# Ultrastructural Changes in the Nephron and Renal Protein Cleaving Function in Suckling Rabbits with Experimental Cholera

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Immature nephrons and the presence of a proteolytic system mediating extracellular protein digestion in the epithelium of proximal tubules are characteristic features of renal cortical ultrastructure in intact 10-12-day-old suckling rabbits. Cholera infection is accompanied by intensive cleavage of exogenous protein, which starts in podocytes and is completed in the cytoplasm of the proximal tubule epitheliocytes. Overload to nephron associated (in addition to urine production) with intensive extracellular hydrolysis impairs nephrothelium reactivity and increases its vulnerability to cholera exo- and endotoxins and their mediators.

**Key Words:** *kidney; acid phosphatase; cholera; ultrastructure*

The intestine in mammals remains biochemically immature during milk feeding [12]. Enzyme activity in the small intestinal epitheliocytes develops only at the end of the neonatal period [8,12]. Due to incompetence of the main stages of digestion, in suckling animals uncleaved milk proteins and exogenous proteins are absorbed into the blood.

In suckling rats, ultrastructural rearrangement characteristic of proteinuria develops in the kidneys: thickening of capillary basal membrane, reduction of podocyte pedicles, and accumulation of protein-containing lysosomes in podocyte cytoplasm. This is paralleled by accumulation of protein-containing electron-dense lysosomes with acid phosphatase (AP) activity in the epithelium of proximal tubule (PT) [4,5].

The development of experimental cholera in suckling rabbits is characterized by potent hypersecretion of fluid and massive desquamation of enterocytes, which creates extra loading of the filtration and particularly proximal portions of the nephron, because of necessity of endogenous cleavage of protein entering with milk during feeding. The aim of the present study was electron-microscopic examination of the kidneys of suckling rabbits with experimental cholera.

## MATERIALS AND METHODS

Experiments were performed on 10-12-day-old suckling rabbits ($n$=23). Experimental rabbits ($n$=17) were intragastrically infected with cholera (18-h *Vibrio El Tor* 5879 culture). To this end, 1 ml 3% sodium bicarbonate was introduced through a polyethylene tube for neutralizing the gastric juice, after which 1 ml Vibrio culture was introduced, and then again 0.5 ml sodium bicarbonate. The infective dose was $10^5$ bacterial cells (by optical opacity standard). After 4 h (during adhesion of *V. cholerae*) and 1 day the animals with clinical signs of cholera (diarrhea, inertness, adynamia) were sacrificed by ether overdosage (8 and 9 rabbits per group, respectively). Controls were treated with 1.5 ml sodium bicarbonate and 1 ml isotonic NaCl and sacrificed at the same terms as experimental animals (3 rabbits per point). Fragments of renal

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cortex were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h at 4°C and treated in 1% $OsO_4$ in the same buffer for 1 h at 4°C. After dehydration in ascending alcohols, the material was embedded in epon 812. General changes were evaluated on semithin sections stained with toluidine blue. Sections made from the same blocks on an LKB 8800 ultramicrotome were contrasted with uranyl acetate and lead citrate and examined under a JEM-100B electron microscope.

## RESULTS

Electron-microscopic examination of the cortical matter of control suckling rabbits showed that epithelial cells of the visceral leaflet of Shumlyanskii—Bowmen capsule (podocytes) were loosely connected to the membrane and therefore some of them lay free in the urinary space (Fig. 1, *a*). Podocytes varied in shape, the majority of them had large nuclei and a narrow cytoplasm rim with scanty organelles. Cells of the cap-

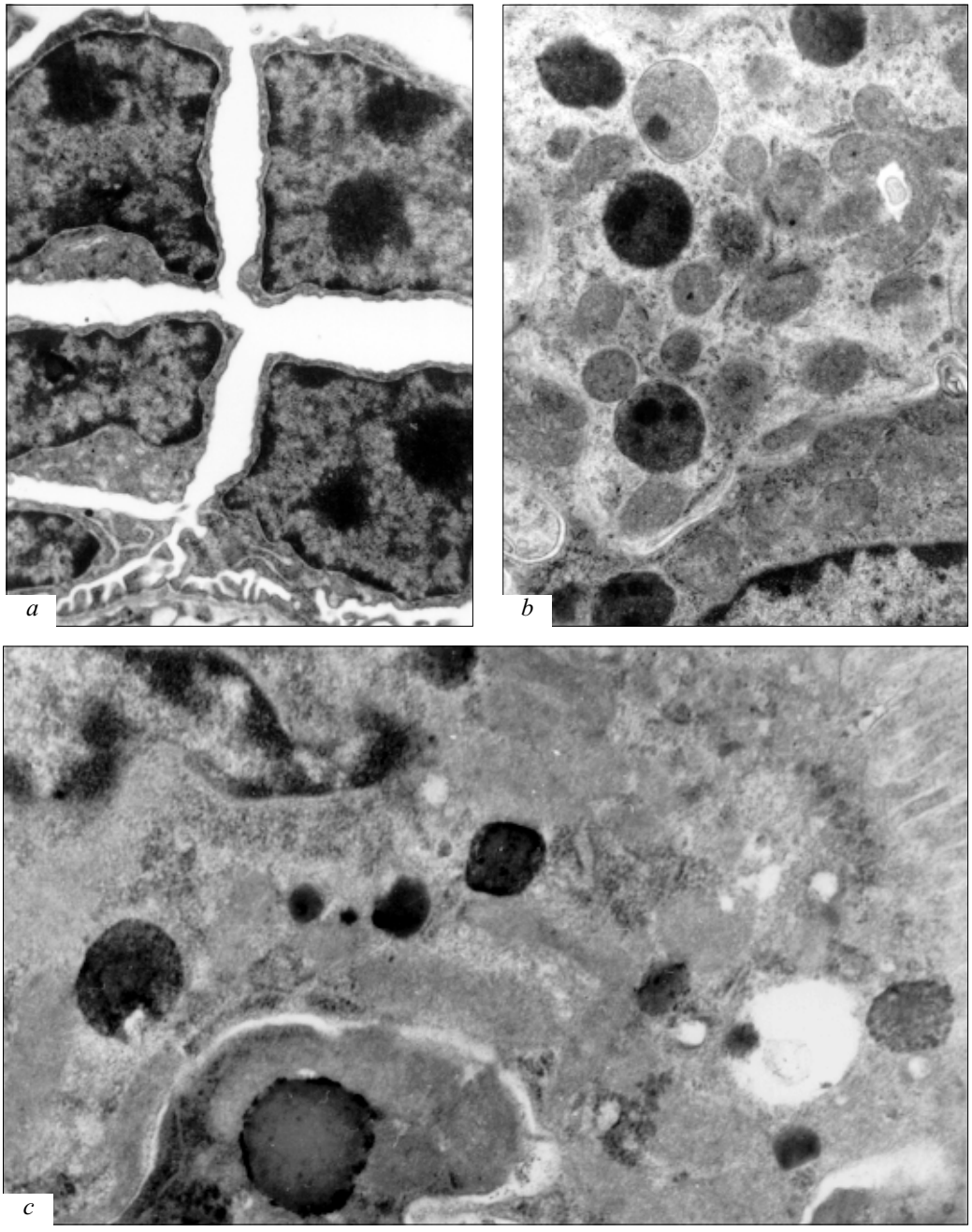


**Fig. 1.** Renal ultrastructure of control suckling rabbits. *a*) podocytes not fixed on basal membrane of glomerular capillaries, ×3000; *b*) epithelial cells of proximal tubules, containing milk cells at different stages of proteolysis, ×10,000; *c*) acid phosphatase activity in the proximal tubular epithelium, ×8000.

sule parietal leaflet and mesangiocytes had no specific features. PT epithelium contained large osmiophilic incorporations — milk protein at different stages of cleavage by lysosomal enzymes (Fig. 1, *b*). Ultracytochemical studies confirmed the presence of AP in the respective protein-containing cytoplasmatic bodies (Fig. 1, *c*).

Hence, incomplete formation of nephrons and the presence of proteolytic system capable of extracellular protein digestion in the PT epitheliocytes is a characteristic feature of renal cortical ultrastructure of suckling rabbits.

The number of podocytes lying free increased during adhesion of *V. cholerae*; sometimes they formed connections of the desmosome type. Podocytes adhering to the basal membrane lost pedicles and spread on it, their cytoplasm contained large vacuoles which sometimes fused and formed a giant cavity with just cell membrane or thin cytoplasm rim along the periphery (Fig. 2, *a*). Swelling of mitochondria and extension of endoplasmatic reticulum was seen in few cells of the capsular visceral leaflet. Death of podocytes as a result of apoptosis was rarely seen. Basal membrane

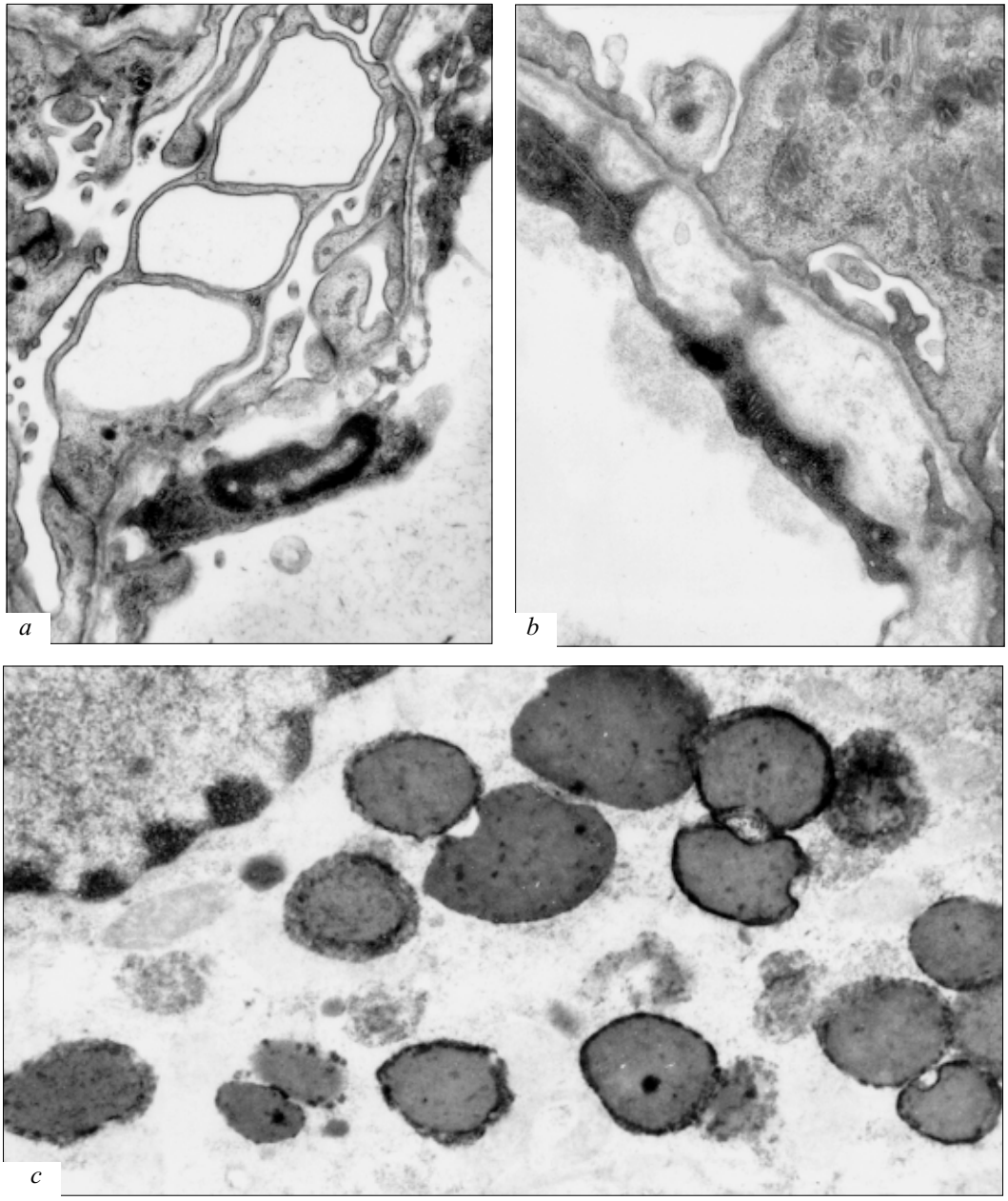


**Fig. 2.** Renal ultrastructure of suckling rabbits during adhesion of *V. cholerae*. *a*) Spreading of podocytes on basal membrane and appearance of large cavities in one of them, ×5000; *b*) changed thickness of basal membrane of glomerular capillary, ×8000; *c*) high activity of acid phosphatase in proximal tubular epitheliocyte, ×6000.

become thickened with uneven contours on the *lamina rara interna* side at the expense of impregnation with a plasma-dense substance (Fig. 2, *b*). Epithelial cells swell into capillary lumen and sometimes formed sort of arcades. Mesangiocytes were unchanged, some of them formed contacts with the endothelium and their processes even reached the vascular lumen (interposition).

Like in control animals, round osmiophilic bodies with high AP activity were seen in lysosomes of PT epitheliocytes (Fig. 2, *c*). However we should like to emphasize that hydrolytic activity of lysosomes in the proximal part of the nephron was essentially higher during adhesion of *V. cholerae* compared to the control.

Hence, adhesion of *V. cholerae* is associated with thickening of glomerular capillary basal membrane

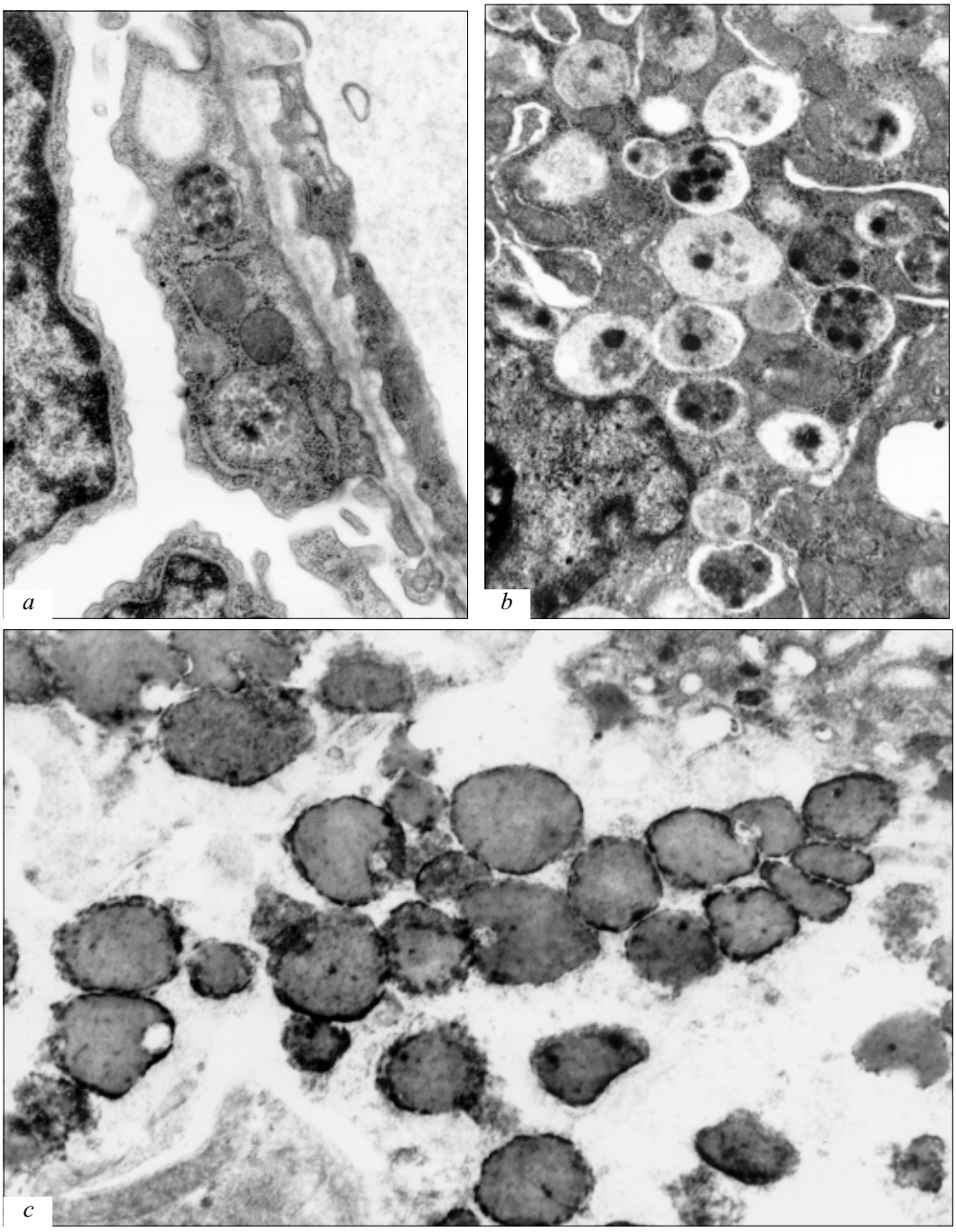


**Fig. 3.** Renal ultrastructure of suckling rabbits with cholera. *a*) cleavage of milk proteins in podocyte cytoplasm, ×10,000; *b*) cleavage of proteins in proximal tubular epitheliocyte cytoplasm, ×6000; *c*) high activity of acid phosphatase in lysosomes involved in proteolysis in the proximal tubular epithelium, ×8000.

and marked activation of the lysosomal system in renal PT epithelium, similar to that observed during protein loading.

After 24 h (in cholera) irreversible changes of enterocytes in the small intestine [2,3,6] were paralleled by ultrastructural changes in nephrons, primarily essential thickening of glomerular capillary basal membrane, flatening of podocytes, and accumulation of numerous protein-containing lysosomes in their cytoplasm and loss of pedicles (Fig. 3, *a*). Many cells of the visceral leaflet, lying free in the capsule lumen, also contained protein incorporations. The size of these incorporations and their osmiophilia varied within a wide range, which suggests that digestion of exogenous proteins entering with maternal milk starts in podocytes.

Interestingly that myelin-like figures originating from visceral leaflet epithelium and endothelium were situated in the immediate vicinity to podocytes and endothelial cells, in the urinary space and capillary lumen, respectively. Exocytosis of intracellular incorporations was paralleled by clasmatosis of podocyte cytoplasm sites, although not expressed. It started with the formation of macroprocesses resembling microvilli, or round swelling of plasmalemma, which then protruded into the capsule lumen. The endothelium contained, apart from myelin figures, microvesicular formations, previously observed under the effects of purified choleragen [1], histamine [10], leukotriene $C_4$, and endotoxin [9]. It seems that such the reaction in the endothelium is really explained by functional activity of cell surface, playing both a compensatory-adaptive and pathogenetic role under pathological conditions [10].

The number of protein-containing lysosomes sharply increased in the PT epithelium 24 h after infection (Fig. 3, *b*). Proteins entering the epitheliocyte cytoplasm were further degraded by lysosomal enzymes (Fig. 3, *c*).

Hence, biochemical immaturity of suckling rabbit intestine is largely compensated by intracellular digestion of proteins in the kidney. Extra loading of the nephrons due to intracellular hydrolysis (apart from urine production) impairs nephrothelium reactivity and makes it susceptible to the effects of choleric exo- and endotoxin and their mediators.

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