Ultrastructural Changes in the Interstitial Cells of the Renal Medulla during Endotoxin Shock

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Ultrastructural modifications of renomedullar interstitial cells are described at different stages of experimental endotoxemia in rats and dogs. The dynamics of changes occurring in organelles, lipid granules (precursors of prostaglandins), and medullar capillaries is presented. It is suggested that the alterations in these granules during endotoxin shock are associated with the synthesis, accumulation, and secretion of their precursors in interstitial cells.

Key Words: *lipid granules; interstitial cells; endotoxin shock*

It has recently been recognized that the arachidonic acid derivatives thromboxane A_2 and prostaglandin I_2 play an important role in polyorganic insufficiency in endotoxin shock (ES) and septic shock (SS) (the clinical equivalent of ES); the blood content of these compounds increases markedly during ES and SS [1,8,11]. In endotoxemia, thromboxane A_2, synthesized in macrophages, lymphocytes, and platelets, acts as a potent vasoconstrictor and induces platelet aggregation and the release reaction, i.e., it initiates blood coagulation. Prostaglandin I_2, produced by vascular endothelial cells, acts as an antagonist, and accordingly dilates blood vessels and prevents platelet aggregation [11,12].

Although prostaglandins and their precursors are found in almost all organs and tissues, they have been reliably identified as lipid inclusions only in the interstitial cells (IC) of the renal medulla [3,9]. In the kidney, the content of prostaglandin E_2 is the highest [10]; during endotoxemia its blood level increases markedly, which leads to lung edema and pulmonary hypertension, induces local and systemic hemodynamic changes, and adversely affects the function of various organs [12].

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In light of this, our objective was to study the ultrastructure of the renal medulla at different stages of ES.

MATERIALS AND METHODS

Experiments were performed on 39 mongrel dogs weighing up to 10 kg and 42 male rats weighing 200 g. The animals were maintained on the standard vivarium diet and had free access to water. *E. coli* endotoxin was injected intravenously: dogs were given 5 mg/kg and rats received 2 mg/100 g body weight in the caudal vein. The animals were euthanized with a Nembutal overdose after 30 min (initial stage of ES), 5 h (intermediate stage), and 3 days (late stage). Control animals (9 dogs and 9 rats) were administered equal volumes of normal saline. All the animals (control and experimental) were deprived of food and water 12 h before sacrifice. Pieces excised from the inner part of the renal medulla were fixed in 2.5% glutaraldehyde on phosphate buffer, postfixed in 1% OsO_4, dehydrated in ascending grades of ethanols, and embedded in Epon 812. Morphological changes were assessed under a Diavar (Reichert) light microscope on semithin (1-2 μ) sections stained with Toluidine Blue. Sections cut in an LKB 8800 ultramicrotome

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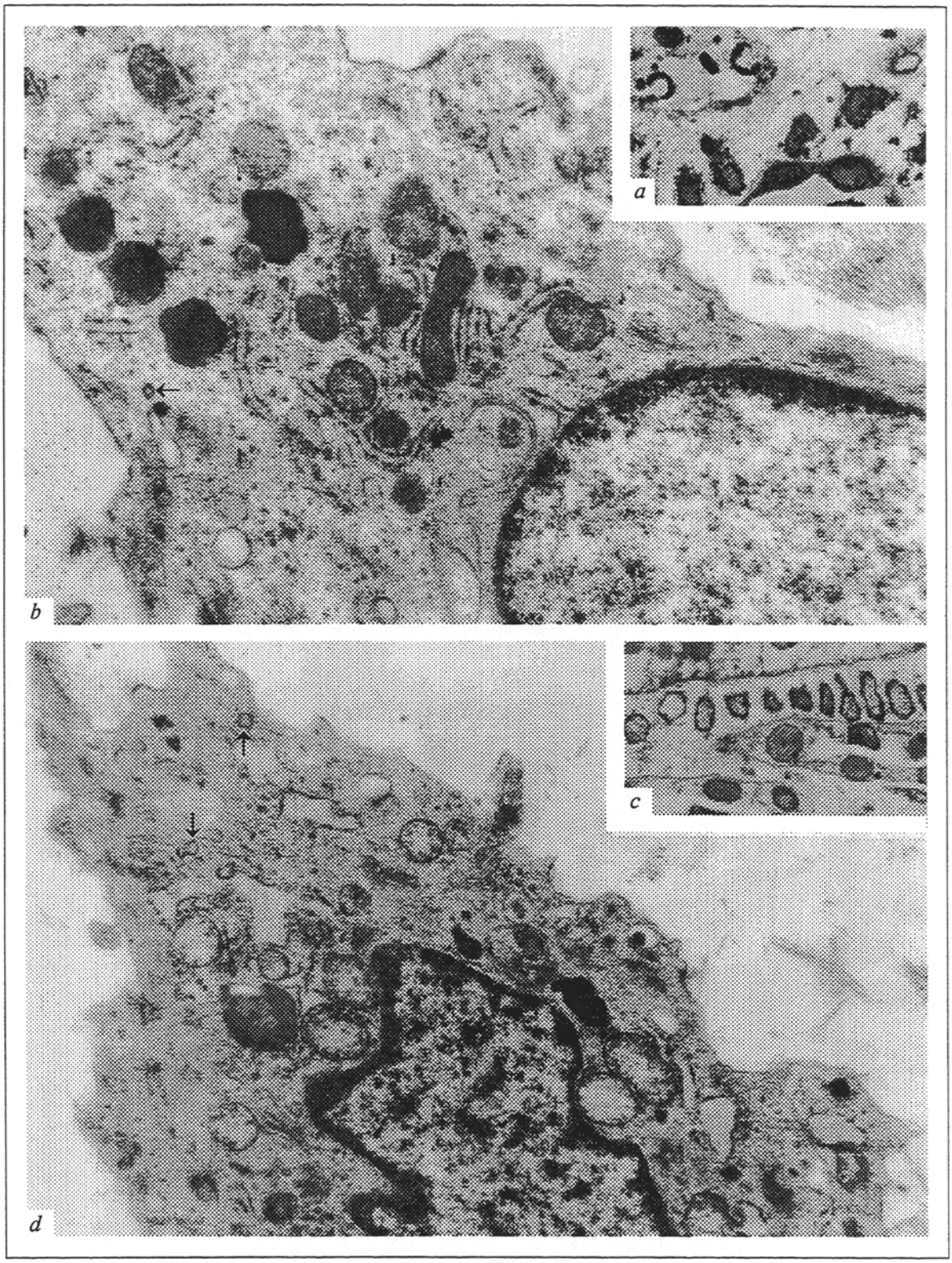

Fig. 1. IC of control rats during the initial stage of ES. *a*) control (semithin section). Cells with numerous of lipid granules in the cytoplasm and lateral processes. ×1600; *b*) fragment of IC of control animal with lipid granules and unchanged organelles (arrows points to an enclosed vesicle). ×6000. *c*) disappearance of lipid granules at the initial stage of shock (semithin section). ×1600; *d*) pronounced decrease in the number of lipid granules, swelling of mitochondria, widening of granular reticulum, and invagination in the nucleus at the initial stage of ES (arrows point to enclosed vesicles). ×6000.

were contrasted with uranyl acetate and lead citrate and viewed in a JEM-100B electron microscope. The number of lipid granules per 50 IC was calculated on control and experimental sections. The results were analyzed using the methods of variational statistics.

RESULTS

Light and electron microscopy revealed no changes in IC from the inner zone of the medulla in control animals (administration of normal saline). Interstitial cells were oriented along the longitudinal axis perpendicular to the pyramid apex (Table 1) and were generally located among collecting tubules, thin limbs of the loop of Henle, and blood vessels (Fig. 1, *a*). Most IC were elongated and had cytoplasmic processes which were long at the poles and short at the lateral surface, so that on transverse sections the cells looked stellate. A specific feature of the cytoplasm was the presence of lipid granules which were sometimes surrounded by a unilayer membrane and were also seen in cell processes (Fig. 1, *b*). In control animals, the number of granules varied inconsiderably (Table 1). Their IC had a well-developed granular reticulum and Golgi apparatus, sparse mitochondria with a dense matrix, and a few enclosed vesicles. The nuclei often had a widened perinuclear zone; chromatin was concentrated along the inner layer of the karyolemma. The cells contained occasional primary lysosomes.

At the initial stage of ES (30 min) capillary plethora and occasional hemorrhages were seen in the medulla and juxtamedullar zone. Some thin segments of the nephron contained exfoliated endothelium of convoluted tubules. This was accompanied by dystrophic changes and a sharp decrease in the number of lipid granules ($p<0.001$), to the point of their disappearance (Fig. 1, *c*). Generally, the granules looked like bodies of irregular shape with reduced osmiophilia. The topography of the lipid granules also changed: they were located at the plasma membrane or in the processes making contact with the basal membranes of capillaries and tubules. The cytoplasm of IC contained swollen mitochondria with an osmiophobic matrix and shortened cristae, widened cisternae of granular endoplasmic reticulum, and nuclei with an undulated karyolemma and shallow invaginations (Fig. 1). There were no changes in the Golgi apparatus. The number of secondary lysosomes was increased in some IC. The number of enclosed round or ovoid vesicles with characteristic "ciliation" was increased, and they could be found anywhere in the cytoplasm.

A unique feature of the granules was that they consisted of triglycerides and fatty acids containing up to 70% arachidonic acid and could serve as a depot of prostaglandin precursors [4,10]. It is known that the contents of the granules are released under the action of phospholipase A_2, whose activity increases 11-fold in rabbits during ES [14] and 16-fold in patients with SS [13]. Arachidonic acid is converted into a number of active metabolites: prostaglandins, thromboxanes, and leukotrienes, which play a key role in the pathogenesis of ES and SS [8].

At the intermediate stage of ES (5 h) the content of lipid granules still remained markedly lowered ($p<0.001$, Fig. 2, *a*). This often coincided with the appearance of a light zone evenly surrounding the entire granule or a part of it. Many IC contained no lipid granules. The length of the contacts between their processes and capillaries increased (Fig. 2, *b*). It is noteworthy that the cytoplasm of these cells contained large cavities surrounded by a unilayer membrane (Fig. 2, *c*). Generally, these cavities were electron transparent, but some of them contained a homogeneous flaky material and occasional small vesicles. The perinuclear zone was more or less evenly widened, but sometimes lacunae with myelin figures inside were seen. Myelin figures were more frequent in the vicinity of the Golgi complex and in the zone of secondary lysosomes. A relationship could be

TABLE 1. Changes in the Number of Lipid Granules in IC of the Renal Medulla at Different Stages of Endotoxin Shock and at the Late Stage of Endotoxemia ($M \pm m$)

Time after endotoxin administration	Number of lipid granules per cell		
	intact (n=3)	control (n=9)	experiment (n=30)
30 min		6.10±1.17	2.10±0.37*
5 h	5.81±0.72	5.46±0.41	2.31±0.41*
3 days		5.60±0.64	6.28±1.20

Note. Asterisk indicates significance of differences in comparison with the corresponding period in the control ($p<0.001$); n is the number of experiments.

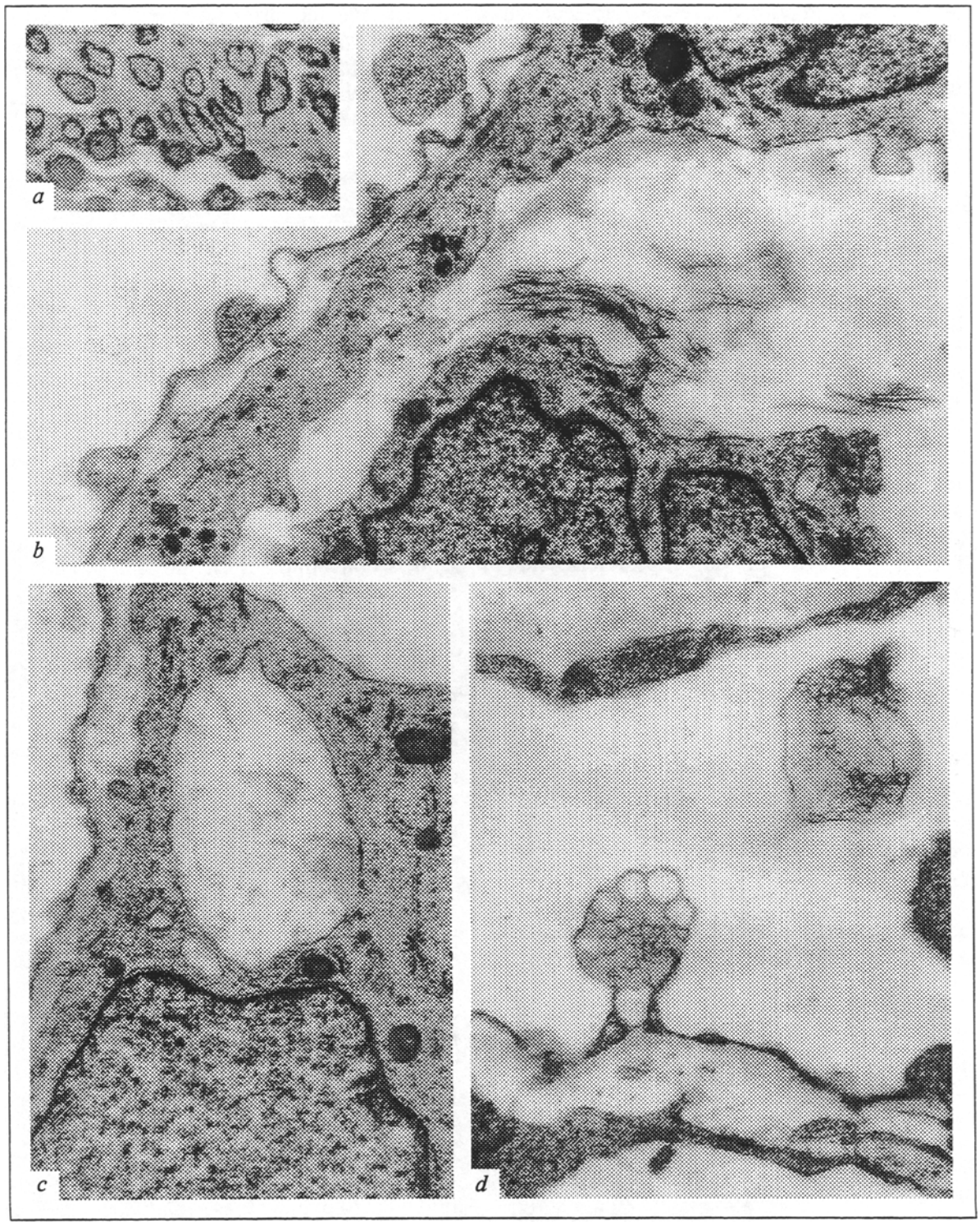

Fig. 2. Intermediate stage of ES. *a*) absence of lipid granules in IC (semithin section) ×1600; *b)* increase in length of the contact between IC processes with reduced lipid granules and a capillary. ×5000; *c*) formation of a large cavity in the cytoplasm of IC. ×6000; *d*) microvesiculization of endothelial cell. At right: clasmatosis of vesiculated segment of endothelium. ×8000.

traced between the activation of lysosomes and the formation of myelin figures. In medullar vessels, endothelial cells were desquamated or disintegrated into small fragments, which exposed the basal membrane. The formation of microvesicles that were released into the circulation as a result of clasmatosis was another response of the vascular endothelium (Fig. 2, *d*).

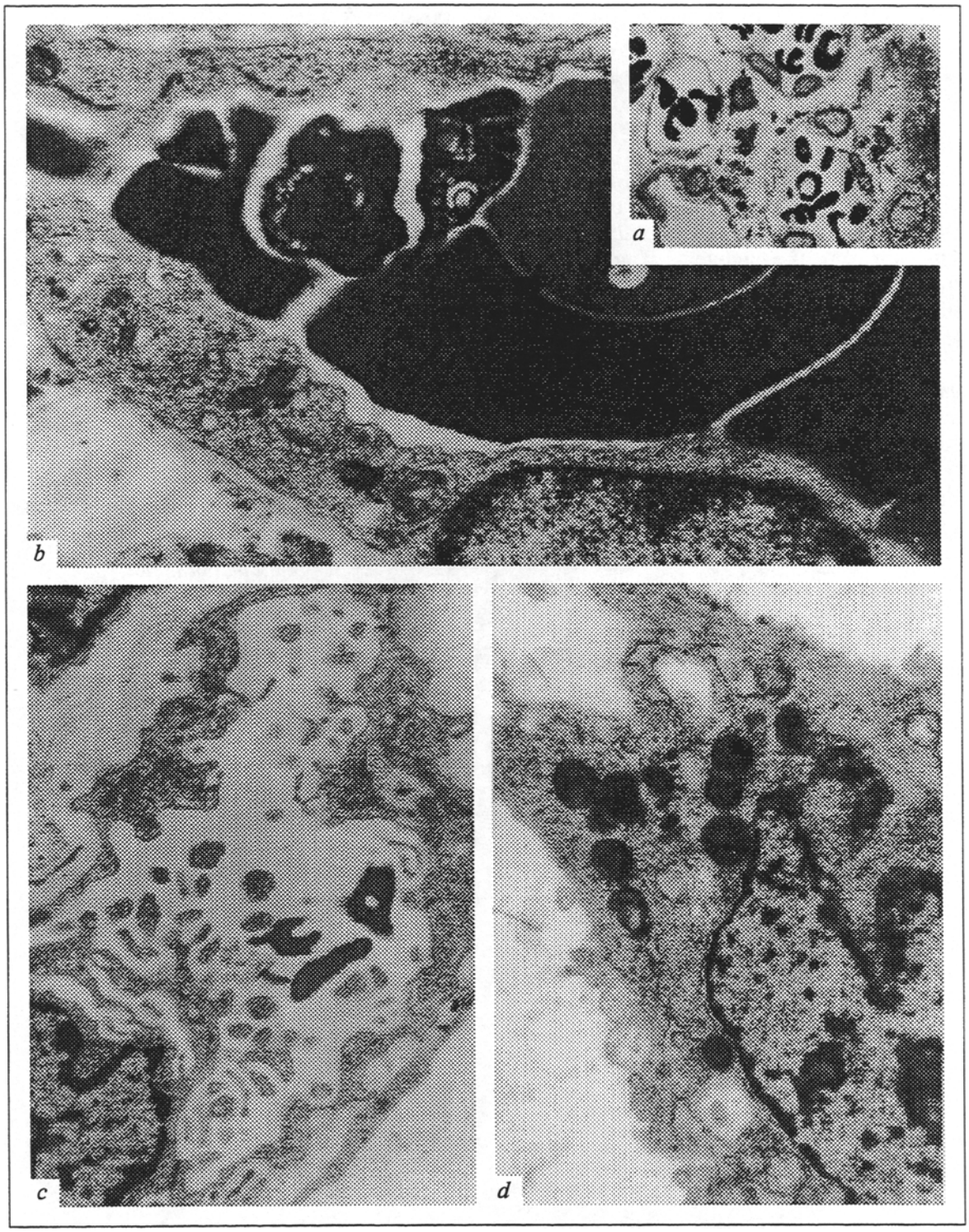

Fig. 3. Late stage of endotoxemia. *a*) appearance of granulation in IC and widening and plethora of capillaries in the renal medulla (semithin section). ×1600; *b*) erythrocyte aggregation in capillary lumen. ×6000. c) pronounced microclasmatosis of endothelial cell. ×6000; *d*) appearance of numerous lipid granules in IC. ×6000.

A similar endothelial restructuring with the formation of grapelike vesicles was observed in anesthetized dogs after intracoronary infusion of leukotriene C_4 [3]. In addition, intravascular changes occurred in the capillaries surrounded by IC. These changes were similar to those observed in the microcirculatory bed of other components of rat and dog kidney [6,7].

At the late stage of ES (3 days) the following microcirculatory disorders were observed: plethora of medullar capillaries and hematorheological alterations - stasis, erythrocyte aggregation, and sludge (Fig. 3, *a*, *b*). A pronounced microclasmatosis was noted in some erythrocytes (Fig. 3, *c*). The number of lipid granules in IC had increased to 6.28±1.20 (5.81±0.72 in intact rats) (Table 1, Fig. 3, *d*). Dystrophic processes were replaced by reactive shifts indicating the recovery of cell function. Since 90% of arachidonic acid metabolites are inactivated by the endothelium of pulmonary capillaries [5], the prognosis and course of ES are strongly dependent on lung function [7].

Thus, the development of ES is characterized by initial hyperfunction of IC, followed by reversible dystrophic alterations and, at later stages, reactive shifts. Variations of the level of prostaglandins in ES are probably associated with the synthesis, accumulation, and secretion of their precursors in IC. The microcirculatory disorders occurring in the renal medulla result from hematorheological shifts and damage to the vascular endothelium.

REFERENCES

1. L. N. Kvochina, V. N. Kotsyuruba, A. A. Moibenko, and L. A. Grabovskii, *Fiziol. Zh.*, **35**, № 3, 43-49 (1989).
2. A. A. Moibenko, Yu. N. Kolchin, V. N. Bulakh, and A. E. Sorochinskii, *Byull. Eksp. Biol. Med.*, **111**, № 2, 120-123 (1991).
3. M. A. Pal'tsev, M. Kh. Tur'yanov, S. G. Pak, *et al.*, *Ibid.*, **93**, № 4, 103-105 (1982).
4. Yu. V. Postnov and Yu. L. Perov, *Arkh. Pat.*, **34**, № 6, 70-75 (1972).
5. I. R. Tupeev and O. A. Gomazkov, *Pat. Fiziol.*, № 1, 78-82 (1984).
6. N. G. Kharlanova and E. A. Bardakhch'yan, *Fiziol. Zh.*, **38**, № 4, 56-62 (1992).
7. N. G. Kharlanova, Yu. M. Lomov, and E. A. Bardakhch'yan, *Ibid.*, **39**, № 2-3, 56-62 (1993).
8. N. G. Kharlanova, Yu. M. Lomov, and E. A. Bardakhch'yan, *Vrach. Delo*, № 9, 32-38 (1993).
9. B. N. Tsibel' and L. Yu. Raevskaya, *Arkh. Anat.*, **102**, № 11-12, 58-62 (1991).
10. S. O. Bohman, *Prostaglandins*, **14**, № 6, 729-744 (1977).
11. E. M. Camporesi, S. Oda, H. J. Fracica, *et al.*, *Circ. Shock*, **29**, № 3, 229-244 (1989).
12. E. Svartholm, D. Bergquist, A. Hedner, *et al.*, *Acta Chir. Scand.*, **154**, № 1, 133-139 (1988).
13. P. Vadas, J. *Lab. Clin. Med.*, **104**, № 7, 873-881 (1984).
14. P. Vadas and J. B. Hay, *Canad. J. Physiol. Pharmacol.*, **61**, № 5, 561-567 (1983).