the wound result in active release of products of these reactions into the plasma [3]. Excess of AOF and LPO products during the development of burn shock leads to failure of antioxidant defense (oxidative stress [11]), drop of endogenous antioxidant level, and visceral dysfunctions, primarily of the kidneys (subjected to ischemia, reperfusion, toxic injury by tissue degradation products and overloading as a result of water-electrolyte imbalance) and lungs (because of increased intrapulmonary blood volume resultant from greater centralization of circulation).

The delay of infusion therapy determines severe course of burn shock with sharp disorders of homeostasis fraught with rapid decompensation of vital organs (each hour of delay increased the probability of a lethal outcome by 10%) [9,10].

SOD preparations (EC 1.15.1.1.) occupy a special place among the drugs normalizing the levels of AOF and LPO products. SOD is a redox enzyme, the basic component of antioxidant defense of all pro- and eukaryotic cells [4]. We studied the effect of erysod (PES), derived from human erythrocytes, on the course of burn shock in rats with extensive burns of the skin with consideration for immediate and delayed start of antishock infusion therapy.

MATERIALS AND METHODS

The study was carried out on 66 male albino Wistar rats of the same age (210-220 g) from Rappolovo Breeding Center of Russian Academy of Sciences. The animals were divided into 11 equal groups: intact for evaluation of normal values and 10 experimental ones. All animals were kept under the same conditions. Deep burns of 20% body surface on the back were repro-

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TABLE 1. Content of MDA (pmol/mg tissue) in Rat Tissues after Deep Burn of 20% Body Surface under Conditions of Immediate Antishock Therapy ($M\pm m$, n=6)

Organ	Normal value	After 12 h			After 24 h		
		control	LA	LA+PES	control[x]	LA	LA+PES
Kidney	31±4	75±4	74±3	68±5 (-8)	—	84±12	71±9 (-15)
Liver	44±9	56±11	46±5	40±7 (-13)	—	55±4	42±4[+] (-24)
Heart	28±3	52±2	44±2*	40±4* (-9)	—	41±4	39±2 (-5)
Lung	25±2	53±9	41±3	33±4*[+] (-20)	—	47±3	37±2[+] (-21)

Note. [x]1 of 6 rats survived (Fig. 1). Here and in Table 2: $p<0.05$: **vs.* control; [+]*vs.* LA; difference in comparison with LA in percent is shown in parentheses.

duced in experimental animals as described previously [2]. Body surface area (in cm^2) was estimated by the formula $S=12.54\times M^{0.66}$, where M is body weight in kg. Skin depilated with 10% Na_2SO_3 was exposed to KG220-1000-4 lamp (1 kW) during 30 sec through a 6×10 cm mask. The lamp was placed at a distance of 3 cm from the skin (anesthesia: 70 mg/kg calypsol and 1 mg/kg droperidol intramuscularly).

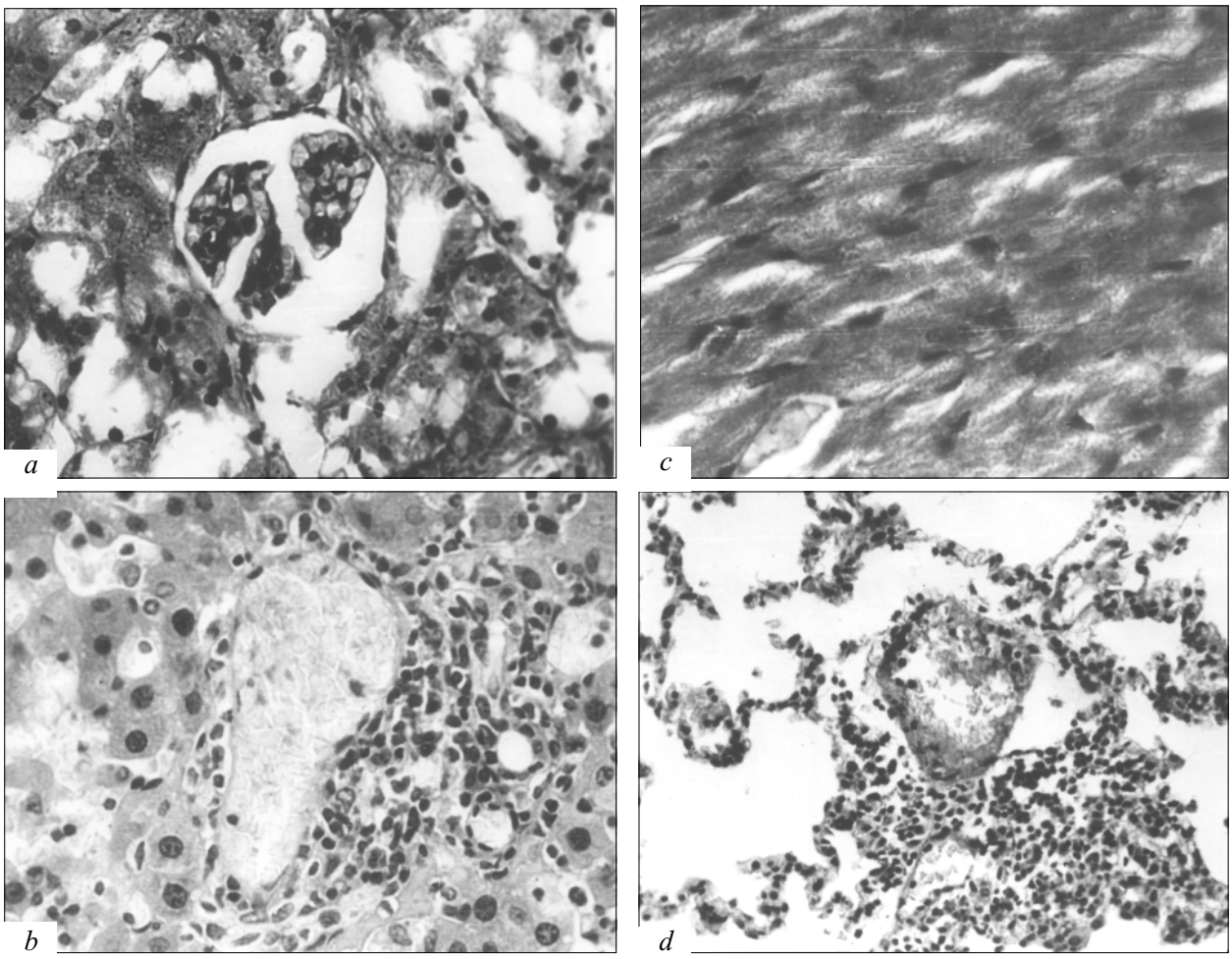

Fig. 1. Tissue structure 24 h after deep burn of 20% body surface in rats receiving no treatment, ×400. *a*) kidney: the volume of the glomerulus is decreased because of stretching of the capsular cavity; the epithelium of the external leaflet of the glomerular capsule is sharply flattened; glomerular capillaries are dilated, endothelium is flattened; mesangial cells are hypertrophic; proximal and distal compartments of the nephron are unevenly stretched, with flattened, destroyed, or desquamated epithelium; *b*) liver: the architectonics of hepatic lobule is impaired; infiltration round the central vein, its lumen is dilated and filled with desquamated cells and compact secreta; biliary tubules are dilated; hepatocyte cytoplasm contains small vacuoles, with high tinctorial properties; capillaries in the lobules are dilated, part of them are in a state of stasis; *c*) heart: interstitial edema; stratification of part of myofibrils; microcirculatory disorders (stasis); *d*) lung: alveoles are stretched, with individual desquamated cells in lumens; capillaries are dilated, with stasis; endothelium is flattened or desquamated.

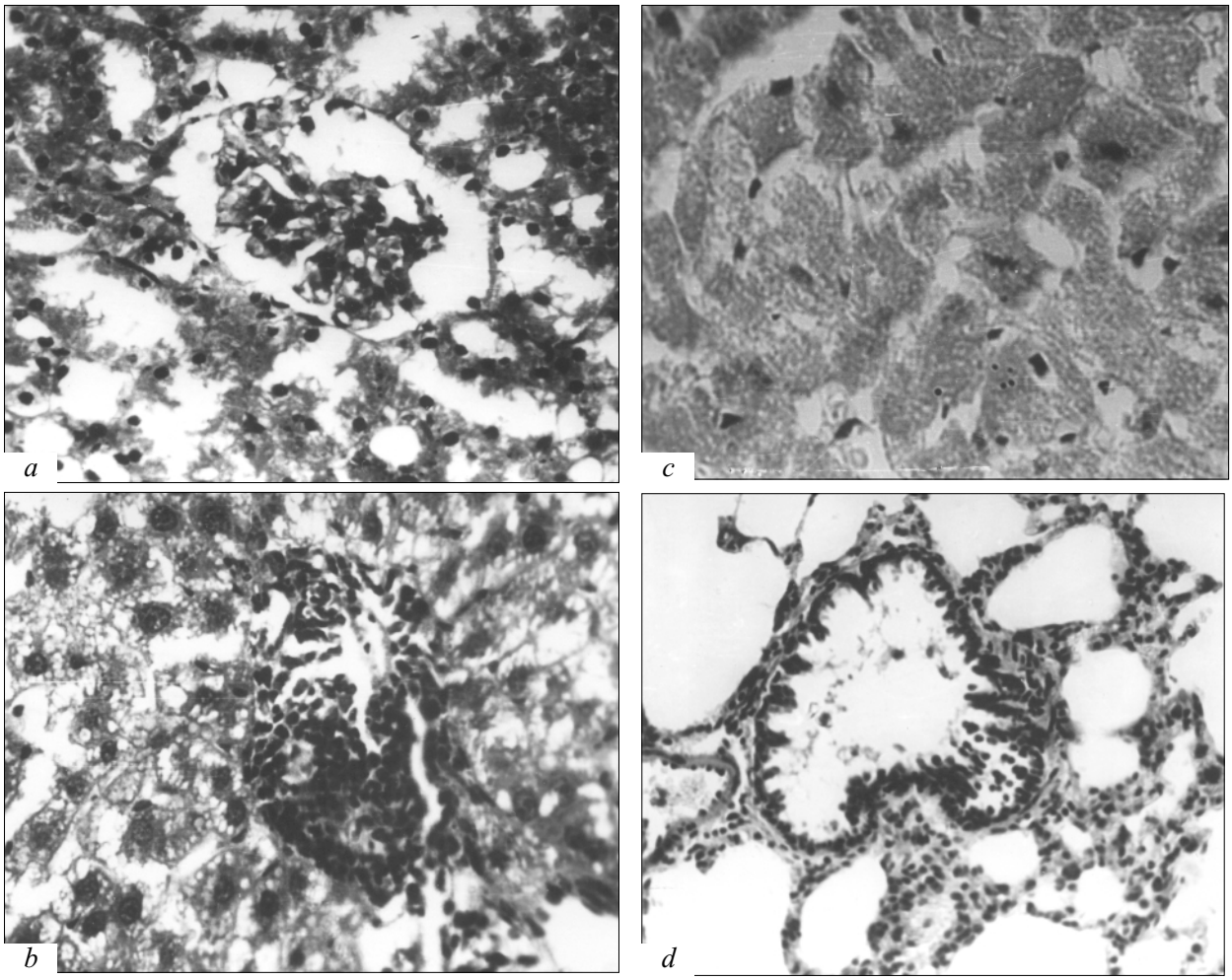

Fig. 2. Rat tissue structure 24 h after deep burn of 20% body surface after 6-h delay of lactosol infusion, ×400. *a*) kidney: the volume of the glomerulus is moderately decreased because of stretching of the capsular cavity; the epithelium of the outer leaflet of the capsule is flattened; glomerular capillaries are moderately dilated, endothelium is flattened; mesangial cells are hypertrophic; necrobiosis of the proximal and distal compartments of the nephron epithelium; *b*) liver: infiltration round the central vein; the structure of hepatic cords is impaired; hepatocyte cytoplasm is clarified or sharply vacuolated; part of hepatocytes are lyzed; capillaries in the lobules are sharply dilated; *c*) heart: moderate interstitial edema; cardiomyocytes retain cross striated structure; *d*) lung: alveoles are stretched, partially ruptured; capillaries and veins are plethoric, with stasis; endothelium of respiratory bronchioles is edematous; slight infiltration round vessels.

Antishock therapy was carried out by infusion of either lactosol (LA) alone or erysod solution in LA (LA+PES) in a dose of 100 μg PES per rat during 24 h or 0.47 mg/kg/day. The volume of infusion (ml/day) was estimated by the formula $V=4M\times P$, where M is body weight in kg and P is burn area in percent of body surface area [5]. In order to carry out infusion therapy, the animal was lowered with its head down, the skin

TABLE 2. Content of MDA (pmol/mg tissue) in Rat Tissues 24 h after Deep Burn of 20% Body Surface under Conditions of Delayed Antishock Therapy ($M\pm m$, n=6)

Organ	LA		LA+PES	
	6 h delay	12 h delay	6 h delay	12 h delay
Kidney	95±14	109±12°	98±16° (3)	106±12° (-3)
Liver	69±7	78±14°	50±4+ (-28)	74±10° (-5)
Heart	66±8°	80±11°	42±7+ (-36)	82±8° (3)
Lung	63±12°	86±14°	61±6° (-3)	88±10° (2)

Note. °$p<0.05$ compared to the conditions of immediate antishock therapy (Table 1).

on the median surface of the lower third of the hip was pricked, and the needle was passed (with small amounts of fluid preceding it) intraperitoneally.

The state of the viscera in rats with burns which received antishock therapy immediately (less than 10 min) after the injury was studied in 3 pairs of animal groups). Pair No. 1 received no treatment, pair No. 2 received LA, and pair No. 3 received LA+PES. The animals of 3 groups (one from each pair) were decapitated 12 h after the injury, the others 24 h after the injury. The impact of delay of antishock therapy was studied in 2 pairs of animal groups. In one pair infusion of LA or LA+PES was started 6 h after the injury, in the other pair 12 h after the injury; all animals were decapitated 24 after burns were inflicted.

The intensity of LPO processes in rat viscera (kidneys, liver, heart, lungs) was evaluated by measuring MDA content in tissue by spectrophotometry by the reaction with TBA [1]. Biopsy specimens for histological analysis were fixed in 10% neutral formalin (24 h, 24°C), dehydrated in ethanol of ascending concentrations (from 40 to 100%), and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and examined under an Opton microscope.

Empirical mean and dispersion were estimated for each group, after which the control-experiment pairs were checked for coincidence (with 0.95 probability) of the true means suggesting the equality of the true dispersions (Student's *t* test).

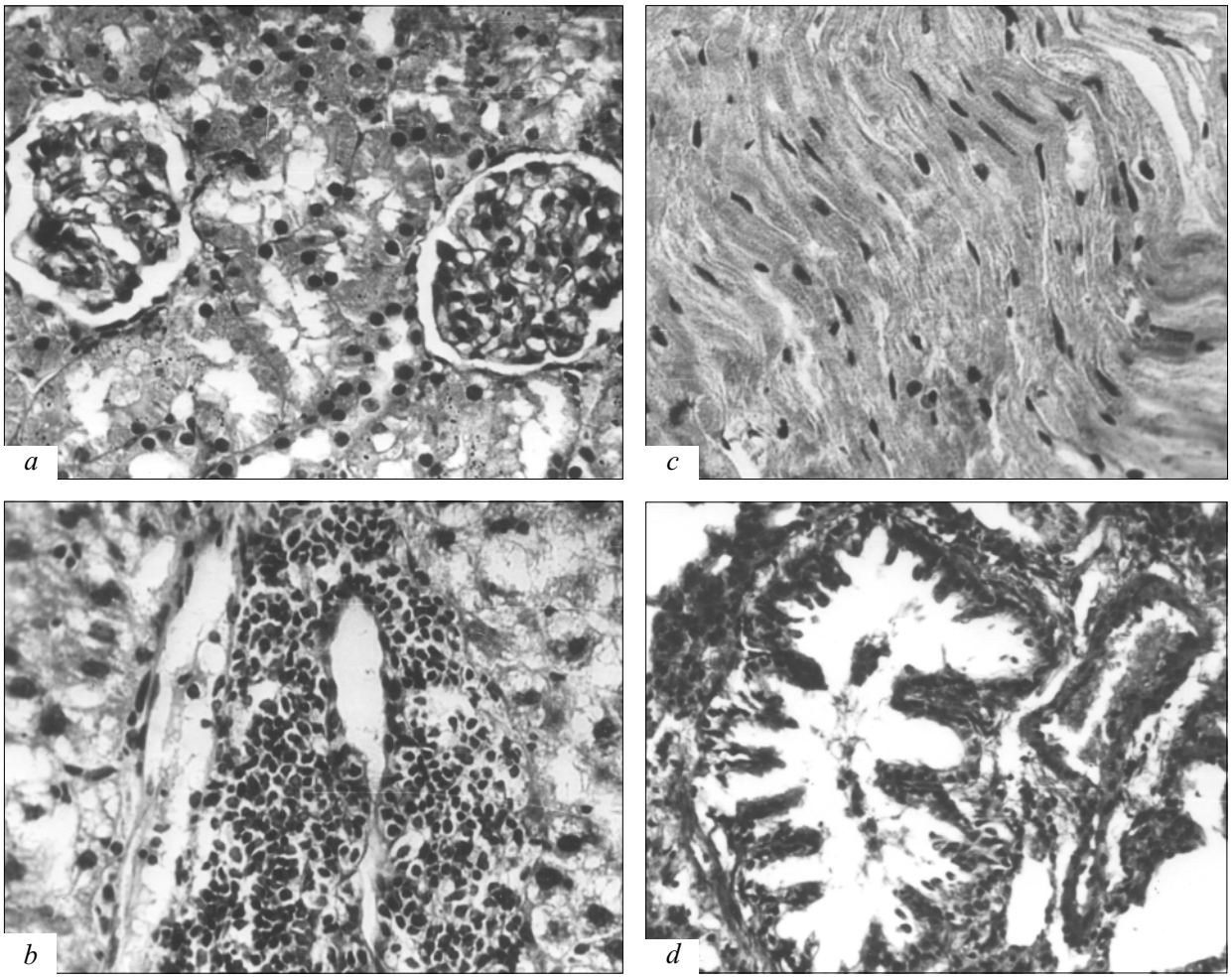

Fig. 3. Rat tissue structure 24 h after deep burn of 20% body surface after 6-h delay of lactosol and erysod infusion, ×400. *a*) kidney: the size of the renal glomerulus is normal, the renal corpuscle occupying the greater part of the glomerular volume; volume of capsular cavity is moderately decreased; the epithelium of the external layer of the capsule is sharply flattened; glomerular capillaries are moderately dilated, endotheliocytes are vacuolated; mesangial cells are scanty; proximal and distal compartments of the nephron vary greatly; the cytoplasm is vacuolated, apical compartments are destroyed and epitheliocytes of the nephron lining are desquamated; *b*) liver: abundant infiltration round the central vein of the hepatic lobe; the radial disposition of hepatic girders is impaired; hepatocyte cytoplasm is sharply vacuolated; *c*) heart: moderate interstitial edema; cross striated structure of cardiomyocytes is clearly seen; *d*) lung: alveoles are stretched and contain desquamated epitheliocytes; epithelium of respiratory bronchioles exhibits high tinctorial properties, some bronchioles contain compact homogenous substrate; slight infiltration of connective tissue walls.

RESULTS

The content of MDA in tissues of control animals 12 h after injury 1.3-2.4-fold surpassed the normal. This increase was significant in the kidneys, heart, and liver ($p<0.05$, Table 1). These data differ from the results of other investigations: a 2-fold increase in MDA content in the kidneys and lungs was observed 2 h after injury in rats with burns of 25% body surface [8] and significant increase in MDA level in the lungs was detected 1 h after the injury, the peak being observed 24 h after injury [7]. In one study (mice, burns of 30% body surface) an essential increase of MDA level in the liver was observed only 4 h after the injury, while significant differences were detected only 2 days after the injury [6].

When infusion therapy was started immediately, MDA level in all organs (except the kidneys) decreased essentially after 12 h in comparison with the control, the difference being significant for the lungs (LA+PES) and heart (LA and LA+PES) (Table 1). Infusion of LA+PES ensured more pronounced (in comparison with LA alone) suppression of LPO processes in all cases, the differences being significant for the liver (after 24 h) and lungs (after 12 and 24 h).

If infusion therapy was delayed, MDA content in all organs increased (after 24 h) with prolongation of the delay, the highest levels of MDA being detected in the kidneys and liver, which could be due to essential intracellular damage of these organs during burn shock (Table 2). After 6-h delay LA+PES suppressed LPO processes in the liver and heart more intensely than LA (the difference being significant); in other cases the results of LA and LA+PES treatment differed negligibly.

Morphological changes were most pronounced in the kidneys, heart and lungs of control animals (Fig. 1): severe lesions, such as necrosis and degeneration of the organs, and pronounced microcirculatory disorders (endothelial and vascular membrane abnormalities, plethora, and stasis) were found. Antishock therapy more (LA+PES infusion, Fig. 3) or less (LA infusion, Fig. 2) reduced these changes, the effect being the greater, the sooner was infusion therapy was started. Minimum morphological changes were observed in rats treated with LA+PES immediately or 6 h after injury. The differences between LA and LA+PES were significant for the groups in which infusion was started immediately and 6 h after it (Figs. 2 and 3) and negligible for the groups in which treatment was started 12 h after injury.

Hence, addition of SOD preparations to antishock therapy notably reduced the intensity of LPO processes in tissues, if the treatment was started immediately or 6 h after thermal injury; such treatment decreased visceral injuries during the acute period of the injury and prevented the development of burn disease complications.

REFERENCES

1. V. G. Gavrilov, A. R. Gavrilova, and L. M. Mazhul', *Vopr. Med. Khim.*, **33**, 118-122 (1987).
2. N. I. Kochetygov, *On Experimental Reproduction of Thermal Burns* [in Russian], Leningrad (1964).
3. E. I. L'vovskaya, *Disorders in Lipid Peroxidation Processes in Thermal Injury and Pathogenetic Validation of Therapy with Plasma Antioxidants*, Abstract of Doct. Med. Sci. Dissertation, Moscow (1998).
4. E. B. Men'shikov and N. K. Zenkov, *Uspekhi Sovrem. Biol.*, **113**, 442-455 (1993).
5. V. Rudovskii, V. Nazilovskii, V. Zitkevich, and K. Zinkevich, *Theory and Practical Treatment of Burns* [in Russian], Moscow (1980).
6. S. Kawai, J. Komura, Y. Asada, and Y. Niwa, *Arch. Dermatol. Res.*, **280**, 171-175 (1988).
7. D. Konukoglu, O. Cetinkale, and R. Bulan, *Burns*, **23**, 541-544 (1997).
8. D. Saitoh, Y. Okada, T. Takahara, *et al.*, *J. Exp. Med.*, **174**, 31-40 (1994).
9. M. Sakurai, H. Tanaka, T. Matsuda, *et al.*, *J. Surg. Res.*, **73**, 24-27 (1997).
10. H. Tanaka, H. Matsuda, S. Shimazaki, *et al.*, *Arch. Surg.*, **132**, 158-161 (1997).
11. Z. F. Xia, R. E. Barrow, L. D. Broemeling, and D. N. Herndon, *J. Burn Care Rehabil.*, **13**, 530-537 (1992).