NEPHROTOXIC EFFECTS OF TETANUS TOXIN: AN ULTRASTRUCTURAL STUDY

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V. SOTTIURAI and A. A. FEDINEC. Nephrotoxic effects of tetanus toxin: an ultrastructural study. *Toxicon* 13, 415–421, 1975.—Generalized nephrotoxicity was observed in the rat as early as 6 hr after intramuscular injection of 1000 MLD of tetanus toxin. The most striking morphological changes, including swelling and rupture of the mitochondria, sloughing of the microvilli, and dissolution of cells, were found exclusively in the renal proximal tubule. Acute tubular necrosis with degeneration of the organelles and disruption of the cell membrane were also encountered throughout the entire nephron.

Since these observations parallel those reported in patients with complicated acute tubular necrosis secondary to nephrotic syndrome and chemical toxicity, perhaps the kidney is one of the major targets in tetanus intoxication. It is possible that injury to the nephron results in electrolyte imbalance that can contribute to the neuromuscular manifestation accompanying tetanus toxicity.

INTRODUCTION

REVIEW of the clinical signs, symptoms and clinical laboratory data of tetanus patients reveal that pulmonary edema, uremia and abnormal blood chemistry are frequently observed in benign and fatal tetanus patients (KLOETZEL, 1963; ADAMS *et al.*, 1966). No sound explanations have yet been offered regarding the etiology of these clinical manifestations. The kidney is an organ of multiple functions and a *sine qua non* in maintaining electrolyte homeostatis of the body. Damage to this organ can alter the delicate balance of electrolytes and produce sequelae resembling those encountered in tetanus patients. In light of this possible correlation, this study was undertaken (1) to determine whether tetanus toxin inflicts damage upon the nephron, (2) to demonstrate ultrastructural morphology of tetanus intoxicated nephrons, and (3) to correlate renal morphological findings with the clinical symptoms.

MATERIALS AND METHODS

Fourteen adult Albino male rats of Sprague–Dawley strain weighing between 250 and 300 g were used in this experiment. The animals had been kept on diet of commercial pellets and water *ad libitum*. Temperature and space allowed for movement was kept uniform. Pentobarbital sodium (Diabutal®), 50 mg per kg, injected intraperitoneally was used as surgical anesthesia.

Purified tetanus toxin was kindly provided by William C. Latham of the Institute of Laboratories, Massachusetts Department of Public Health, Boston, Massachusetts (LATHAM *et al.*, 1965). The purified toxin (Batch CPTxn-21-AS-II) contained 8×10^7 mouse minimum lethal doses (MLD's) and 1000 I.U.Lf doses per ml. The toxin was lyophilized, reconstituted with sterile saline, and the toxicity reconfirmed by bioassay in mice immediately prior to use in the experiment (KING and FEDINEC, 1973).

Experimental rats were injected into the left gastrocnemius muscle with 1000 mouse MLD per 0·05 ml of tetanus toxin, and under surgical anesthesia bilateral kidneys were removed from the animals in pairs at

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6, 12, 16, 24 and 32 hr after toxin injections or before demise (40–45 hr). Kidneys removed from uninjected rats served as controls.

Renal tissue from the cortex, medulla, and papilla was separated and diced to < 0·5 mm³ while being fixed for 2 hr in a ½ strength Karnovsky fixative containg 2% potassium pyroantimoniate (for purpose of Na + Ca precipitation) in 0·1 M potassium phosphate buffer at pH 7·4. The fixative was followed by 6 hr wash in pH 7·4, 0·1 M potassium phosphate buffer and fixation for 2 hr in 2% OsO₄. After fixation the tissues were rinsed for 10 min with 0·5 M maleic acid, pH 5·2 and stained for 1 hr in 0·5 M maleic acid containing 0·5% uranyl acetate at pH 5·2. The tissues were then washed with 0·5 M maleic acid, dehydrated in methanol, and embedded in Epon 812. Sections 1–2 µm thick were stained with methylene blue. Thin sections were stained with uranyl acetate and lead citrate, and examined at 75 KV in a Hitachii HU 11A electron microscope. This report is restricted to morphological observations in the electron microscope; the localization of Na and Ca will be reported subsequently.

RESULTS

Morphological changes in the rat nephrons were detectable with transmission electron microscopy less than 6 hr after i.m. injection of tetanus toxin (1000 mouse MLD). However, there was no noticeable progressive morphological damage after the initial insult. Comparison of the ultrastructural changes of the nephron at 6 hr after tetanus toxin injected with those prior to the animals' demise (40–45 hr) did not reveal significant differences. The morphological changes of the tetanus intoxicated rat nephron can be summarized according to regions of the nephron as follows:

Glomerulus

The constituents of the glomerulus appeared normal. There was no consistent evidence of intracellular and intercellular swelling and capillary congestion (Fig. 1). The podocytes and their foot processes appeared normal except for some thickening or fusions of foot processes. Pedunculated evaginations containing inclusion bodies were observed in the podocytes (Fig. 2). The basement membrane showed intermittent thickening, loss of the trilaminar structure (Figs. 2–4) and evidence of edema. The intracellular organelles appeared normal in morphology and number. Endothelial cells and their fenestrations were preserved and intact (Fig. 2–4).

Proximal tubule

The proximal tubule sustained the most severe damage. Some cells were disrupted, many were denuded of microvilli and a few had indistinct cell contours (Fig. 5–7). The intercellular spaces were widened (Fig. 5). The basement membrane demonstrated differing degrees of thickening and pleomorphic changes (Fig. 7). Damage and changes in the organelles were striking. The endoplasmic reticular were markedly distended and transformed to vaculoid structures. The mitochondria underwent several changes consisting of vacuolation, swelling, loss of cristae, disruption and laminar body formation (Figs. 5 and 7).

Randomly distributed vesicles of differing sizes and empty intracellular spaces were numerous (Figs. 5–8). The nuclear bags were distended. The golgi complexes were few and moderately dilated in the intermembranous space (Figs. 6–7).

Thin segment

Injury to this portion of the nephron was less striking. The epithelia were generally intact. Changes encountered were mostly confined to the subcellular level. The intercellular tight junctions were intermittently disassociated and the intercellular space widened (Fig. 9). The basement membranes were displaced peripherally and interrupted by empty spaces indicative of intercellular edema (Figs. 9–11). Mitochondria were swollen, plump

and contained very few cristae. Space between golgi complex were dilated. Intracellular vesicles were abundant and widely distributed.

Distal tubule

Cellular changes are less discernible in this area; the majority of intracellular organelles were well preserved. However, disruption of cell membrane, vacuolated mitochondria, loss of microvilli and intracellular empty spaces were not uncommonly observed (Figs. 12 and 13).

Collecting tubule

Very little ultrastructural changes were detectable in this portion of the nephron except minor degenerative transformation of the mitochondria and vacuolation exhibited by some cells (Fig. 14). The most consistent finding was intertubular edema and laminar bodies.

DISCUSSION

The kidney, because of its rich blood supply, its ability to concentrate substances, and its excretory function, is especially vulnerable to adverse effects of nephrotoxic agents.

Nephrotoxic agents can inflict damage on several entities of the kidney: the glomerulus, the tubules, the blood vessels, and the interstitium. Tetanus toxin, in addition to its neurotoxic effect, is also a nephrotoxic exotoxin. Although ultrastructural changes resulted from tetanus toxin involved the entire nephron, the proximal tubule sustained the most severe damage. The degree of injury varied from mitochondria degeneration to disruption of the cells as depicted in Figs. 5–8.

Ultrastructural observations of the nephron in our study are in consonant with clinical symptoms in patients with complicated acute tubular necrosis secondary to nephrotic syndrome, chemical toxicity and toxin intoxication (MUEHRCKE, 1969). Perhaps correlation can be made between the ultrastructural observations and tetanus patient with regard to the pathogenesis and etiology of some of the abnormal clinical findings in blood chemistry, hypotension, and pulmonary edema.

Selective damage to the proximal tubules by the tetanus toxin with minimal morphological changes of the glomerulus and moderate degenerative change in the remaining nephron are puzzling. However, such findings are in accord with the clinical manifestation and the clinical laboratory results reported in the literature. The physiological roles of the proximal tubule are numerous. Those that may relate to the clinical findings in tetanus include degradation of insulin and other hormone; tubular reabsorption of filtered proteins, electrolytes, glucose, organic acids and bases, BUN, and other elements.

Tetanus toxin is probably handled by the kidney like other proteins. It is filtered through the glomerulus, reabsorbed by the proximal tubule, and broken down into amino acids and other constituents (4–7) (ELIASCH *et al.*, 1955; ERICSSON, 1964; MILLER and PALADAE, 1964; OLIVER, 1954). Based on this assumption of how tetanus toxin is handled by the nephron, it is highly suggestive that the proximal tubule because of its role in degradation of tetanus toxin is apt to sustain more damage than the remaining portion of the nephron.

Damage inflicted on the proximal tubule resulting in renal epithetial cell degeneration and disruption, as depicted in Figs. 5–8, can seriously impair many functional roles assigned to these cells. Therefore observation of elevated blood urea nitrogen by JACKSON (1962)[*]

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[*]JACKSON, B. G. (1962) Problems in the technique of intermittent pressure respiration in the treatment of tetanus. M.D. Thesis, University of London.

and high potassium by SMYTHE and BULL (1959) and GARLAND (1959) with low urine levels of these two substances could indicate injury to the renal tubules and failure to maintain the normal balance of these substances. Prerenal deviation of water and excessive protein catabolism can also present azotemia. Similarly abnormal electrolytes can be attributed to the incapacitation of the injured proximal tubule to maintain electrolytes balance (Figs. 5–8). Pulmonary edema and peripheral edema are other common complications in tetanus. Renal tubular impairment secondary to damage by tetanus toxin can certainly account for such sequellae. Hypoalbuminemia in tetanus reported by HOLLOWAY (1970) is probably the consequence of impairment of the proximal tubule in protein reabsorption subsequent to tetanus nephrotoxicity, although injury to the glomerulus *per se* can enhance protein filtration and augment further protein loss.

Hyponatremia has been reported to be often associated with fatal tetanus by KLOETZEL (1963). This could be ascribed to the effects of severe proximal tubular damage, as this segment is responsible for more than 90% of the electrolyte reabsorption. Hypoglycemia and ketonuria (KLOETZEL, 1963) are other complications not uncommonly encountered in tetanus patients. These conditions were indicative of profound disturbance in metabolism with insufficient caloric intake because intravenous infusion of hypertonic glucose solution seemed to mitigate such complications. However, proximal tubular dysfunction can also contribute to such disturbance. The convoluted segment of the proximal tubule has been demonstrated to metabolize as much as 35% of the serum insulin (SOTTIURAI, 1974). Impairment of this segment of the nephron can therefore prolong the effect of insulin and produce hypoglycemia.

Tetanus toxin, long regarded as a neurotoxic agent, can inflict severe damage on the nephron, particularly the proximal tubule. Since the proximal tubule is the primary site concerned with tubular reabsorption, secretion, and degradation of certain hormones, it is logical to assume that impairment of this portion of the nephron can affect renal function and alter the normal plasma composition to an extent that will interfere with the homeostatis of the body. It is also important to note that manifestation of clinical symptoms relating to abnormal blood chemistry secondary to nephrontoxicity as a rule precedes neuromuscular symptoms. Whether the neuromuscular symptoms are a byproduct of prolonged and profound electrolyte abnormalities or whether tetanus is primarily a neuromuscular disease is a very interesting question and warrants further study. However, our data are more inclined to suggest that perhaps tetanus is a sequellae of a multiorgan disease with the kidney in particular. This view is supported by observations of KIRILENKO et al. (1964) that ^{131}I-tetanus toxin was more concentrated in mouse and guinea pig kidneys than other organs. FEDINEC (1967) autoradiographically demonstrated tritium labeled tetanus toxin in mouse renal tubule.

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FIG. 1. A PORTION OF A GLOMERULUS FROM RAT, 6 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN.

There were no significant morphological changes noted. Endothelium (Ed); lumen of capillary (Ca); foot process (Fp); basement membrane (Bm) urinary space (U). ×20,000.

FIG. 2. A PORTION OF A RAT GLOMERULUS, 6 hr AFTER i.m. INJECTION of 1000 MLD TETANUS DEPICTING SOME DEGENERATIVE CHANGES IN THE FOOT PROCESS (Fp) OF A PODOCYTE.

A pedunculated evagination with inclusion bodies (PIB) was demonstrated. The basement membrane (Bm) had lost its trilaminar feature. Fenestrations (Fe) of an endothelial cell were intact. ×15,000.

FIG. 3. A SECTION OF A RAT GLOMERULUS, 43 hr AFTER i.m. INJECTION OF 1000 MLD OF TETANUS TOXIN.

There were no striking morphological changes except a few laminar bodies (Lb) in a foot process (Fp). The basement membrane (Bm) was uniform in thickness and had lost its trilaminar feature. Fenestrations (Fe) of the endothelial cells were intact. The urinary space (U) was normal in size. Lumen of a capillary (Ca). Red blood cell (Rbc). ×13,000.

FIG. 4. A SMALL SECTION OF A RAT GLOMERULUS, 43 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN, DEPICTING NORMAL APPEARANCE OF FOOT PROCESS (Fp) AND FENESTRATIONS (Fe) OF AN ENDOTHELIAL CELL.

The basement membrane (Bm) appeared slightly thickened with some evidence of edema (e). Lumen of a capillary (Ca). ×60,000.

FIG. 5. A TRANSVERSE SECTION OF A RAT PROXIMAL TUBULE, 6 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN.

Striking and severe morphological changes in the cell organelles and cell contour were demonstrated. Mitochondria (M) were dense. Numerous lysosomes (Ly) were demonstrated. Intercellular spaces (Ics) were dilated. Large vesicles (V) were noted. Multiple intracellular empty spaces suggested cell swelling. Some cells were ruptured with intracellular contents extruding into the lumen of the tubule. Sloughing of microvilli (Mi) was also observed. The basement membrane (Bm) was intact and uniform in width. ×6000.

FIG. 6. A PORTION OF A RAT PROXIMAL TUBULE 43 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN.

Mitochondria (M) were relatively well preserved and slightly swollen. Occasional laminar bodies (Lb) were seen. There were numerous intracellular vesicles (V). The golgi complex (Gc) was slightly distended. Nuclear bags (Nb) were enlarged. Nucleus of proximal tubule (NPT). ×15,000.
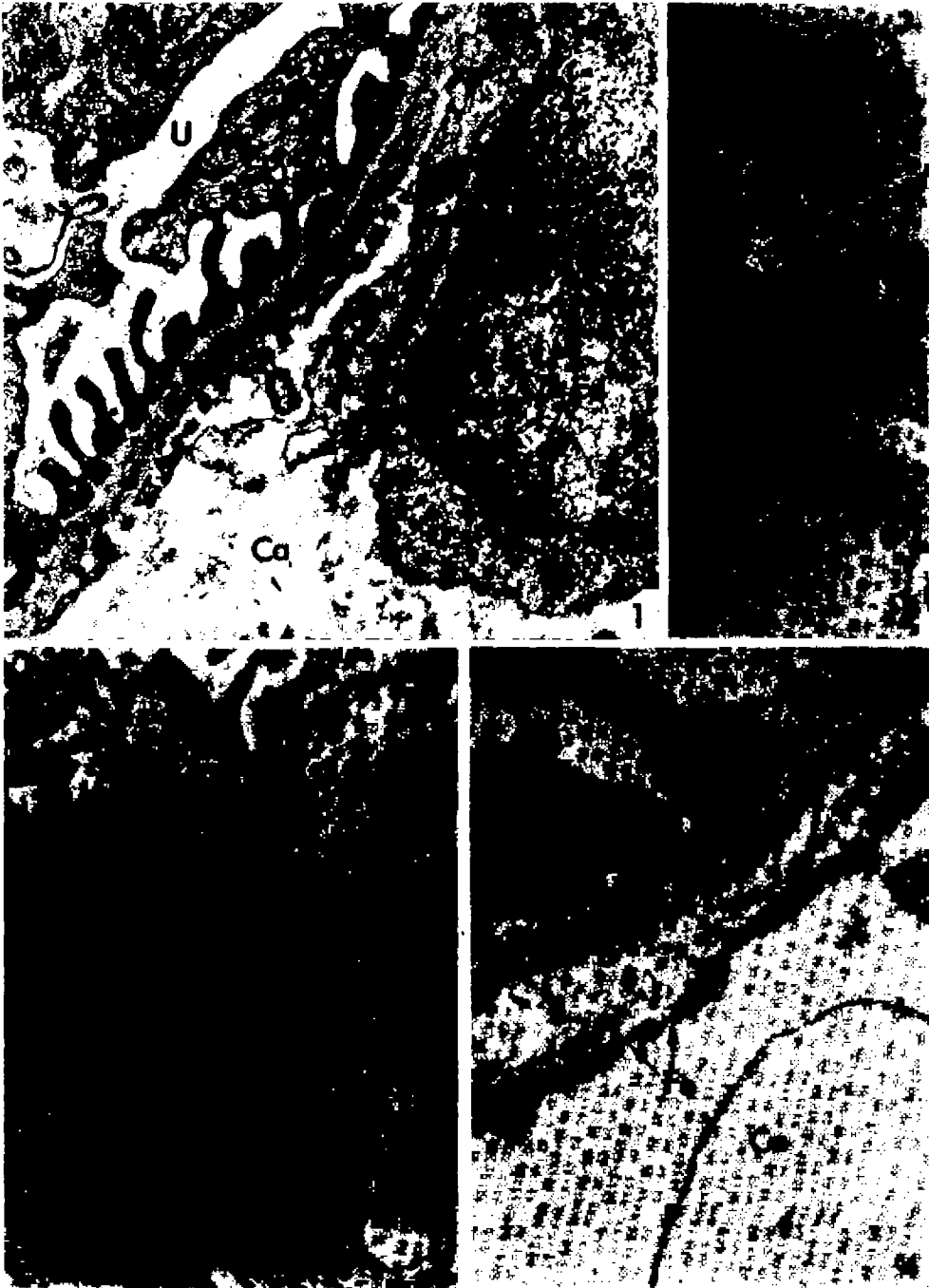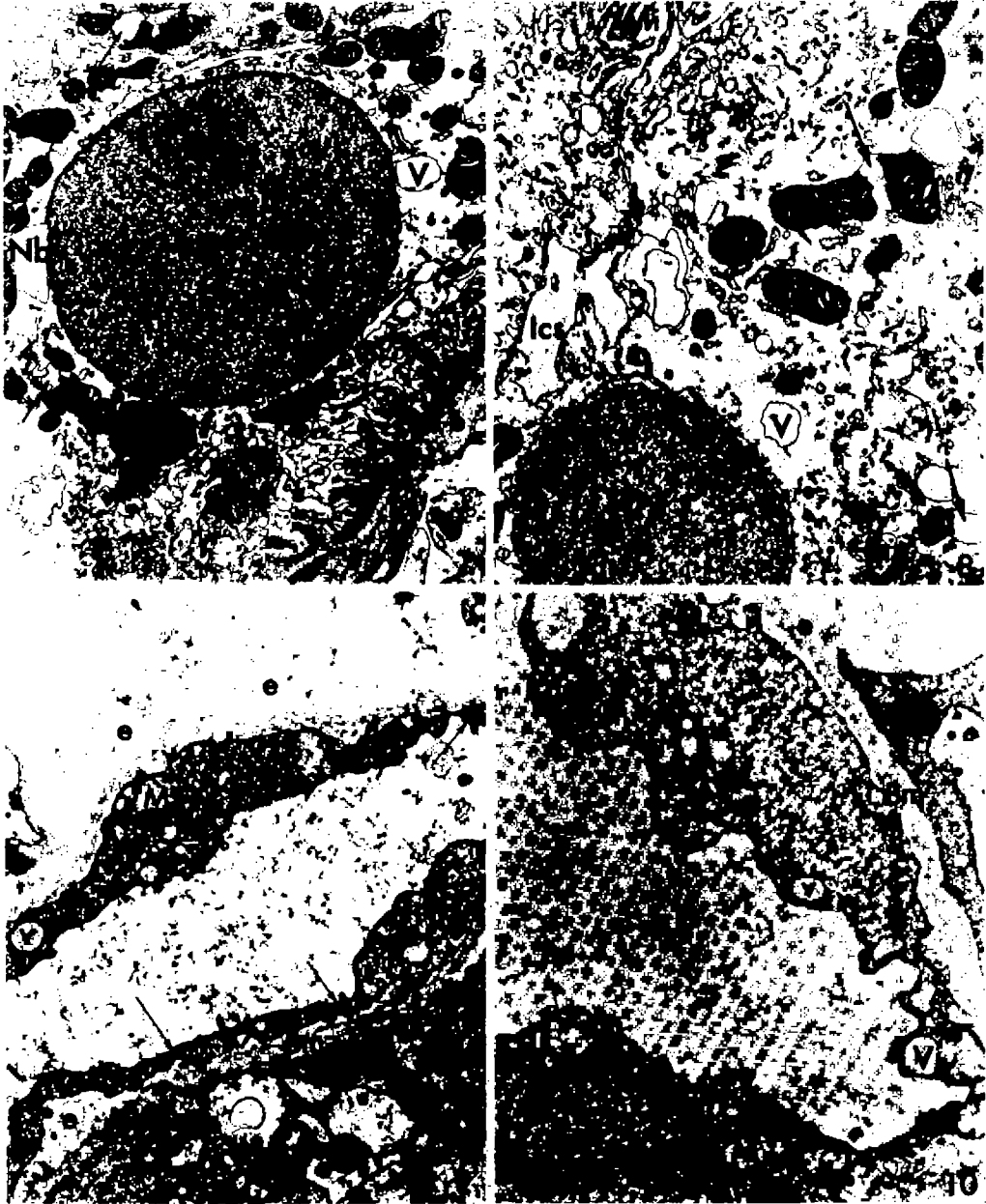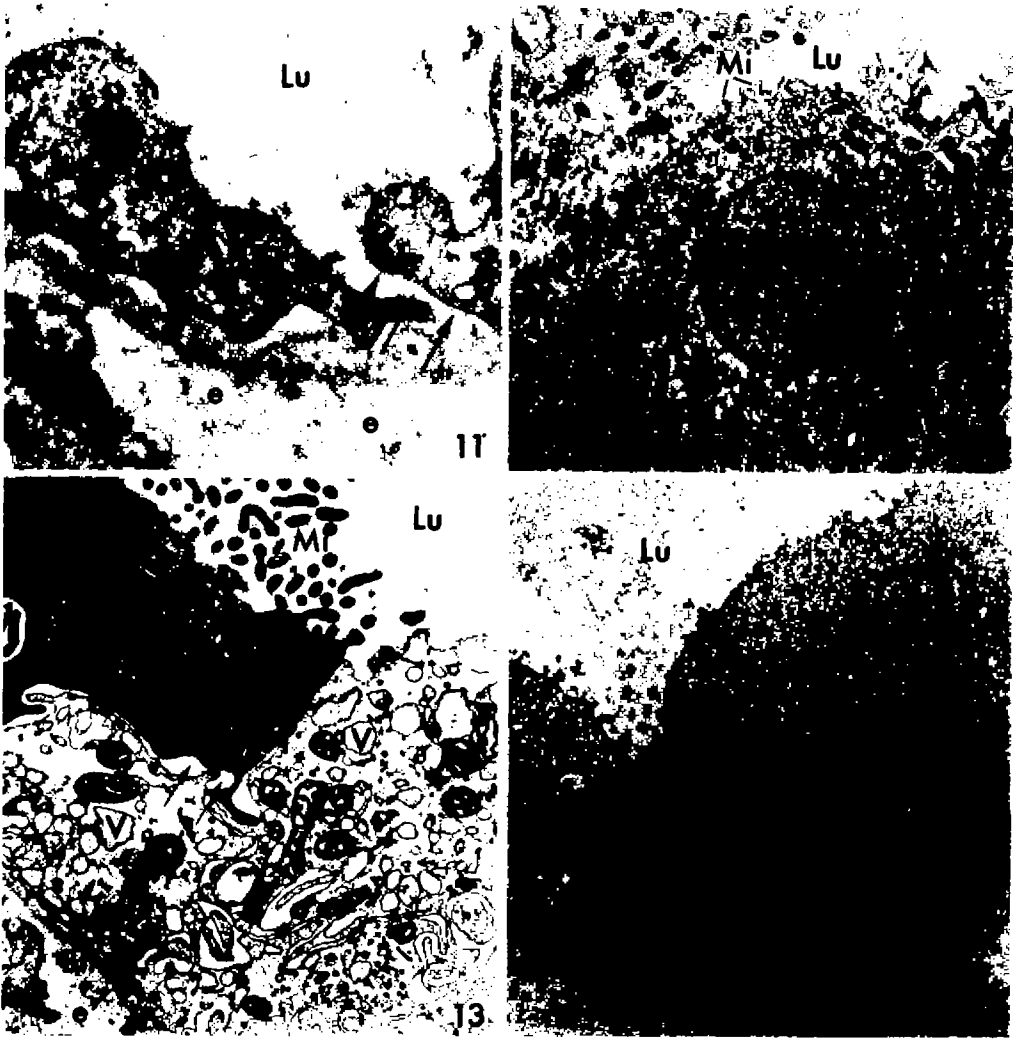
FIGS. 1–4
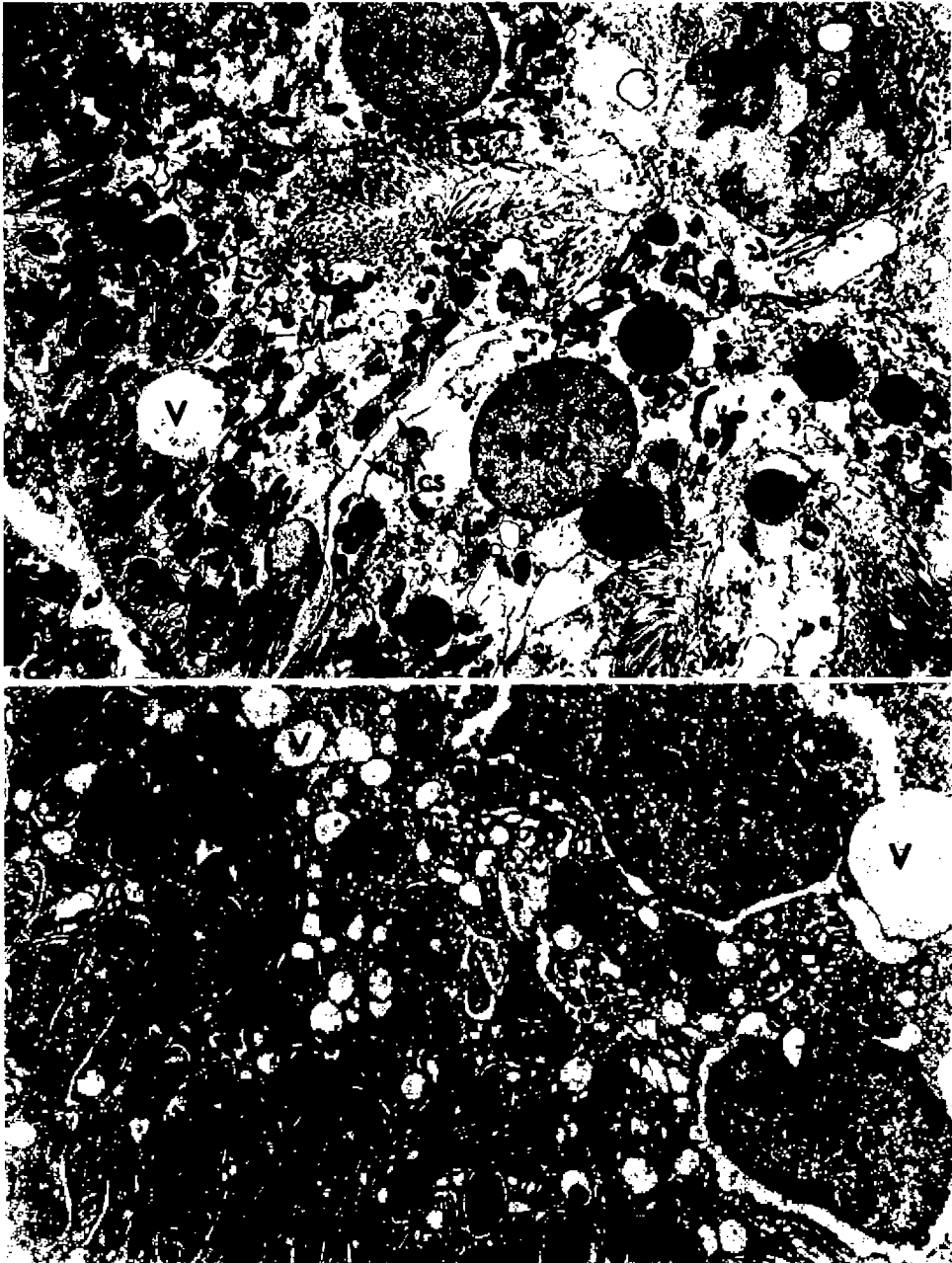
FIGS. 5–6

FIGS. 7–10

FIGS. 11–14

FIG. 7. A SECTION OF A RAT PROXIMAL TUBULE, 7 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN DEMONSTRATING A DILATED NUCLEAR BAG (Nb).
The mitochondria (M) were dense with infrequent vacuolation (tips of arrow). Occasional lysosomes (Ly) and vesicles (V) were observed. The basal infoldings (Bi) and the basement membranes (Bm) were distorted and pleomorphic. There was evidence of edema in the interstitium (e). Nucleus of a proximal tubule (NPT). ×15,000.

FIG. 8. A CROSS-SECTION OF A RAT PROXIMAL TUBULE 6 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN.
The intercellular spaces (CS) were dilated with numerous intracellular empty spaces suggestive of cellular swelling. Mitochondria (M) were dense with occasional vacuolation (arrows). There were few vesicles (V). Microvilli (Mi). Nucleus of a proximal tubule (NPT). ×20,000.

FIG. 9. A PORTION OF A TRANSVERSE SECTION OF A RAT THIN SEGMENT 6 hr AFTER i.m. INJECTION of 1000 MLD TETANUS TOXIN.
Mitochondria (M) contained very few cristae and appeared swollen. The basement membrane (Bm) was slightly displaced peripherally by interstitial edema (e). Single membrane vesicles (V) lumen of thin segment (Lu). Interepithelial tight junction (arrows) were intact. ×20,000.

FIG. 10. A PORTION OF A TRANSVERSE SECTION OF A RAT THIN SEGMENT, 42 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN, SHOWING CHANGES IN INTRACELLULAR ORGANELLES.
Some mitochrondria (M) were swollen and had very few cristae; some had dilated vacuoles (arrow). Vesicles (V) were also numerous. The basement membrane (Bm) was slightly displaced by intercellular edema (e). Lumen of a thin segment (Lu). Nucleus of a thin segment (NT). ×20,000.

FIG. 11. A SMALL SECTION OF A RAT THIN SEGMENT, 6 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN, DEPICTING DISASSOCIATION OF TIGHT JUNCTION BETWEEN THE CYTOPLASMIC PROCESSES OF TWO RENAL EPITHELIA (ARROWS).
The basement membrane (Bm) was irregular in thickness and markedly distorted. Interstitial edema (e) was also demonstrated. Tubular lumen (Lu). ×40,000.

FIG. 12. A CROSS-SECTION OF A DISTAL TUBULAR CELL 42 hr AFTER i.m. INJECTION OF 1000 MLD OF TETANUS TOXIN.
The cell membrane appeared swollen with abundant empty spaces. Only a few mitochondria were preserved. Mitochondria (M) showed dense matrix and cristae. Some demonstrated vacuolation changes (arrow). The adjacent cells showed ruptured cell membranes. Microvilli (Mi). Tubular lumen (Lu). ×7000.

FIG. 13. A PORTION OF A TRANSVERSE SECTION OF A RAT DISTAL TUBULE, 42 hr AFTER i.m. INJECTION OF 1000 MLD TETANUS TOXIN, COMPARING TWO CELLS SUSTAINING DIFFERING DEGREES OF INJURY AND DAMAGE.
The cell at the lower right was more severely impaired with multiple vesicles (V) empty spaces and nerve degenerative changes with rupture of cell membrane; whereas the cell to the left retained its microvilli (Mi) and the intracellular organelles appeared normal. Mitochondria (M); basement membrane (Bm). Arrow points to intermittently dilated intercellular spaces. Edema (e) was found in the interstitium. ×25,000.

FIG. 14. A PORTION OF A COLLECTING TUBULE IN TRANSVERSE SECTION DEMONSTRATING DIFFERING DEGREES OF MORPHOLOGICAL CHANGE BETWEEN TWO ADJACENT CELLS 42 hr AFTER i.m. INJECTION OF 1000 MLD OF TETANUS TOXIN.
The intercellular spaces were normal in width (arrow). Mitochondria (M) were normal in the cell on the right. Laminar bodies (Lb) and vesicles (V) were found in the adjacent cell to the left, the laminar bodies were believed to be degenerating mitochondria. The basement membrane (Bm) was normal in thickness and morphology. Fenestrations (Fe) of the nearby blood vessel appeared normal. Strong evidence of interstitial edema was noted. Nucleus of collecting tubules (Nc). ×8000.