46. PHARMACOLOGY OF CRYSTALLINE CROTOXIN.
IV. NEPHROTOXICITY

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Renal lesions identical to those originally found in crush syndrome (haemoglobinuric nephrosis, lower nephron nephrosis or tubulorhexis) have been described in autopsy and biopsy tissue (1, 2, 5) from human cases of snakebite caused by Crotalus durissus terrificus (South American rattlesnake). Similar lesions, according to Amorim and coworkers (3) can be experimentally produced by injecting dogs with the crude venom of this snake. Identification of the component or components of the venom responsible for such histopathologic picture is highly desirable. A study was, therefore, undertaken of the renal lesions produced in dogs by crototoxin, one of the main toxins of the venom. Besides motor paralysis, this venom component elicits albuminuria, haemoglobinuria and oliguria in dogs as it was shown in a previous paper (7).

MATERIAL AND METHODS

The histopathological study was made on the kidneys of twenty dogs intravenously injected with doses of 0.102, 0.128, 0.160, 0.200 and 0.250 mg/Kg of crototoxin. The kidneys were removed one to nine days after crototoxin administration from animals under sodium pentobarbital anaesthesia or from dogs recently died of crototoxin intoxication.

Pieces of the kidneys were fixed in Bouin’s fluid, or in 10% formalin in phosphate buffer (pH 7.0), included in paraffin, cut and stained by haemotoxylin and eosine or Masson’s trichrome method. PAS, and benzidine reaction for haemoglobin detection (5), were also made.

RESULTS

Both glomeruli and renal tubules showed pathological alterations. There was some correlation between intensity of lesions and the dose of crototoxin injected, the lesions being more marked in the kidneys of those animals which received higher doses. There were, however, some variations not correlated to the doses.

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The renal lesions showed focal distribution: injured areas were always found to be separated by apparently normal ones.

It was possible to recognize some differences between early lesions, which occurred in the first four days following crototoxin injection, and late lesions, observed after the fourth day. Such differences concern the component of the nephron most attained by the lesions. In early lesions, the renal tubules were less attained than the glomeruli; in late ones, tubular lesions predominated.

**Early lesions**

*Glomerulus.* The lesions most constantly observed were capillary congestion, thickness of the basement membrane, deposit of PAS positive material between the capillary loops (Fig. 1) and nuclear pycnosis of some glomerular cells.

![Fig. 1 — Section of kidney of dog injected with crototoxin. PAS reaction. Early lesions. Cross section of glomeruli shows thickness of the basement membrane and some amorphous PAS positive material among the glomerular loops.](image)

*Proximal tubule.* Degenerative lesions were present in some epithelial cells of the proximal convolutions. The brush border of the damaged cells disappeared at the same time that an alteration of the normal distribution as well as a numerical reduction of the mitochondria occurred (Fig. 2). The cytoplasm of the damaged cells appeared vacuolated and the nucleus was sometimes pycnotic.

It must be emphasized that the degenerative lesions reached only some tubular cells, in very restricted areas. These areas were situated chiefly in the internal zone of the renal cortex, corresponding to the terminal portion of the proximal convoluted tubule.
Inside the tubules, it was frequently found PAS positive and haematic casts.

**Distal tubule.** Degenerative lesions similar to those described for the proximal tubular cells were found in distal tubules. However, the injured areas were smaller and the damaged cells less numerous than those in the proximal convolutions.

**Collecting ducts.** The collecting duct cells were apparently normal. In the collecting duct lumen, some PAS positive haematic casts could be seen.

**Interstitial tissue.** Some areas of oedema near the injured tubules were observed.

**Late lesions**

**Glomerulus.** The glomeruli showed in most cases a normal aspect. However, thickness of the basement membrane could be observed in a few glomeruli.

**Proximal tubule.** The proximal tubules were intensely injured. Although focal in character, the lesions had a tendency to become diffuse through confluence of neighboring injured areas.

The lesions predominated in terminal segments of the proximal tubules. However, cells in the initial ones were also sometimes damaged.
Figs. 3 and 4 — Section of kidney of dog injected with crotoxin. Masson's trichrome stain. Late lesions. The proximal tubules show strongly swollen cells without brush border. The distal tubule cells as well as those of some proximal convolutions present only minor lesions. In this instance, the cells keep their brush border and mitochondria pattern.
The following alterations could be seen in the epithelial cells of the damaged tubules: disappearance of their brush border, a great decrease in the amount as well as an irregular pattern of the mitochondria, swelling and microvacuolar degeneration of the cytoplasm. When intense this degenerative process gave a foamy aspect to the cells (Figs. 3, 4, 5 and 6). The intensely vacuolated cells showed nuclear pycnosis and necrosis. Some PAS positive granules could be observed in the cytoplasm of the vacuolated cells.

PAS positive casts were seen in the lumen of the proximal tubules.

Distal tubule. The lesions were qualitatively identical to those seen in the proximal tubules. However, they were much less intense, cellular necrosis being rarely seen. Disorganization of the mitochondria pattern and slight microvacuolization of the cytoplasm of the epithelial cells (Figs. 4 and 5) were the predominant alterations observed.

The initial portion of the distal tubules appeared more intensely damaged than the distal one.

Collecting ducts. Collecting ducts did not show lesions. In their lumen some PAS positive casts could be seen.

Interstitial tissue. Around the injured tubules there was some chronic inflammatory infiltration and sometimes tissue neoformation.

Fig. 5 — Section of kidney of dog injected with crototoxin. Masson's trichrome stain. Late lesions. Cells of proximal convolutions show intense microvacuolar degeneration. In distal tubules the mitochondria pattern is altered and discreet microvacuolar degeneration of their cells can be seen.
Fig. 6 — Section of kidney of dog injected with crotoxin. Haematoxylin and eosine. Late lesions. Intense microvascular degeneration and cellular necrosis of proximal convoluted tubules can be seen.

DISCUSSION

The histopathological picture shown by the kidneys of dogs injected with crotoxin was not exactly the same as that which Amorim and Mello (1, 2) described in human cases of *C. d. terrificus* snakebite. These authors stated, in effect, that the ascending limb of the Henle’s loop and the distal convoluted tubule were the predominant sites of the degenerative and necrobiotic lesions. In the kidneys from the dogs intoxicated with crotoxin, on the other hand, the most attained segment of the renal tubule was the proximal one. In both cases, however, the lesions presented a focal distribution. Amorim and coworkers (3) also found the ascending limb of the Henle’s loop and the distal convolution to be the prevailing situation of the renal lesions of dogs injected with the South American rattlesnake venom: “The histological lesions of the tubules are principally characterized by degenerative and more serious necrobiotic lesions in the so-called intermediated segment of the nephron, i.e., the ascending branch of Henle’s loop and of the distal or secondary convoluted tubule predominating in the boundary zone of the kidney” (3). However, Rodrigues Lima (6) found in biopsy tissue from human cases of *C. d. terrificus* envenomation that degenerative lesions predominated in the proximal convolutions while necrotic ones were more frequent in the distal tubules: “Nos nossos casos, o néfron foi lesado como um todo, embora as lesões degenerativas tenham preferência por túbulos proximais, enquanto que a necrose e a regeneração ocorreram com mais freqüên-
cia no segmento distal" (6). The degenerative lesions of the tubular epithelium of the proximal convolutions were, as described by Rodrigues Lima (6), of the same type of those we found in the kidneys of dogs injected with crototoxin: “Estas lesões (the degenerative lesions) têm aspectos de microvacúolos que rechacem o núcleo para a periferia, tomando total ou parcialmente o citoplasma celular ou apresentam fina granulação citoplasmática com borramento dos limites celulares. Estes aspectos foram vistos com maior frequência nos túbulos contornados proximais” (6).

Three main factors are probably at work in generating the lesions found in envenomation caused by C. d. terrificus snakebite, and in crototoxin intoxication. They are (1) nephrotoxic substances, (2) intravascular haemolysis and (3) shock. The haemolytic activities of crototoxin and of the venom are nearly the same. They are due to their phospholipase A activities, i.e. to lyssolecithin formation. Thus the factor mentioned in (2) can not explain the differences observed in the renal histopathologic picture of the two conditions.

Lyssolecithin besides being haemolytic is toxic to renal epithelial cells. It is formed by crototoxin, which is composed of crotactin (a polypeptide toxin) bound to phospholipase A (4), and by the venom through its content of crototoxin and, perhaps, free phospholipase A. The local formation of lyssolecithin at the surface of the tubular epithelium could be one of the causes of tubular lesions, mainly those of proximal convolutions. This local formation of lyssolecithin seems to be very probable since crototoxin attains very high concentrations in the kidneys [a fact which has been revealed in experiments with 131I-labelled crototoxin (unpublished data)]. Thus the nephrotoxic factor would play a more important role in crototoxin intoxication than in the envenomation caused by C. d. terrificus venom. This hypothesis explains why the lesions of proximal convolutions are prevalent in crototoxin damaged kidneys.

Shock is the main factor responsible for renal ischaemia, which, in turn, is the cause of the lesions typical of tubulorhexis. Crototoxin was found to be much less potent than the venom in eliciting hypotension and haemoconcentration (3), i.e., the venom is much more active in producing shock. This fact seems to explain why the renal histopathologic picture of the envenomation is more close than that of crototoxin, to the histopathologic picture of the acute renal failure known as lower nephron nephrosis or tubulorhexis.

**Summary**

An investigation of the renal lesions produced by crototoxin was carried out on the kidneys of twenty dogs intravenously injected with this substance. The kidneys were removed for histological section one to nine days after crototoxin administration from animals under pentobarbital anaesthesia or from dogs recently died of crototoxin intoxication.

Early lesions which occurred in kidneys of dogs within the first four days following crototoxin injection, differed somewhat from late lesions observed after the fourth day. In early lesions the renal tubules were less attained than the glomeruli which showed capillary congestion, thickness of the basement membrane, deposit of PAS positive material between the capillary loops and nuclear pyknosis of some glomerular cells. The degenerative lesions of the proximal convolutions reached only some epithelial cells in very restricted areas. Damaged cells in distal convolutions were still less numerous. In late lesions, tubular damage...
predominated, the segment of the tubules most attained being the proximal one. They showed intense microvacuolar degeneration as well as nuclear pycnosis and necrosis of many epithelial cells.

Early or late lesions showed a focal distribution. However, in late lesions those of the proximal convolutions had a tendency to become diffuse through confluence of neighboring injured areas.

The renal histopathological picture of crotoxin intoxication was compared with that presented by kidneys of human beings bitten by the South American rattlesnake (Crotalus durissus terrificus) and of dogs injected with its venom. An hypothesis was formulated to explain the differences observed.

References