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Histopathological changes in avian kidney caused by *Bothrops insularis* (jararaca ilhôa) venom and a phospholipase A_2-containing fraction

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Summary. The histopathological changes induced in avian kidney by the intramuscular injection of *Bothrops insularis* (jararaca ilhôa) venom and its phospholipase A_2 (PLA_2)-containing fraction were examined. Acute experiments (3 h and 24 h) with *B. insularis* crude venom (20 μg and 80 μg) or its PLA_2-contaning fraction (10 μg and 40 μg) resulted in significant structural damage to the kidneys of 5-12-day-old chicks. Histopathological analysis indicated that the venom and its fraction acted on the renal tubules and glomeruli. The morphological changes, although widespread, varied in intensity from cell to cell, and from tubule to tubule in venom-injected chicks. The tubular and glomerular changes produced by the venom and its PLA_2-containing fraction may be the result of a direct cytotoxic effect potentiated by ischemia-related disturbances in the regional hemodynamics. The venom and its fraction affected more segments along reptilian-type nephrons than along mammalian ones. This divergent sensitivity to the venom and its fraction may reflect the species-specific characteristics of *B. insularis* snake, an example of geographical isolation influencing its diet which is almost exclusively avian.

Key words: Avian, Kidney, Histopathology, *B. insularis* snake venom

Introduction

B. insularis is an endemic species of pit viper restricted to Queimada Grande Island, a rocky island 40 miles off the southeastern Brazilian coast (Amaral, 1924). In contrast to continental *Bothrops* species, *B. insularis* feeds mainly on birds (which are abundant on the island) rather than on rodents. As a result this species is much more arboreal than continental members of the genus. *B. insularis* is hermaphroditic with a tendency towards the extinction of males (Amaral, 1918, 1919, 1924).

There is no record of human envenoming by *B. insularis* and little is known about the composition of the venom of this species.

The venom of *Bothrops* snake species frequently cause local necrosis of the skin and muscle at the site of the bite and also produce coagulation disturbances, myolysis, hemolysis, vascular damage and haemorrhage. These actions, together with the systemic release of hypotensive factors and acute renal failure contribute to the severe clinical picture in envenomed persons (Rosenfeld, 1971; Vargaftig et al., 1974; Da Silva et al., 1979a,b; Amaral et al., 1985; Kamiguti et al., 1991).

Cogo et al. (1993) used electrophysiological and myographic techniques to show that avian skeletal muscle was more sensitive to the pre-synaptic and post-synaptic actions of *B. insularis* venom than was mammalian muscle. A fraction which accounts for most of the pharmacological effects of crude venom was purified by a combination of gel filtration on Sephadex G-150 and ion exchange chromatography on DEAE-Sephadex (Cogo et al., 1998). Peak IV of the latter chromatography contained all of the PLA_2 activity of the venom. When injected into the left pectoral muscle of young chicks, both the venom and its PLA_2-containing fraction produced ultrastructural alterations in the muscle fibers and blood vessels (Cruz-Höfling et al., 1992).

In this study, we investigated the pathogenesis of the early renal changes which occur after the intramuscular (i.m.) injection of *B. insularis* crude venom or its PLA_2-containing fraction in young chicks. In particular, since the avian kidney is functionally intermediate between that of mammals and reptiles (Sokabe and Ogawa, 1974) and has both mammalian- and reptilian-type nephrons, we examined whether there was any difference in their sensitivity to *B. insularis* venom.

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Material and methods

Male chicks, 5-12 days old (HY- Line W36 lineage) and weighing 45-50 g, were supplied by Granja Ito S/A (Campinas, SP). The birds were fed standard avian chow and had free access to tapwater. Lyophilysed *B. insularis* venom was provided by the Instituto Butantan (São Paulo, SP). The PLA_2-containing fraction was isolated from the venom by gel filtration on Sephadex G-150 equilibrated with 0.1M phosphate-buffered saline (PBS), pH 7.2. This fraction was further purified by ion exchange chromatography on a DEAE-Sephadex column equilibrated with 0.1M ammonium bicarbonate buffer, pH 8.0 (Cogo et al., 1998).

Venom (20 or 80 μg/0.1 ml Krebs solution) or PLA_2-containing fraction (10 or 40 μg/0.1ml Krebs solution) was injected i.m. into the left pectoral muscle of the chicks. Three and 24 h later, the chicks (n=2 for each time and dose) were anesthetized with sulfuric ether and sacrificed by intracardiac perfusion with 100 ml of 2 % glutaraldehyde in 0.1M phosphate buffer, pH 7.4. The kidneys were removed, sectioned sagitally and immersed either in Bouin's fixative for routine processing and paraffin embedding or in Karnovsky fixative for Araldite 502 (Polysciences) embedding. Paraffin sections (5 μm) stained with hematoxylin-eosin (HE) or Masson's Trichrome (MT) and plastic sections (1 μm) stained with toluidine blue (TB) were examined by light microscopy. Kidneys from control birds injected i.m. with 0.1 μl Krebs solution and sacrificed 3 and 24 h later (n=4) were processed in an identical way.

Results

Clinical and gross observations

The birds injected with 80 μg of venom or 40 μg of PLA_2-containing fraction, showed head-drop and respiratory distress soon after the i.m. injection but were still alive a few hours later, with no behavioural abnormalities. Lower doses of venom and the PLA_2-containing fraction produced milder clinical effects but the birds became prostrate. At all venom and fraction doses there was haemorrhage at the site of injection as well as in the thoracoabdominal cavity (except for 10 μg of PLA_2-containing fraction). The brownish coloured kidneys in the retro peritoneum contrasted to the pale pink colour of the control animals. Some of the chicks injected with venom also showed lung haemorrhage.

Histological findings

Controls

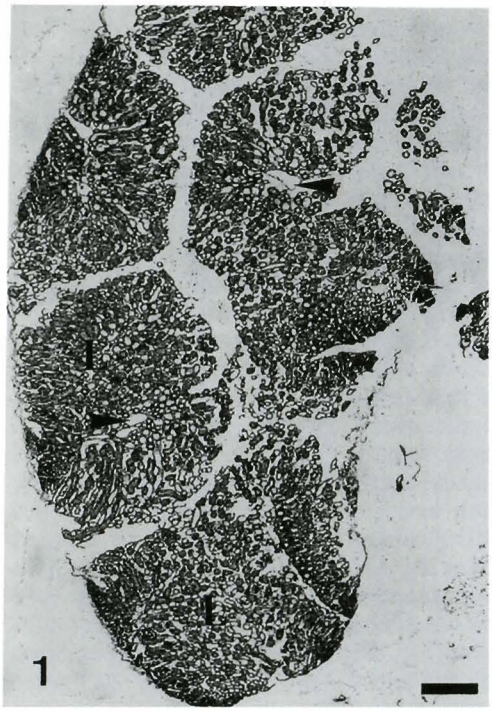
In normal (control) kidneys, the renal parenchyma was divided into lobules with the limits between the outer cortical region and the inner medullary region hardly distinguishable (Figs. 1-3). Indeed, depending on the plane of the section, small islets of medullary tissue were found interposed with the cortical tissue indicating there was an intermingled pattern between cortex and medulla (Figs. 2, 3). Transverse sections showed that well-delimited hexagonal-shaped cortical lobules surrounded a central vein (Fig. 1). Sagittal sections showed that each renal lobule had a mushroom-like appearance composed of a domed-cortex and a medullary cone or renal pyramid, the base of which was located at the corticomedullary limit whereas the tip extended towards the branches of the ureters (Figs. 2, 3). Avian kidneys have two types of nephrons: a reptilian-like type located in the cortex with smaller glomeruli and no loops of Henle and a looped mammalian-type with greater juxtamedullary glomeruli (see Johnson, 1974). Histological identification of the two nephron types is difficult. The marginal clusters of highly basophilic tubules seen in these kidneys (Fig. 3) probably represent embryonic remnants that commonly persist in the avian kidney up to six weeks of age (Riddel, 1987).

There was considerable similarity between the histological characteristics of the nephrons of normal young chicks and those of mammalians. In the cortex, most of the convoluted tubule profiles were proximal tubules. These had a brush-border eosinophilic epithelium whereas distal tubules had a paler colour and smaller cubic cells and diameter (Fig. 4). The renal corpuscles showed a central cluster of mesangial cells around which the glomerular capillary loops overlapped by exuberant podocytes were arranged. A narrow filtration space was interposed between the podocytes and the outer squamous parietal epithelium (Fig. 5). Collecting ducts of different size and loops of Henle belonging to mammalian-type nephrons were observed in the medullary cones (Figs. 3, 6). All of these structures had a normal appearance in the chicks injected with Krebs solution.

Venom (20 and 80 μg)

Within 3 h after the injection of either doses of venom but especially after 24 h, there were autolytic changes in the renal cortical parenchyma, particularly in the proximal convoluted tubules and cortical loops of Henle (Fig. 7). With 20 μg of venom, the distal segments were morphologically unchanged. However, normal-looking nephron segments were interposed among affected ones, indicating that the lesions have a patchy pattern. The changes included separation of proximal tubule epithelial cells from the basement membrane and from each other, apical cytoplasmic blebs, accentuation of apical eosinophilia and multi-vacuolation, in addition to variable degrees of disintegration of segments of the loops of Henle in the renal cortex (Figs. 7, 8). Cellular or amorphous casts were also observed in the lumen of proximal tubules (Fig. 7) which were deep-stained in blue with TB (Fig. 8). By 24 h, the venom caused the proliferation of glomerular mesangial cells and the accumulation of blood cells and/or plasma protein-like amorphous material in the capillary loops. The filtration

space of Bowman's capsule as well as the glomerular capillaries in a number of corpuscles were hardly identifiable (Figs. 8, 9). Capillary ballooning and capillary microaneurysms were seen at this stage and generally appeared as capillary dilatations which progressed to form wide capillary channels (Figs. 8, 10). Cell detachment and luminal hyaline casts, probably proteinaceous in nature, were a common finding in the proximal tubules 24 h after 80 mg of venom (Fig. 10). The distal segments were relatively unaffected by this venom dose after 24 h. The alterations in the medulla were also less prominent than those in the cortex (Fig. 11). Medullary oedema was less evident than in the cortex (Fig. 11). But the collecting ducts of the renal medulla were more sensitive to the venom than the medullary loops of Henle which were also more



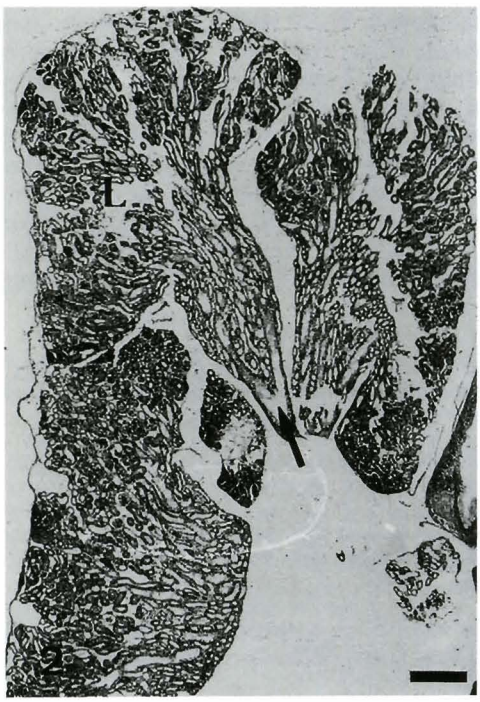
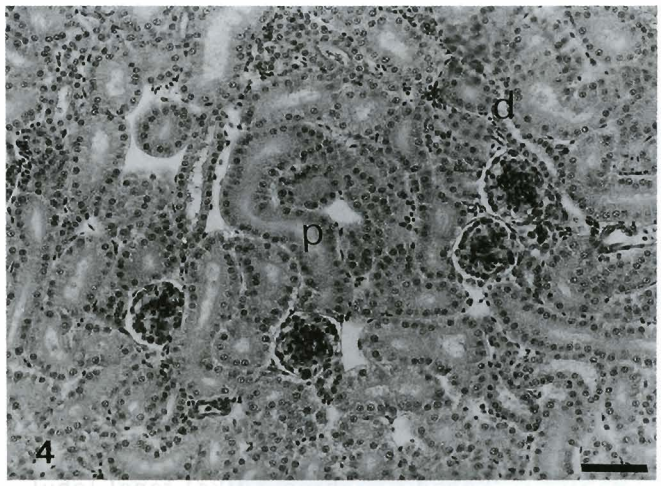


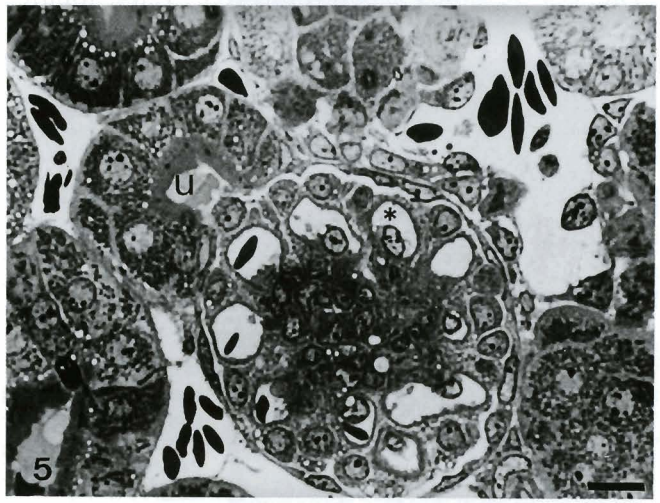
Fig. 1. Histological appearance of kidneys from chiks injected with saline (controls). Paraffin sections, H.E. Cross-section of well-defined renal lobules (L), the nephron components of which are arranged around a central vein (arrowheads). Bar: 200 μm.

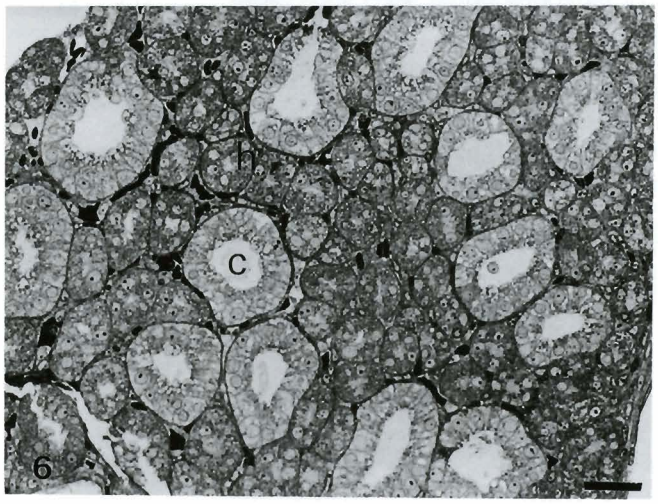
Fig. 2. Histological appearance of kidneys from chiks injected with saline (controls). Paraffin sections, H.E. Longitudinal section of a renal lobule (L) showing a mushroom-like appearance; arrows: two collecting ducts opening into a small calyx. Bar: 100 μm.

Fig. 3. Histological appearance of kidneys from chiks injected with saline (controls). Paraffin sections, H.E. A view of an entire lobule extending from the renal capsule (C) to the papilla (P). co: cortex; m: medulla; b: basophilic tubules (embryonic remnants). Bar: 200 μm.

preserved than the cortical branches (Figs. 7, 8, 11). In addition, a moderate interstitial inflammatory infiltrate (not seen 3 h after 20 μg of venom), and extra vascular blood cells were also observed (Fig. 11). These findings indicate that extent of damage depends on the dose of venom.







PLA-containing fraction (10 and 40 μg)

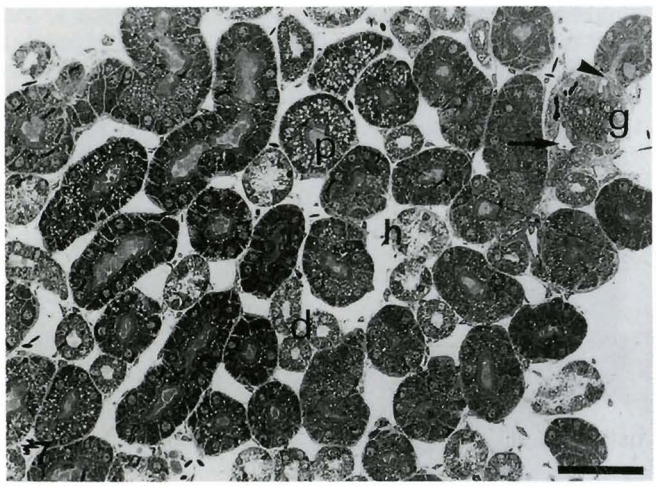
The normal renal architecture was disturbed apparently because of the greater interstitial haemorrhagic effect of the fraction (Fig. 12). The PLA_2-containing fraction caused glomerular compactness leading to structurally ill-defined podocytes and filtration spaces, as well as the presence of capillary thrombi, microaneurysms and mesangial hypercellularity (Figs. 12-14). Marked tubulorhexis affected practically all segments of the nephrons, except distal tubules (Figs. 14-16). Swollen proximal tubules showed various foci of brush-border discontinuities, denuded areas deprived of basement membrane, heterogeneous staining in the cells of the same tubule, luminal cell debris (Figs. 13-16), vacuolation and cell shedding (Figs. 14, 15). In the cortex, the changes in the distal cells were mild compared to the cells of the proximal tubule and cortical loops of Henle (Fig. 13). Cortical portions of the loops of Henle were lined by a discontinuous epithelium with damaged dark cells and cell debris in the lumen (Figs. 13, 14), but the thin medullary branches showed little or no histological alterations (Fig. 16), a finding similar to that for the whole venom. The collecting ducts showed disintegrating apical cytoplasm, intercalating dark cells, disruption of the basal cell membrane and nuclei and cells either obliterating the duct's lumen or located abnormally within the duct lining (Fig. 16). The usually well-defined cell membranes of collecting ducts (see controls, Fig. 6) were barely-visible after treatment with the PLA_2 fraction (Fig. 16). The extent of the alterations produced by the fraction did not differ 3 h or 24 h after either of the doses, suggesting a rapid onset of damage to the renal parenchyma. These results also indicate that in the doses used the PLA_2-containing fraction was more potent in inducing vasculotoxic effects than the whole venom. As with the venom, the severity of the effects was proportional to the dose used. In contrast to the venom, interstitial edema was more visible in the medulla with the PLA_2-containing fraction. However, haemorrhaging was extensive in the vicinities of the ureter branches at the tip of the medullary cones (Fig. 17), and elsewhere (Figs. 12, 13). The PLA_2-containing

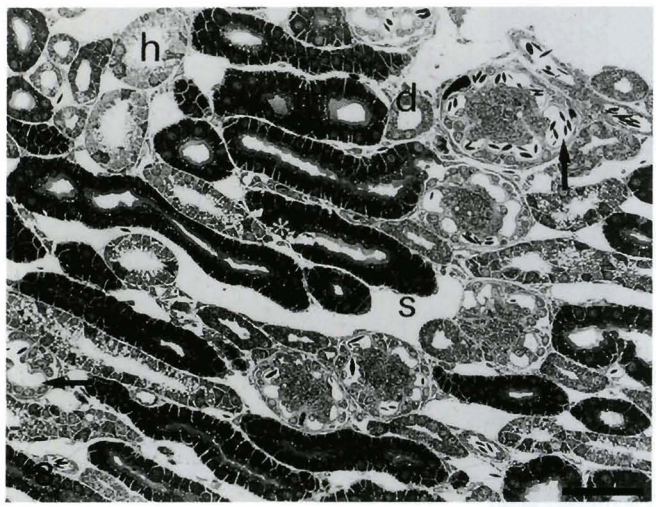
Fig. 4. Saline-injected chicks (controls). Paraffin-embedded section of the renal cortex showing four glomeruli interspersed among proximal (p) and distal (d) tubules. H.E. Bar: 50 μm.

Fig. 5. Saline-injected chicks (controls). A renal corpuscle sectioned at the urinary pole (u) where the proximal tubule emerges. Note the Bowman's capsule with its parietal layer and the visceral layer with the peculiar arrangement of podocytes (arrowheads) encircling the glomerular capillary loops (*). Intraglomerular mesangial cells are present in the middle. Araldite section, TB. Bar: 10 μm.

Fig. 6. Saline-injected chicks (controls). Normal appearance of the outer strip of the medullary zone. C: collecting ducts; h: medullary limbs of the loops of Henle. Bar: 25 μm.

fraction caused marked cell desquamation which resulted in discontinuities of the prismatic epithelium lining the ureters (Fig. 18). Necrotic cell debris, hematic casts and proteinaceous material were also detected in the lumen of the ureters after treatment with the PLA_2-containing fraction (Fig. 18). Similar effects, but less pronounced, were observed in the ureters 3 h after 80 μg of venom (Fig. 19).





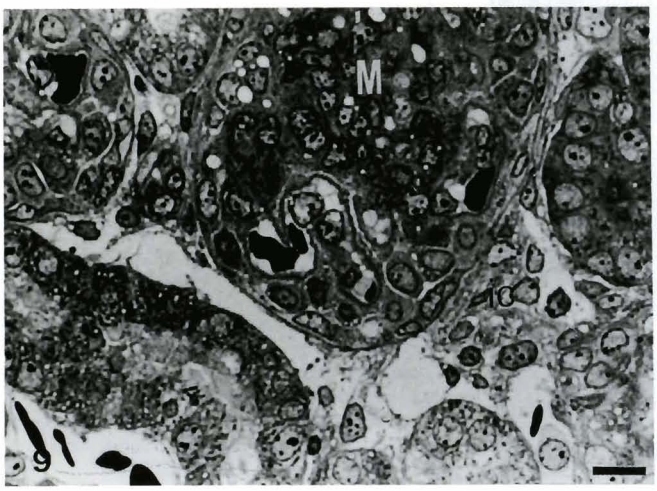


Fig. 7. Morphological alterations in the kidneys of chicks injected with *B. insularis* venom. Araldite-embedded sections, TB. Three hours after 20 μg. Note the interstitial edema, the vacuolated cells in the proximal (p) tubule, the desintegrating loops of Henle (h) and the preservation of distal (d) tubules. g: glomerulus showing the urinary (arrowhead) and the vascular (arrow) poles. Bar: 50 μm.

Fig. 8. Morphological alterations in the kidneys of chicks injected with *B. insularis* venom. Araldite-embedded sections, TB. Twenty four hours after 20 μg. A survey of the cortical region showing very dark proximal tubules, one of them ruptured (*), as well as normal-looking distal (d) tubules, loops of Henle (h) with heterogeneous cell types and edema-induced interstitial spaces (s). Note also the capillary microaneurysms (arrows) and the hypercellular core of the glomeruli. Bar: 50 μm.

Fig. 9. Morphological alterations in the kidneys of chicks injected with *B. insularis* venom. Araldite-embedded sections, TB. A renal corpuscle with a very proliferative mesangium (M), absence of capillary loops and capsular space and topographical distortion of podocytes. Note also the interstitial hypercellularity (ic). Bar: 10 μm.

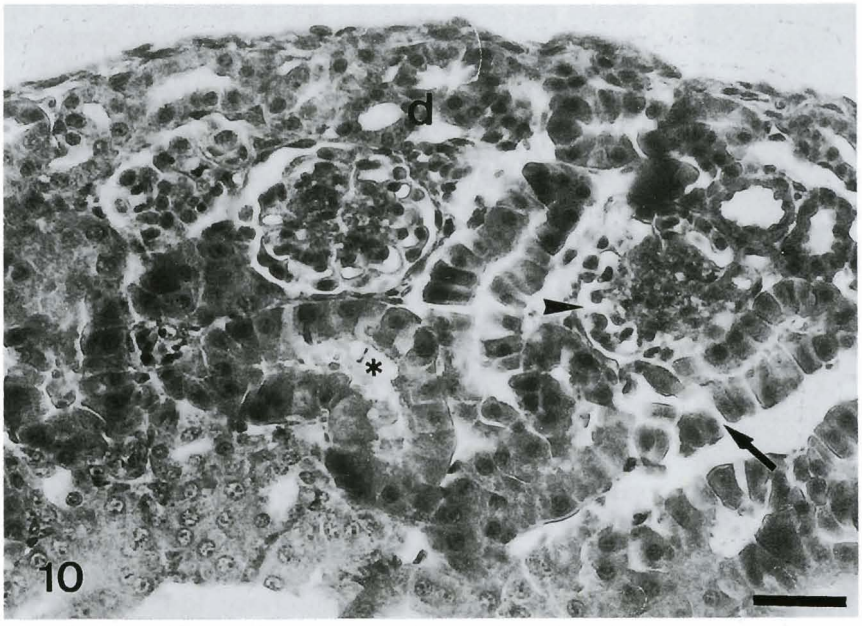
Discussion

Renal complications have important consequences on the clinical course of envenoming following snakebite in humans (Amorim and Melo, 1954; Chugh et al., 1975; Aung-Khin, 1978; Da Silva et al., 1979a,b; Date and Chastry, 1982; Amaral et al., 1985; Burdmann et al., 1993).

The i.m. injection of *B. insularis* venom produced time- and dose-dependent changes in the proximal tubules and loops of Henle and in the corpuscles of the renal cortex but distal tubules were preserved. Medullary components, mainly the limbs of the loops of Henle, were also relatively unaffected. Johnson and Skadhauge (1975) proposed that the greater the number of medullary cones in avian kidneys the greater the fraction of mammalian-(looped) type nephrons and the lower the number of reptilian (non-looped) nephrons. This organization is linked to effective water conservation. The dome-shaped (mushroom-like) renal lobules described above indicate that the kidney of young chicks has few medullary cones but a large amount of cortex associated with each cone, indicating that reptilian-type nephrons greatly outnumber mammalian-type ones. The low proportion of kidney of mammalian-type nephrons suggests that the kidney of young chicks may be inefficient at conserving water, or that this kidney is not suited to dry habitats. Johnson and Skadhauge (1975) also pointed out that the relative thickness of the renal medulla is one of the osmoregulatory factors involved in the ability of the avian kidney to concentrate urine.

In this work, the medullary loops of Henle (belonging to mammalian-type nephrons) were more resistant to the toxic effects of the venom, than the collecting ducts. Similarly, the cortical loops of Henle (of the reptilian- or mammalian-type nephrons) were

more damaged than the medullary loops of Henle. We hypothesize that the cortical loop branches of the mammalian-type nephrons were less affected than the corresponding reptilian-type ones, in a manner similar to the medullary counterpart. Such a distinct intensity of effect of *B. insularis* venom was observed by Cogo et al. (1993) in skeletal muscle depending on the species. The authors observed that mammalian muscle of mice is less sensitive to the pre- and post-synaptic effects of *B. insularis* venom than the avian muscle (young chicks). Similar results were obtained with the PLA_2-containing fraction of the venom that shows that avian preparation was more sensitive to the fraction than was the mammalian one, with the blockade induced being both dose- and time-dependent (Cogo et al., 1998). In the current work, a preponderance of effects would be directed to the reptilian-type nephrons, independent of their cortical or medullary localisation. This would partially explain why the tubular alterations caused by the venom was generally patchy. An explanation for that would be the presence of loops of Henle belonging to the reptilian-type nephrons in the cortex. As for proximal and distal segments of both type of nephrons which would also respond differently to the venom. The two types of nephrons perform distinct functional roles and variations in tubular ion transport in the birds (Johnson,



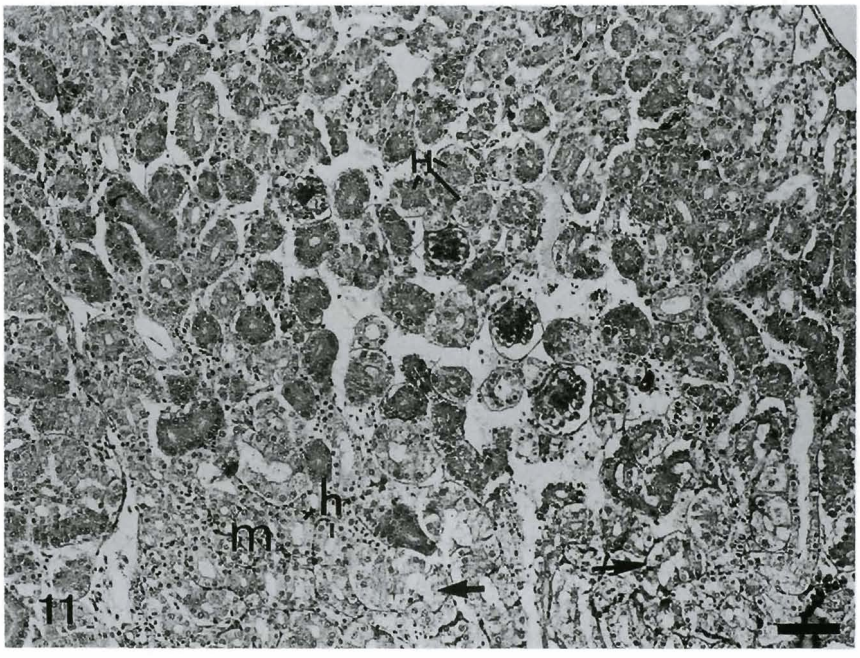


Fig. 10. Morphological alterations in the renal cortex 24 h after 80 μg of *B. insularis* venom. Paraffin sections, HE. Cortex zone showing proximal tubules with cell detachment (arrows) and hyaline casts (*) obliterating the lumen. Note the compactness of the renal corpuscles along with glomerular capillary microaneurysms (arrowhead). Bar: 50 μm.

Fig. 11. Morphological alterations in the renal cortex 24 h after 80 μg of *B. insularis* venom. Paraffin sections, HE. Note the marked cortical interstitial edema and inflammatory infiltrate, and the tubular degeneration and compactness of the mesangium associated with an enlargement of the renal corpuscles. m: medulla region; arrow: degenerated collecting ducts; h: medullary Henle limbs; H: cortical damaged Henle limbs. Bar: 50 μm.

1974).

The PLA_2-containing fraction of the venom produced similar effects 3 h and 24 h after a dose of 10 μg. The first changes noted involved the renal circulation. The PLA_2 fraction apparently produced extensive thrombus deposition in the glomeruli and leads to the accumulation of blood cells in the renal interstitium. Although the hemorrhaging caused by the venom was not so extensive as that seen with the PLA_2 fraction the changes affecting the nephrons in both cases were similar. Both the venom and PLA_2 fraction produced glomerular capillary ballooning and microaneurysms. The formation of microaneurysms in response to habu venom (*Trimeresurus flavoviridis*) initiates with mesangiolysis, e.g. failure of the system supporting the capillary tuft (Uiker and Kriz, 1995). This is followed by distension of the capillaries which subsequently fuse with each other, leading to glomerular microaneurysm. This process obviously involves concomitant glomerular endothelial defects. Similar lesions have been reported after envenoming by *Crotalus* (Pearce, 1909, 1913) and *Agkistrodon* (Sakurai et al., 1986) snake venoms, and also occur in experimental and human glomerulopathies (Uiker and Kriz, 1995).

Infarctation is not normally seen in the avian kidney, perhaps because of the dual blood supply to the tubules

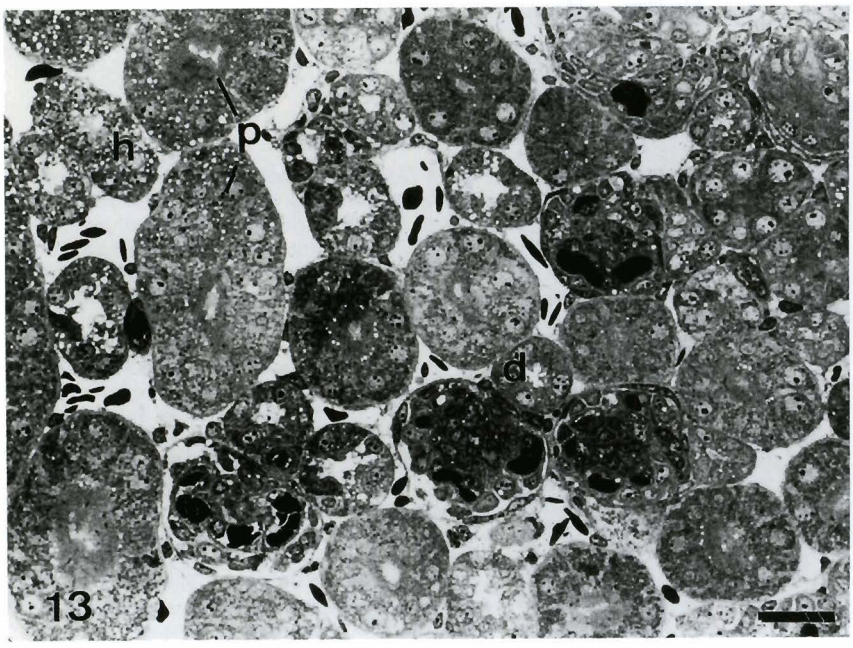


Fig. 12. The morphological appearance of kidneys from chicks 3 h after injection with the PLA_2-containing fraction. Paraffin section, HE. An overview of the cortex showing congested renal corpuscles, hemorrhage (H) and abundant inflammatory infiltrate (I). Bar: 100 μm.

Fig. 13. The morphological appearance of kidneys from chicks 24 h after injection with the PLA_2-containing fraction. Araldite section, TB. Hypercellularity of five congested renal glomeruli. Note the edematous tubular cells and the different degrees of tubular degeneration of loops of Henle (h) and proximal tubules (p). d: normal-looking distal tubules. Bar: 20 μm.

(see Riddel, 1987). Blood will reach the tubules from the renal portal circulation, even if the arterial blood supply is obstructed at or before a renal glomerulus. The alterations produced by *B. insularis* venom and its PLA_2-containing fraction extended to the glomerular structure. There was also evidence of mesangial proliferation 24 h after 80 μg of venom, along with congestion of the capillary loops and these glomeruli disappearance from Bowman' space. If a circulatory detour is in fact provided via the portal circulation, the additional tubular changes seen with lower doses of venom or PLA_2 fraction most probably did not result from glomerular circulatory collapse, i.e., glomerular ischemia could be excluded as a cause of further tubular damage. In this case, the renal tubule alterations caused by the venom or PLA_2-containing fraction may be secondary to anoxic effects or may be the result of a direct nephrotoxic action, or both. The electrophoretic profile of the PLA_2-containing fraction of *B. insularis* venom (F-IV) showed four polypeptide chains all with molecular masses of 14 – 17 kDa (Cogo et al., 1998), which seems to exclude the presence of hemorrhagic toxins in the fraction, known to have higher molecular masses in the order of 25 to 65 kDa (Borkow et al., 1993; Gutiérrez et al., 1995; Sánchez et al., 1995). However, the PLA_2-containing fraction of *B. insularis*

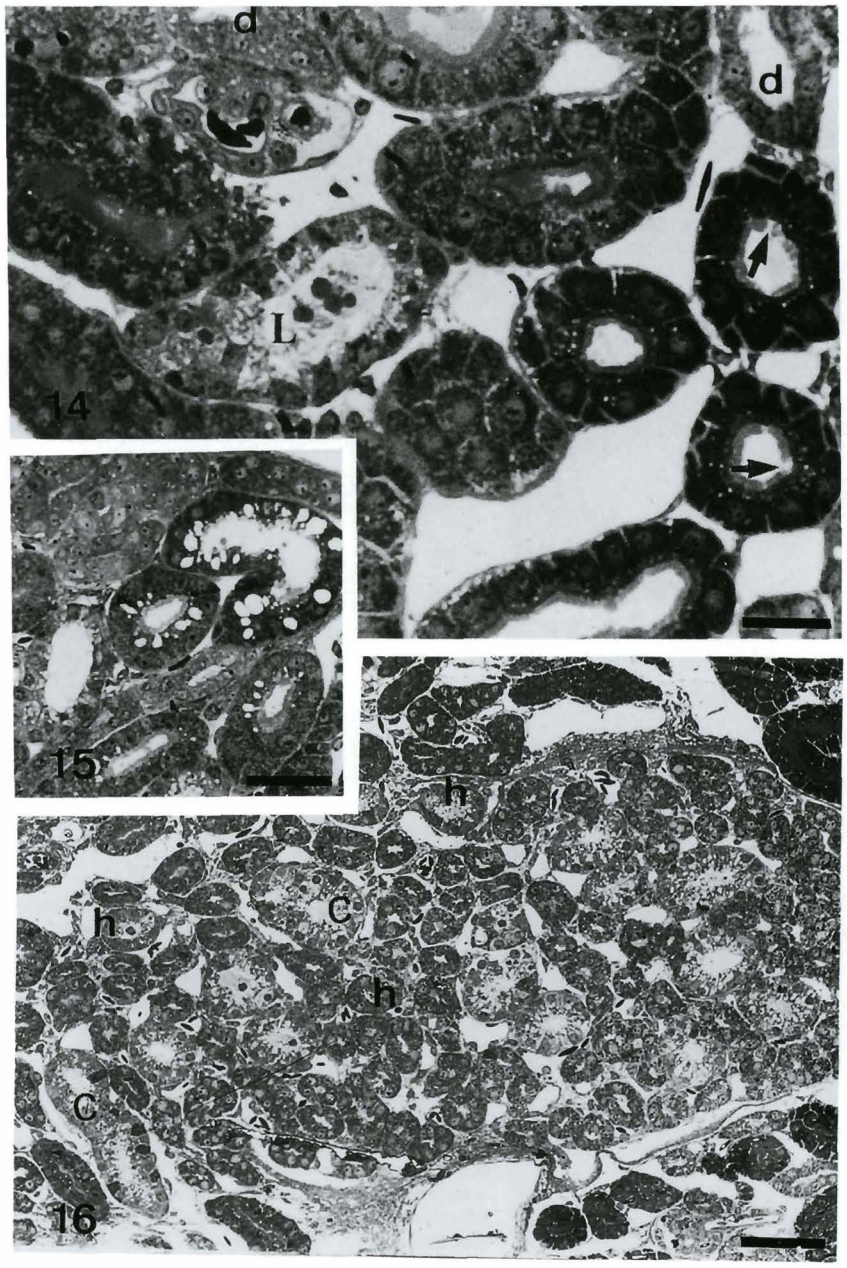


Fig. 14. Details of the cortical and medullar alterations caused by the PLA_2-containing fraction. Araldite sections, TB. Twenty-four hours after 40 μg showing very dark proximal tubules with brush border discontinuities (arrows) and two highly degenerated tubules (loops of Henle) with luminal casts and wall disintegration (L). d: normal distal tubules. Bar: 20 μm.

Fig. 15. Details of the cortical and medullar alterations caused by the PLA_2-containing fraction. Araldite sections, TB. Twenty-four hours after 10 μg. Note the large apical vacuoles causing loss of the brush border of the proximal convoluted tubules. Bar: 30 μm.

Fig. 16. Details of the cortical and medullar alterations caused by the PLA_2-containing fraction. Araldite sections, TB. Three hours after 10 μg. Cross-sections of well-preserved loops of Henle (h), and of degenerated collecting ducts (C), some of them with cell remnants in their lumen. Bar: 20 μm.

venom produced marked haemorrhage in the renal parenchyma of young chicks. This effect probably might be caused by desestabilization of the membrane phospholipids in response to the PLA_2 activity. This effect would lead to the weakening of the endothelium, and extravasation of blood cells causing hypotension and the impairment of glomerular filtration, thus contributing to physiopathological changes in renal function. A decrease in blood pressure and in fractional sodium excretion associated with histopathological changes have been reported for rats treated with *Bothrops* jararaca snake venom (Rezende et al., 1989).

Other tubule alterations seen in the present investigation, such as tubule cell detachment, loss of apical brush border, formation of apical cytoplasmic blebs, or the appearance of clear vacuoles, cell swelling and the presence of hyaline casts and amorphous proteinaceous masses in the lumen of tubules (and ureters) are common signs of renal injury and dysfunction, particularly of acute tubular necrosis (see Racusen, 1992). The physiological sequelae of such structural changes include increased leakage of fluid across the walls of damaged nephrons, tubular obstruction, decreased glomerular permeability and a decrease in renal flow. The venom also caused renal cortical interstitial edema which probably contributed both to the tubular and glomerular damage. The interstitial edema of the medulla may have contributed to the damage in collecting ducts.

Bothrops snake venoms have predominantly proteolytic, coagulant and hemorrhagic activities (Furtado et al., 1991). The venom of *B. insularis* contains fibrinolytic and coagulant activities which are higher than in other species of *Bothrops* (Mebs, 1970). Fraction I-SII-R of the venom contains a thrombin-like activity capable of hydrolysing the α- and ß-chains of fibrinogen (Selistre and Giglio, 1987), thus giving rise to fibrin deposits (Seegers and Ouyang, 1978).

Aung-Khin (1978) reported that the renal failure seen after envenoming by *Vipera russelli*, the venom of

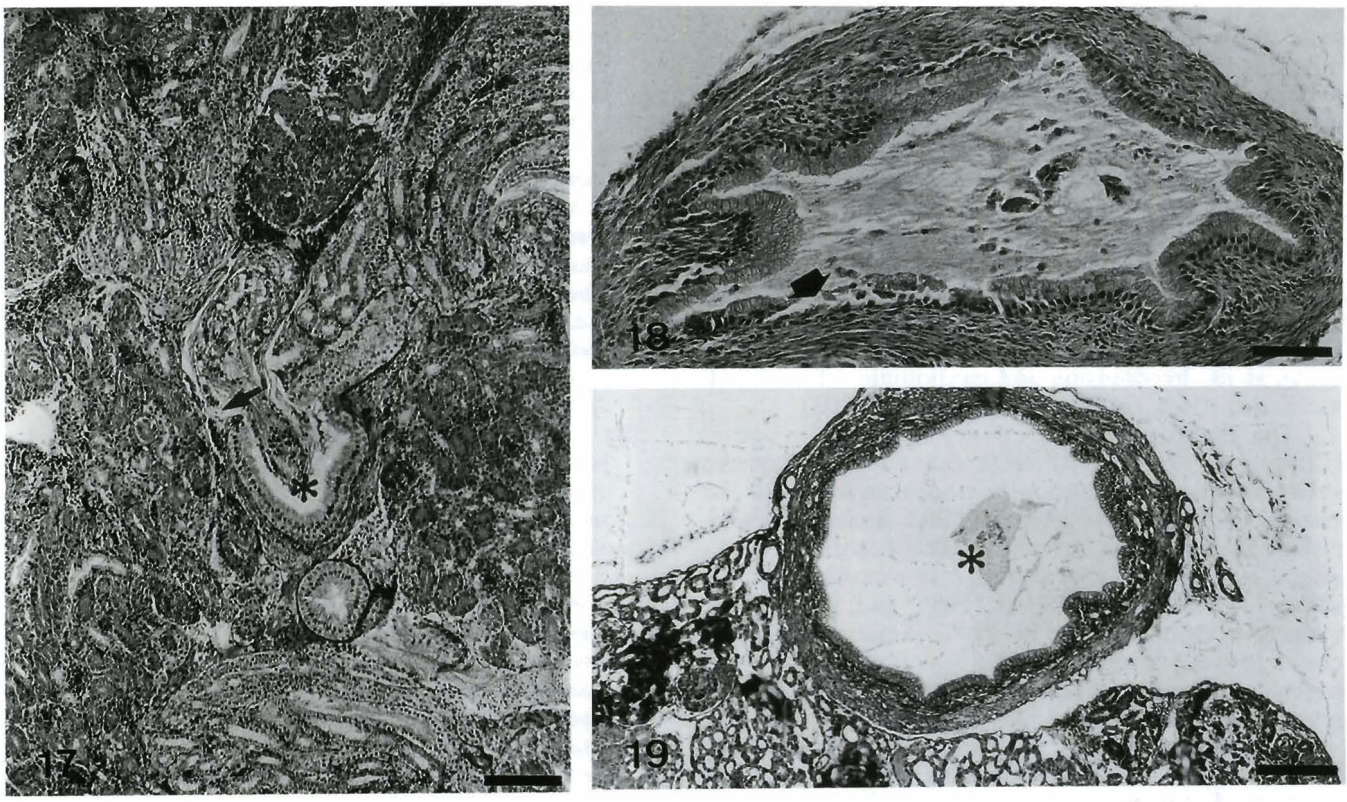


Fig. 17. Morphological alterations in the excretory portions of the kidney and ureter after the injection of *B. insularis* venom or a PLA_2-containing fraction. Paraffin sections, TM. Three hours after 40 μg of PLA_2 fraction showing intense hemorrhaging in the medullary zone. Note the cluster of detaching cells bulging into the calyx in the centre of the micrograph (*) rupture of the epithelium (arrow). Bar: 100 μm.

Fig. 18. Morphological alterations in the excretory portions of the kidney and ureter after the injection of *B. insularis* venom or a PLA_2-containing fraction. Paraffin sections, TM. Twenty four hours after 10 μg of PLA_2 fraction showing rupture of the epithelium lining the ureter (thick arrow) and the presence of detached cells and exudate in the lumen. Bar: 50 μm.

Fig. 19. Morphological alterations in the excretory portions of the kidney and ureter after the injection of *B. insularis* venom or a PLA_2-containing fraction. Paraffin sections, TM. Three hours after 80 μg of *B. insularis* venom. Note the comparatively better-preserved ureter epithelium. Some free cells in the lumen (*). Bar: 100 μm.

which has a strong coagulant effect, was attributable to the obstruction of glomerular capillaries by coagulated material which caused ischaemia-induced tubular necrosis and further renal collapse. Recently, it was shown that a single intravenous injection (0.4 mg/kg) of *B. moojeni* snake venom in rats caused a marked decrease in the glomerular filtration rate and a sustained rise in tubule sodium excretion which peaked 5 h and 16 h after venom administration (Boer-Lima et al., 1999). There was no significant change in arterial blood pressure. These physiological changes paralleled the morphological changes in the glomeruli and nephron tubules (Boer-Lima et al., 1999).

B. insularis venom contains bradykinin-potentiating factors (Cintra et al., 1984). In addition, five edema-inducing toxins, one of them myotoxic and three of them hemorrhagic, were described by Selistre et al. (1990). This venom also possesses PLA_2 activity (Cogo et al., 1998) capable of increasing the release of creatine kinase (CK) from chick biventer cervicis preparations (Cogo et al., 1993). CK has been used as a marker of membrane rupture in numerous pathological situations. PLA_2 hydrolyses membrane phospholipids and causes the tubule and glomeruler membranes to become leaky. In addition, alteration of the phospholipid composition can cause membrane dysfunction. When the cell membrane is injured, it loses its function as a barrier to calcium influx, with the result that the intracellular Ca^{2+} exceeds the normal physiological concentration, supposedly triggering calcium-dependent cell-changes and eventually cell death. PLA_2 induces a broad-spectrum of physiological alterations, including neurotoxicity, myotoxicity, the induction and/or inhibition of platelet aggregation, and hemolysis, edema, coagulopathy, convulsions, hypotension and cardiotoxic effects (Kini and Evans, 1989; Gutiérrez and Lomonte, 1995).

The combination of edema induced-ischemia (Chapman, 1968), the effects caused by vasoactive substances such as histamine and bradykinin in the venom, the direct myonecrotic effect, hemoglobinuria, myoglobinuria and/or hematuria, fibrin deposition, PLA_2 effects on mitochondrial structure and electron transfer in cardiac muscle (Vidal et al., 1966) and, possibly the membrane rupture caused by PLA_2 (Cogo et al., 1993) could provide stimuli for the development of renal lesions. Although a multi-factorial mechanism, may be involved in the renal changes described here, specific interactions between *B. insularis* venom or its PLA_2 fraction and renal tissue cannot be excluded perhaps involving differentiate effects on mammalian or reptilian nephron segments.

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