Ultrastructural Analysis of Endotoxin-Induced Transepithelial Pinocytosis in the Distal Part of the Nephron

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The development of endotoxin shock (ES) is accompanied by a sharp drop of arterial pressure due to blood congestion in the celiac region, a decrease of the venous return to the right ventricle [2,5,11], and ultrastructural myocardial damage [2]. The last affects above all the auricle cardiomyocytes secreting atrial natriuretic peptide [6]. In its turn, a decrease of the systemic blood flow leads to renal hypoperfusion and a marked reduction of the glomerular filtration rate. In consequence of these features, diuresis is reduced by 40 % compared to the control [10,12]. The ultrastructural changes in the filtrative and reabsorptive part of the nephron may be one more factor in diuresis reduction [3]. Vasopressin (also called antidiuretic hormone) is another important regulator of water-salt metabolism. The antidiuretic hormone (ADH) is secreted by giant neurons of the anterior hypothalamus and stored in the posterior lobe of the pituitary gland (neurohypophysis). It is released into the blood flow to regulate water reabsorption in the distal parts of the nephron and collecting tubules. Our previous investigations demonstrated the intensification of neurosecretory granule formation in neuron perikaryons of the supraoptic nucleus, synchronized with forced neurosecretion release in the axovasal synapses of the neurohypophysis after 30 min and 5 h i.v. injection of endotoxin to rabbits and dogs [1]. According to later biochemical investigations, the concentration of ADH in dog plasma increases 20- and 25-fold after 15 and 30 min endotoxin treatment, respectively, and urine osmomolarity falls by nearly 50% [12]. However, there are no data on ultrastructural changes of the distal part of the nephron at different periods of ES development, nor on the state of the posterior lobe of the pituitary during the late stages of endotoxemia.

The aim of this study was an electron-microscopic investigation of the distal part of the nephron and collecting tubules epithelium of rats and dogs at different periods of ES in order to determine the role of the intracellular fluid-transporting structures. In addition, the correlation between the ultrastructural changes in rat renal tubules and the neurohypophysis in the late period of endotoxemia was studied.

MATERIALS AND METHODS

The experiments were carried out on 39 mongrel dogs of both sexes weighing 8-14 kg, i.v.injected

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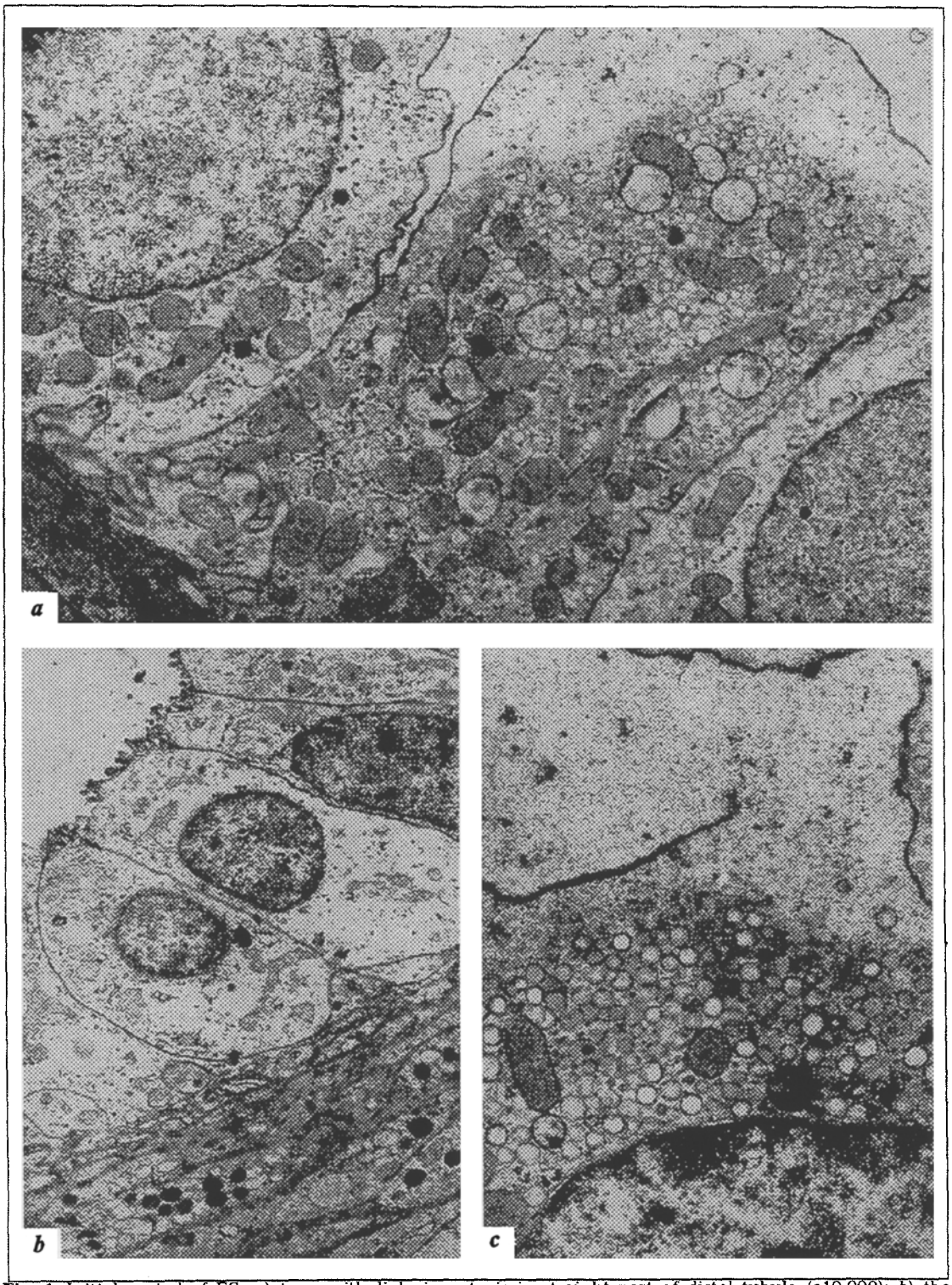

Fig. 1. Initial period of ES. *a*) transepithelial pinocytosis in straight part of distal tubule (×10,000); *b*) the absence of micropinocytic vesicles in macula densa epithelial cells (granules are seen in juxtaglomerular cells, (×5000); *c*) apical plasmalemma destruction in distal tubule epithelial cell (×20,000).

with 5 mg/kg *E.Coli* endotoxin. The same number of rats weighing 200-250 g received 2mg/100g endotoxin in the caudal vein. After 30 min (initial period of ES), 5 h (intermediate period of ES), and 3 days (late period of endotoxemia) the animals were killed with a lethal dose of nembutal.

Control animals were injected with an equal volume of saline. Each experimental series was performed on 10 animals of both sexes. The control group consisted of 3 animals per series. In all experimental series 5 pieces from different parts of the kidney were dissected. In addition, specimens from

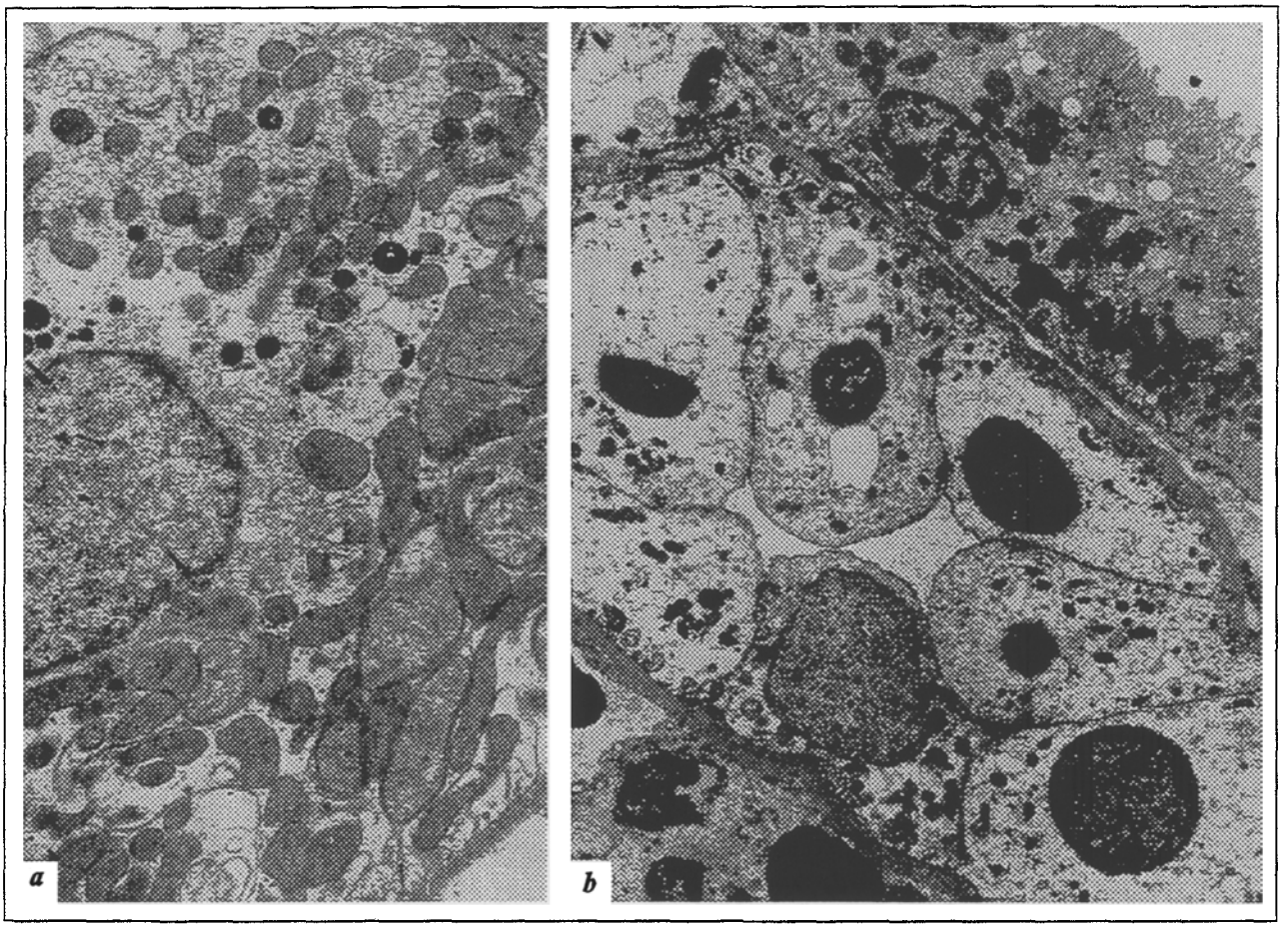

Fig. 2. Intermediate period of ES. *a*) transepithelial pinocytosis in distal tubule epithelium (×8000); *b*) appearance of pinocytic vesicles in light cells and of vacuoles containing lipid inclusions in dark cells of collecting tubules (×6000).

the posterior lobe of the pituitary were studied in the late period of endotoxemia. The tissue pieces were fixed in glutaraldehyde and postfixed in osmium tetroxide. After dehydration the specimens were embedded in Epon-812. The ultrathin sections obtained with an LKB-8800 ultramicrotome were double-stained with uranyl acetate and lead citrate and examined under a JEM-100S electron microscope.

RESULTS

No ultrastructural damage was detected in the rat and dog kidneys or the posterior lobe of the pituitary after i.v. saline injection. However, numerous micropinocytic vesicles appeared, mostly in the apical and more rarely in the central zone of the nephron's distal part and collecting tubules epithelial cells in the initial period of ES, regardless of the species of animal. It is interesting that such ultrastructural changes are exhibited only in the straight and convoluted portions of the distal tubules epithelium (Fig. 1, *a*) and never in the macula densa epithelium (Fig. 1, *b*). Sometimes several vacuoles coalesce, forming larger vacuoles. In dog nephrocytes this process leads to subapical edema, forcing the pinocytic vesicles away from the plasmalemma (Fig. 1, *a*) and even sometimes destroying the latter (Fig. 1, *c*). Notably, the width of the intracellular spaces remains unchanged during all ES periods in both species.

Ginetsinskii [4] holds that ADH initiates hyaluronidase secretion, depolymerizing the hyalurone complexes and increasing the extracellular water transport. If only this mechanism were involved, the ultrastructural manifestation of the above-mentioned process would be epithelial cell disjunction and intercellular space enlargement. However, despite the activation of hypothalamo-hypophyseal neurosecretory system (HHNS) in endotoxemia [1] and, consequently, massive ADH discharge [12], the width of the intercellular spaces is unchanged, whereas the subapical zone of epithelial cells contains numerous vesicles specialized for transporting fluid.

Judging from our data, the reabsorption of water in the nephron distal part is mainly achieved by way of ADH-induced transepithelial micropinocytosis. This process, very similar to endothelial pinocytosis [7], is active and calls for large-scale energy out put by the mitochondria. The mechanism of nephron epithelial

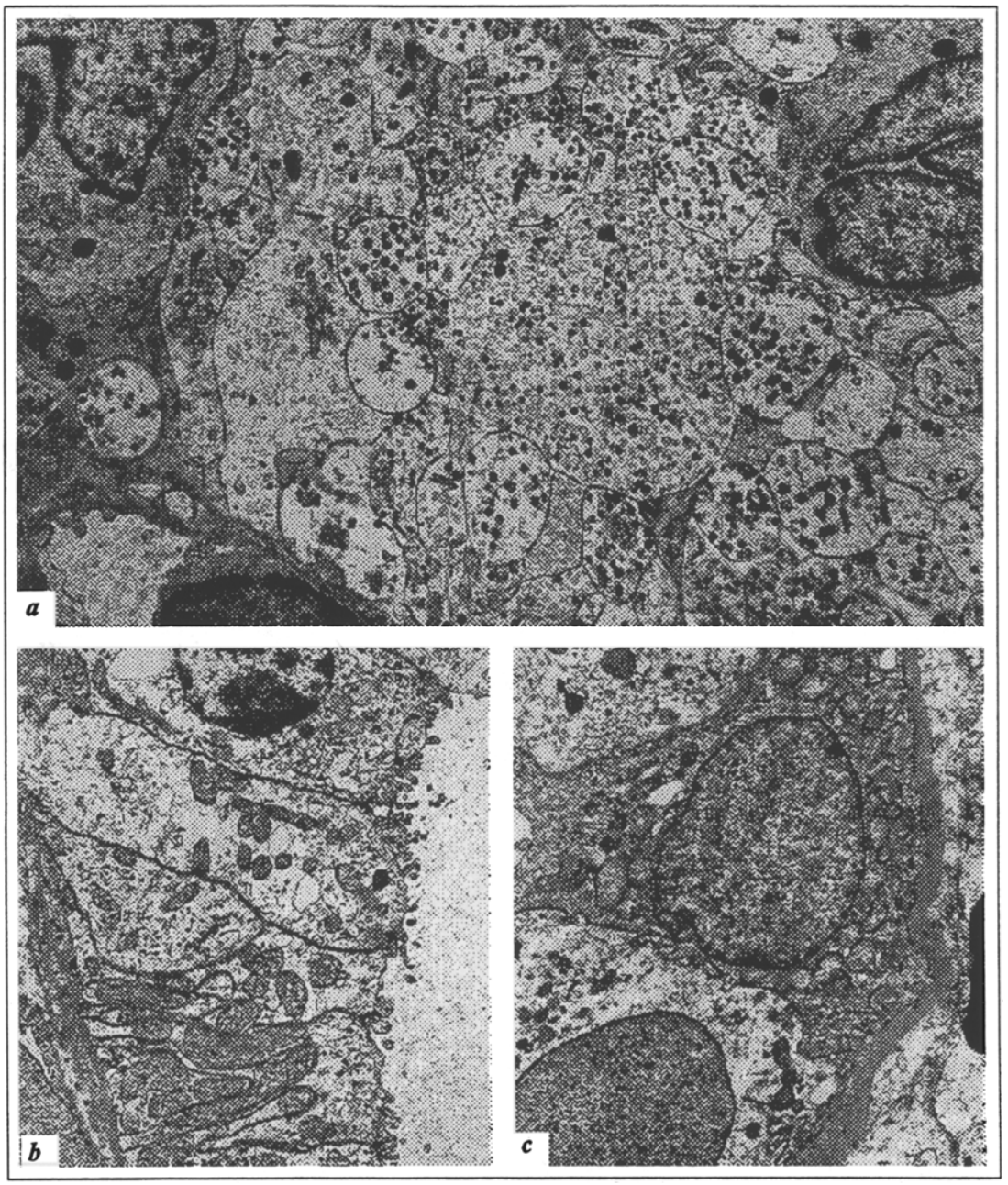

Fig. 3. Late stage of endotoxemia. *a*) numerous secretory granules in neurosecretory axons of posterior lobe of pituitary (×4000); *b,c*) absence of pinocytic vesicles in epithelial cells of distal tubule and collecting tubules (*b* – ×5000; *c* – ×10,000).

transcellular liquid transport is quite universal and has been described in the small intestine, appendix, Peyer's patches, bursa of Fabricius, and so on [8]. Moreover, it has been proved that dye and soluble specific antigens can be transported in pinocytic vesicles from the appendix lumen toward the basal plasmalemma [8,9].

Maximal devastation of the posterior lobe of the pituitary gland in the intermediate period of ES [1] is accompained by ultrastructural nephron changes similar to those in the initial period. The apical zone of the distal tubule epithelium still contains a large number of small vesicles, attesting to a high level of transepithelial micropinocytosis sustained during the first 5 hours after endotoxin injection (Fig. 2, *a*). The basal plasmalemma folds become somewhat smoothed, and the intercellular spaces remain unchanged. There are numerous mitochondria with a dense matrix and destroyed cristae. Pinocytosis is less pronounced in the collecting tubules epithelial cells and is observed mainly in the light cells, whereas the cytoplasm of the dark cells contains large vacuoles filled in part with lipid inclusions (Fig. 2, *b*).

Thus, the structural changes of the distal and collecting tubules are identical in the initial and in-

termediate periods of ES. The material substrate of oliguria is related to prolonged micropinocytosis, which causes long-term reabsorption in the distal part of the nephron.

After 3 days, at the late stage of endotoxemia, numerous neurosecretory granules are seen in the posterior lobe of the pituitary gland (Fig. 3, *a*). There are no ultrastructural features of the endothelium pointing to a state of high permeability for ADH. Such neurosecretory events are in agreement with the ultrastructural state of the renal epithelium. There are no micropinocytic vesicles, and consequently the process of transepithelial pinocytosis is leveled (Fig. 3, *b, c*).

Thus, the ultrastructural changes developing during endotoxemia reveal the reciprocal relationships in the hypothalamo-hypophyseal-renal system. Renal function is dependent on the state of the higher regulatory centers. In the process of ES, distal reabsorption is reduced and neurosecretion is affected according to the feedback principle. The appearance of numerous pinocytic vesicles in the initial and intermediate periods of ES points to a marked increase of facultative reabsorption and is an element of acute renal insufficiency. The disappearance of the vesicles in late endotoxemia is evidence of the restoration of water homeostasis.

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Morphofunctional Features of an Ovary Left after Unilateral Oophorectomy in Various Cycle Phases and in Pregnancy

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It was previously shown that the progeny of unilaterally oophorectomized rats have increased brain mass at the age of 5 days [4] and later differ from the control in the parameters of the sex and lymphoid organs [5,6]. One may suppose that changes in the ovary left after removal of the paired organ contribute to the origin of these deviations, the endocrine functions of the ovary being an important factor de-

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