KIDNEY, MUSCLE AND VASCULAR STRUCTURAL AND ULTRASTRUCTURAL DAMAGES PRODUCED BY TOXIC AGGRESSION OF BEE (*Apis mellifera*) VENOM

Daños Estructurales y Ultraestructurales en Riñón, Músculo y Vasos de Ratón Producido por la Agresión Tóxica del Veneno de Abeja (*Apis mellifera*)

***Gladys Rojas*[1], *Alexis Rodríguez-Acosta*[1], *Héctor J. Finol* [2], *Ghislaine Céspedes*[3] *and Alipio Hernández*[3]**

[1] Section of Immunochemistry, Tropical Medicine Institute. Apdo. 47423. Caracas 1041, Venezuela.
[2]Centre for Electron Microscopy of the Science Faculty. [3] Institute of Pathological Anatomy, Universidad Central de Venezuela, Apdo. 47423. Caracas 1041, Venezuela. C-electrónico: rodriguf@camelot.rect.ucv.ve

ABSTRACT

Bee (*Apis mellifera*) venom produces allergic local reaction and systemic reactions such as acute renal failure. The pathogenic mechanisms occurring in these accidents are still unclear. In this work the LD_{50} bee venom activity on kidney, skeletal muscle and renal vasculature of mice inoculated at 5, 10, 24 and 48 hours was studied. Necropsy specimens were obtained from kidney and sartorial muscles for light microscopy and electron microscopy studies. After 24 hours of exposure there was no evidence of structural changes on the kidney under light microscopy. Nevertheless, muscular tissue revealed lesions characterised by necrosis, phagocytosis and associated focal leukocyte inflammatory infiltration. In addition, mild regenerative activity and increase of the intermyofibrilar spaces in other muscular fibres were observed. From 5 to 48 hours there were ultrastructural changes on the kidney tissue. Showing early cellular swelling, non-uniform enlarging of the parietal or visceral glomerular membrane, tubular cell vacuolisation, capillary wall enlargment, haemoglobin presence in the lumen of the convoluted proximal tubules with later damage of sarcolemmal membrane. Changes in the muscular fibres were characterised by the presence of irregular and hyperchromatic nuclei and the sarcoplasma, augmented endothelial cytoplasm with prolongation toward the capillary lumen, muscular atrophy and partial loss of the sarcomeric striation. Besides to the increase of the intermyofibrillar spaces, prominent triads and pleomorphic mitochondria were evidenced. The subsarcolemmic nuclei were rounded and the nucleoli with diminished electronic density. The subsarcolemmic space was uncover, with abundant polysomes and mitochondria. An increase of the intermyofibrillar spaces, suggesting atrophy, was observed. The mitochondria were pleomorphic and electron dense. In some areas there was neither line M nor band H. Necrosis in muscle fibres was also observed, with the loss of the plasmatic membrane and cellular residues. The capillary endothelium of kidney vessels was altered with increment of the fenestrae. The capillary wall was augmented, with prolongation toward the lumen of the cytoplasm. These changes may be due to interactive effects of the multifactorial action of bee venom components (Phospholipase A2, mellitin, apamin and histamine).

Key words: *Apis mellifera*, bee, structure, ultrastructural damage, venom.

RESUMEN

El veneno de la abeja (Apis mellifera) produce reacciones alérgicas locales y sistémicas. Tal como fallo renal agudo. Los mecanismos patogénicos que se desarrollan en estos accidentes permanecen casi desconocidos. En este trabajo se estudió la actividad del veneno de abejas a dosis LD50 sobre el riñón, el músculo esquelético y los vasos renales de ratones a las 5, 10, 24 y 48 horas. Se obtuvieron necropsias de riñón y músculo sartorio, para estudios de microscopía de luz y electrónica. A las 24 horas de exposición, en microscopía de luz, no se observaron cambios estructurales en riñón. Sin embargo, el tejido muscular reveló lesiones caracterizadas por necrosis, y fagocitosis asociada a infiltración inflamatoria de leucocitos polimorfonucleares. Además, un ligero aumento de los espacios intermiofibrilares y actividad regenerativa fue observada. Desde las 5 hasta las 48 horas usando microscopía electrónica, las alteraciones en el riñón comprendieron tumefacción celular temprana. Engrosamiento no uniforme de la membrana parietal glomerular, vacuolización tubular, agrandamiento de la pa-

Recibido: 03 / 09 / 2001. Aceptado: 12 /12 / 2001.

red capilar, presencia de hemoglobina en las células del túbulo contorneado proximal, con daño de la membrana sarcolémica. Los cambios ultraestructurales en la fibra muscular estaban caracterizados por la presencia de núcleos hipercromáticos irregulares, citoplasma endotelial aumentado con prolongaciones hacia el lumen capilar; atrofia muscular y pérdida parcial de las estriaciones sarcoméricas. Además del incremento de los espacios intermiofibrilares, se observaron mitocondrias pleomórficas y electrón densas con tríadas prominentes. Algunos núcleos subsarcolémicos eran redondeados y los nucleolos alterados. El espacio subsarcolémico estaba descubierto, con abundantes polisomas y mitocondrias. Un incremento de los espacios intermiofibrilares, sugerían atrofia volumétrica. En algunas áreas no se observaron líneas M ni bandas H. También se observó necrosis con pérdida de las membranas plasmáticas y residuos celulares. El endotelio capilar estaba alterado con un incremento de las fenestraciones. La pared capilar se encontraba aumentada, con prolongaciones hacia el lumen del citoplasma. Estos cambios fueron atribuidos a los efectos interactivos de la actividad multifactorial de los componentes del veneno de abeja (fosfolipasa A2, melitina, apamina e histamina).

Palabras clave: *Apis mellifera*, abeja, estructura, daño ultraestructural, veneno.

INTRODUCTION

The bee (*Apis mellifera*) venom is a mixture of peptides, proteins, amines, sugars and volatile components whose action in mammals following a bee attack leads to direct envenomation and/or hyperactivity of the immune system [20].

Regarding *A. mellifera* venom systematic activity, an acute renal failure characterised by acute tubular necrosis, has been reported [7]. However, while direct toxic damage to the tubular structures caused by bee venom has been clearly demonstrated, as far as it is known this is not the case for direct toxicity of the glomerules. In apian studies the pathogenic mechanisms producing acute tubular necrosis are still relatively unknown, although myoglobin and/or haemoglobin toxic action on the tubular cells has been suggested [6]. The studies in animals suggest that the acute renal failure induced by these pigments is predominantly due to intratubular obstruction, mainly by the precipitation of heme pigments (myoglobin and haemoglobin) [4]. It has been demonstrated that bee venom also produces severe muscle tissue lesions [15].

In this work, the systemic envenomation effects on mice kidney, skeletal muscle and vasculature caused by bee venom are reported.

MATERIALS AND METHODS

Venom

A. mellifera venom was purchased from Venom Supplies Pty Ltd, Australia and stored at –70°C until used.

Lethal dose fifty (LD_{50})

The LD_{50} values of *A. mellifera* venom and venom fractions were determined by intraperitoneal injection of mice (18-22 g) and calculated according to the SPEARMAN-KÄRBER method [17]. The number of mice used at each dose level was five.

Animals

Five adult males C57/Bl (18-22 g) were intraperitoneally injected with a lethal dose fifty (LD_{50}) of *A. mellifera* venom (5µg/20g). Five controls received 0.1mL saline soution. Mice were maintained at room temperature for 48 hours with food and water *ad libitum*.

Samples from animals injected with bee venom were obtained from each experimental group and selected according to the previously established times of 5, 10, 24 and 48 h. Animals were killed by neck dislocation. A group of necropsy specimens of kidneys and sartorial muscles obtained from animals injected with crude venom were prepared for light microscopy. They were fixed in buffered formaldehyde solution overnight and then washed in three changes of 70% ethanol. The fixed tissues were then embedded in paraffin blocks and 3 µm sections were taken, mounted with Permount® on glass slides and stained with haematoxilin-eosin for microscopy observation [1].

Preparation of specimens for electron microscopy [13]

Sartorial muscle and kidney biopsies were obtained from mice under anaesthesia (sodium thiopental,25 mg/Kg). Samples were immediately *in situ* fixed with 3% glutaraldehyde and 1% OsO4 (both fixatives diluted in pH 7.4, 320 mM phosphate buffer saline), dehydrated in ethanol and embedded in LX-112 resin (Ladd Research Inc.). Ultrathin sections were stained with uranyl acetate and lead citrate and observed in Hitachi H-300 and H-500 transmission electron microscopes with an accelerating voltage of 100 kV. The investigation complies with the norms taken from the guide “Principles of laboratory animal care” [2].

RESULTS

Light microscopy studies at 24 hours from experimental group of mice inoculated with *A. mellifera* venom and controls did not show alterations in the kidney. But, lesions in muscular tissue characterised by necrosis, phagocytosis, mild regenerative activity and increase of the intermyofibrilar spaces were observed FIG.1A. In addition, associated focal polymorphonuclear leukocyte inflammatory infiltration was noticed FIG. 1B Zenker necrosis was evidenced by coagulation of sarcoplasm and pycnosis of nuclei FIG.1B.

At 5, 10, 24 and 48 hours under transmission electron microscopy, renal and muscular tissue from control groups did not show pathological changes. But, mice from inoculated group with *A. mellifera* venom at 5 hours showed muscular atrophy, partial loss of the sarcomeric striation and increase of the intermyofibrillar spaces, prominent triads and pleomorphic

mitochondria FIG.2. Intramuscular capillaries showed hyperchromatic and identated nuclei and widened endothelial cytoplasm with unfolding into the capillary lumen FIG.3.

At 5 hours mice inoculated with *A. mellifera* venom showed at glomerular level a capillary endothelium alteration with the fenestrae widened. The tubular cells cytoplasm showed changes, the nucleus showed a diminished electronic density of the heterochromatin. Podocytes width was not uniform FIG.4. In the apical cellular region tubule lumen dissapered. Some epithelial cells were swollen with the presence of mitochondria. Lipofucsin granules and large vacuoles were noted FIG.5.

At 10 hours, such as it was observed at 5 hours, an increase of the intermyofibrillar spaces in the muscle with pleomorphic mitochondria was observed, suggesting atrophy. Some areas of segmented atrophy were observed FIG.6.

In kidney, vacuolisation of epithelial cells next to the mitochondria and decrease or loss of microvillies in the apical area were seen FIG.7. Increase of the areas between the interdigitated spaces at basal tubular region was observed. The endothelial wall lost its integrity FIG.8.

At 24 hours, capillary fenestrae and basal membrane width look normal. However, Bowman wall appeared degenerated. The outside basal glomerular membrane look widened. The tubular epithelial cells basal area showed a decrease of interdigitations and irregular mitochondria disposition FIG.9. In the tubular cells a loss of the interdigitacions and free polysomes were observed. An autophagic vacuole was observed. The capillary endothelial cell cytoplasm endoplasmic reticulum was oedematous and enlarged. In some areas, capillary wall was lost. The endothelial cytoplasm showed augmented areas and loss areas, disarrangement of the epithelial cells FIG.10. At 48 hours, the changes in muscle and kidney described above remained.

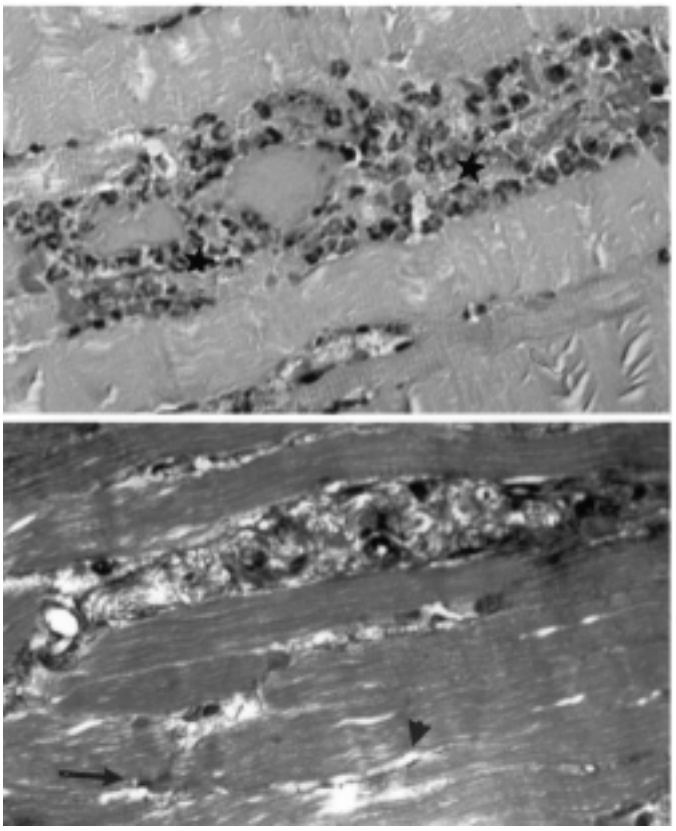

FIGURA. 1a and FIGURA. 1b: IN THIS MUSCLE TISSUE LIGHT MICROSCOPE MICROGRAPH (1A), (ARROW) NECROSIS AND INCREASED INTERMYOFIBRILLAR SPACES (ARROWHEAD) X 120. (1B), FOCAL LEUKOCYTE INFLAMMATORY INFILTRATION (STARS) X 120.

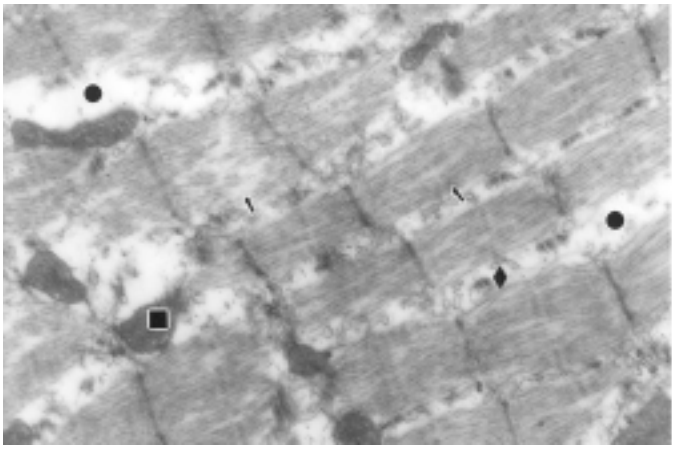

FIGURA 2. IN THIS MUSCLE TISSUE ELECTRON MICROGRAPH, (CIRCLES) WIDENED INTERMYOFIBRILLAR SPACE, (ARROWS) PARTIAL LOSS OF SARCOMERIC STRIATION, H BAND AND M LINE LOST, (RHOMBUS) PROMINENT TRIADS, (SQUARE) PLEOMORPHIC MITOCHONDRIA. X 45,000.

DISCUSSION

A. mellifera venom is constituted by phospholipase A2, hyaluronidase and acid phosphatase proteins and peptides such as mellitin, apamin and the mastocyte degranulating peptide [11]. Other authors [5] reported widespread rhabdomyolysis in massive envenomation of human patients stung repeatedly by the bee *A. mellifera*. Myotoxicity is one of the most perceptible effects induced by the venom of *Apis mellifera* [3]. It is clear that the myotoxic activity of bee venom is induced by more than one constituent [7], but the most important myotoxins existing in bee venom are phospholipase A_2 and mellitin which produce skeletal muscle damage [15]. The findings in the current work with bee venom, the major type of muscle fibre necrosis began with cells having delta lesions followed by the presence of atrophied and densely clumped fibres as well as areas of segmental necrosis. This kind of myonecrosis in-

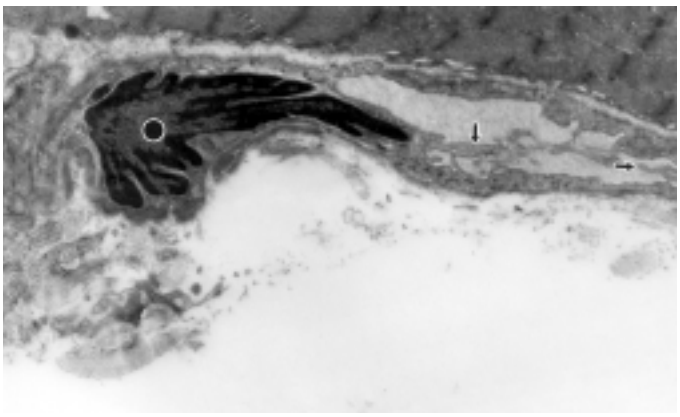

FIGURA 3. IN THIS INTRAMUSCULAR CAPILLARY ELECTRON MICROGRAPH, (CIRCLES) HYPERCHROMATIC NUCLEUS, (ARROWS) WIDENED ENDOTHELIAL CYTOPLASM WITH UNFOLDING INTO THE CAPILLARY LUMEN. X 10,600.

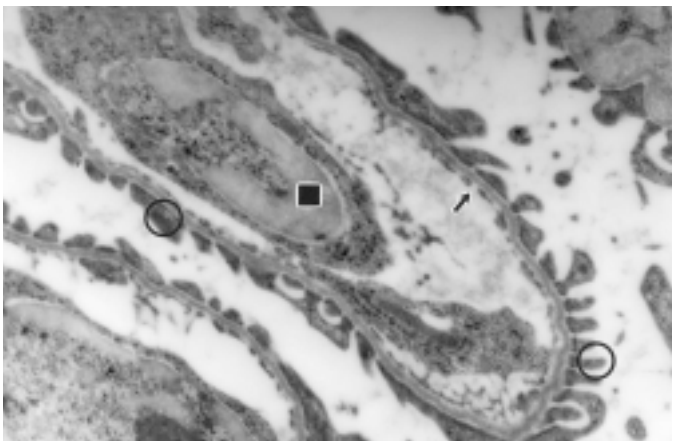

FIGURA 4. IN THIS KIDNEY ELECTRON MICROGRAPH, (ARROWS) WIDENED FENESTRAE, (SQUARE) NUCLEUS WITH DIMINISHED ELECTRONIC DENSITY, (OPEN CIRCLE) PODOCYTES NON UNIFORM. X 24,000.

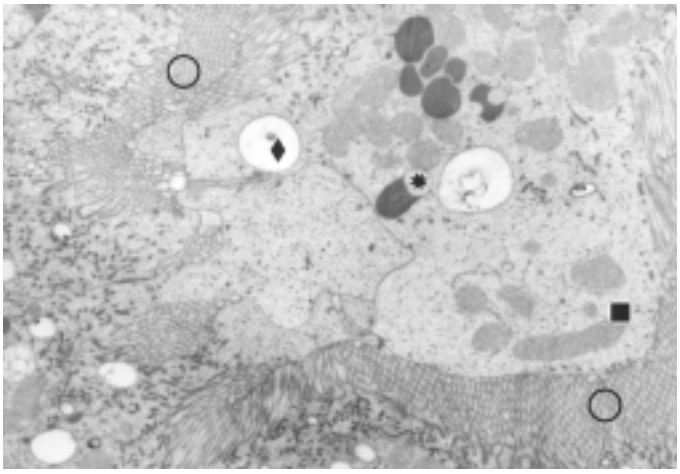

FIGURA 5. IN THIS KIDNEY ELECTRON MICROGRAPH, (OPEN CIRCLE) MICROVILLIES, (STARS) LIPOFUCSIN GRANULES, (RHOMBUS) LARGE VACUOLES, (SQUARE) SWOLLEN EPITHELIAL CELLS WITH MITOCHONDRIA. X 12,000.

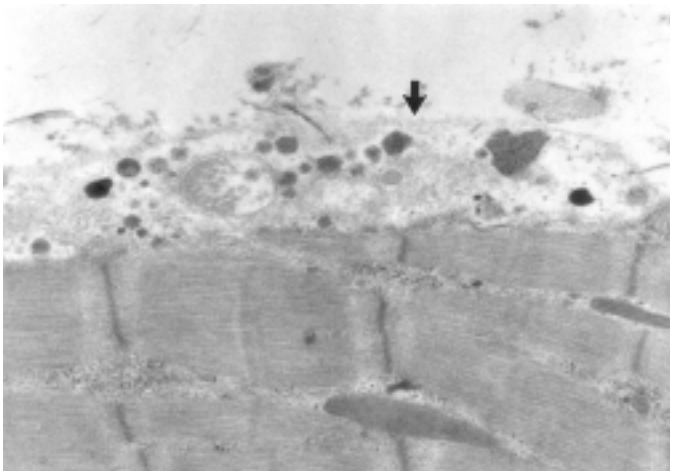

FIGURA 6. IN THIS MUSCLE ELECTRON MICROGRAPH, (ARROW) SEGMENTAL NECROSIS AREA. NOTICE THE PRESENCE OF THE BASAL MEMBRANE, BUT NOT THE PLASMALEMA. X 36,000.

duced by the synergistic effect of phospholipase A2 and mellitin [15] is similar to that caused by taipoxin [12], crotoxin [8] and after injection of crude *Crotalus viridis viridis, Crotalus vegrandis* [16] and *Crotalus atrox* snake venoms [14].

In this work, abnormal capillaries, including non-existence of the capillary wall in some places, enlargement of the rough endoplasmic reticulum and increment of the fenestrae, could also be produced by toxins such as mellitin and phospholipases which would induce fast rupture of the plasmatic membrane with subsequent detriment of its regulation of permeability to ions and macromolecules [9,14, 15]. The phospholipase toxins could cause the dominant form of necrosis observed in our study. This kind of myonecrosis probably develops through an indirect mechanism of action, most likely hypoxia by ischemia following capillary wall damage [10]. Regardless of the actual mechanism, all the above mentioned fibre changes could involve an increased net influx of calcium into cells which triggers the vicious cycle of calcium overload, ATP depletion, leakage and/or activation of hydrolases/proteases, and structural damage resulting in necrosis [19].

Authors [18] suggested that some toxins induce proteases, nucleases and lipases enzymatic activation, which produce cytoskeleton alteration leading to actin and tubulin disruption.

In this work, muscular tissue had associated focal leukocyte inflammatory infiltration. As it has been proposed an allergen might be a chemoattractant for peripheral blood mononuclear and polymorphonuclear leukocytes and could be capable to induce non-infectious inflammatory reactions as a result of its interaction with these sensitive cells [21], as well as the necrotic tissue inducing the local inflammatory response.

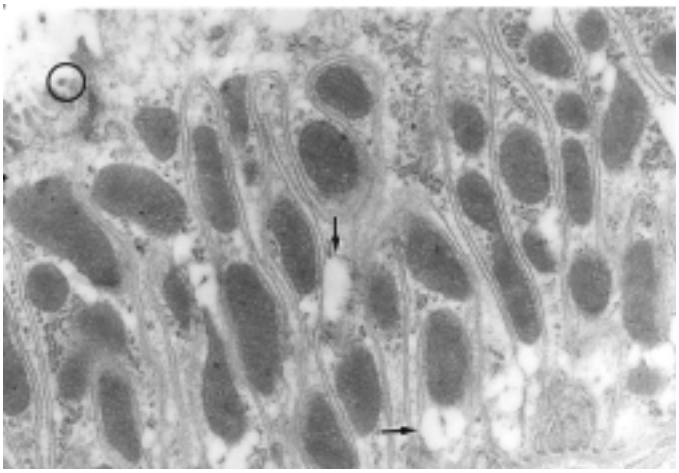

FIGURA 7. IN THIS KIDNEY ELECTRON MICROGRAPH, (ARROWS) VACUOLES NEXT TO THE MITOCHONDRIA IN THE TUBULAR BASAL AREA, (OPEN CIRCLE) DECREASE OF LENGTH AND THE NUMBER OF MICROVILLIES IN THE APICAL AREA. X 30,000.

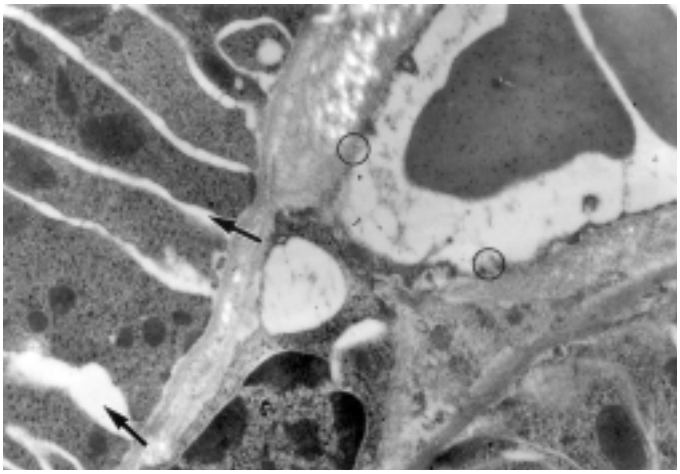

FIGURA 8. IN THIS KIDNEY ELECTRON MICROGRAPH, (ARROWS) INCREASE OF PLASMA MEMBRANE SPACES OF EPITHELIAL CELL BASAL AREAS, (OPEN CIRCLE) ENDOTHELIAL WALL LOST INTEGRITY. X 21,000.

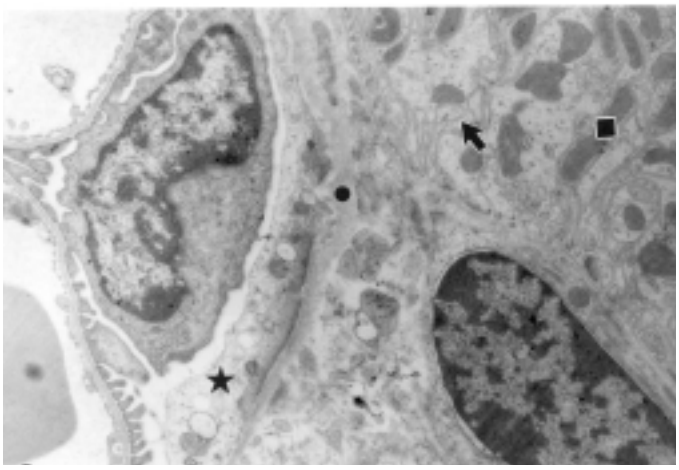

FIGURA 9. IN THIS KIDNEY ELECTRON MICROGRAPH, (STARS) BOWMAN WALL LOOKS DEGENERATED, (CIRCLE) OUTSIDE BASAL GLOMERULAR MEMBRANE LOOK WIDENED, (SQUARE) IRREGULAR MITOCHONDRIA DISTRIBUTION, (ARROWS) EPITHELIAL CELL BASAL AREA SHOWED DECREASE OF INTERDIGITATIONS. X 15,000.

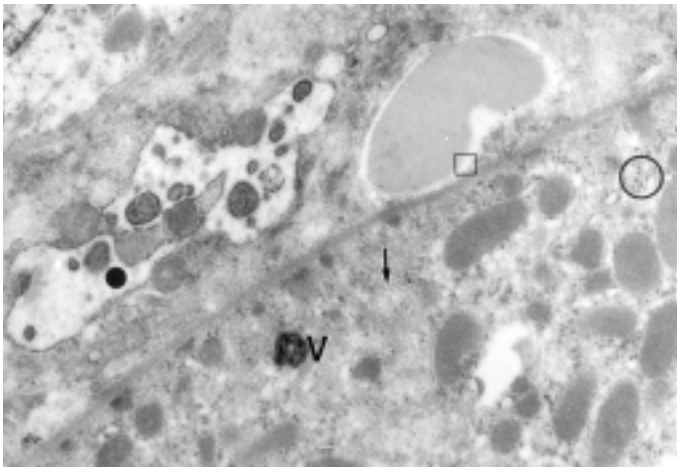

FIGURA 10. IN THIS CAPILLARY ELECTRON MICROGRAPH, (ARROW) LOSS OF INTERDIGITATIONS, (OPEN CIRCLE) FREE POLYSOMES, (V) AUTOPHAGIC VACUOLES, (CIRCLE) ENDOPLASMIC RETICULUM ENLARGED, (OPEN SQUARE) CAPILLARY WALL LOST. X 24,000.

The results of this work indicate that bee venom is directly nephrotoxic to mouse glomeruli and tubular kidney cells in vivo. Human victims of apism also develop severe renal damages with clinical signs and symptoms such as acute renal failure, usually due to acute tubular necrosis. Over the 48 hours following bee stings, acute renal failure may occur mainly due to rhabdomyolysis and myoglobinuria, haemoglobinuria, initial ischemia, or immunopathological mechanisms [20]. Patients who survive for 48 hours with acute tubular necrosis commonly recover over a period of time. Phospholipase A2 acts on the membrane phospholipids, producing the formation of free fatty acids and lysophospholipids that damage the cellular membrane explaining the direct glomerular damage reported in this experiments. Direct and indirect action of the histamine and prostaglandin venom activities could increase capillary permeability producing oedema and hypoxia. Intrarrenal and systemic haemodynamic changes together with myoglobinuria could lead to ischemic damage [6].

Acute renal failure due to acute tubular necrosis following a bee sting is a frequent complication. In [6] electronic microscopy study carried out in envenomed Wistar rats with africanised bee venom, proximal tubule segments showed increment of the intracytoplasmic vacuoles and attenuation of the border in brush and baselateral unfolding. Likewise intracytoplasmic myelin figures with mitochondrial fragments were observed. They suggested that these changes seem to be due to the effects of the venom components (mellitin and phospholi-

pases) acting on the membranes of the tubular cells, and/or to myoglobinuria dependent mechanisms or perhaps an ischemic damage.

In this current work, the disappearance of the rough endoplasmic reticulum and decrease in size and number of the microvillous, as well as the non-uniform widening of the basal membrane and the alteration of the vascular endothelial wall, could correspond to developed lesions caused by ischemic phenomena, with an insufficient sanguine irrigation generally accompanied by hypotension and shock, or through a haemolytic crisis that leads to damage of the epithelial tubular cells, particularly susceptible to anoxia. It has been postulated that the tubular damage produces constriction of the preglomerular arterioles, and that this originates a decrease of the glomerular filtration caused by the activation of the renin-angiotensin system. However, the tubular damage could also by itself produce oliguria, since the tubular debris could block the urine flow and eventually increase the intracellular pressure, diminishing the glomerular filtration [4] that could induce the glomerular and tubular oedema found in this work.

Histamine or similar chemical mediators act between 3 and 30 minutes, increasing the vascular permeability by widening the interendothelial unions and by active contraction of the endothelial cells. The most intense activities produce delayed and prolonged reactions, the increase of permeability lasting from 30 minutes to 10 hours and reaching a peak between 4 to 24 hours. This is in agreement with the results in this work, where capillaries and venules damages findings, resulted in a direct mortal lesion of the endothelium. Based on findings, it makes reasonable to suggest the intervention of histamine and other amines presented in bee venom in one of the several events that take place in the kidney, for instance the early evidence of alterations in the glomerular endothelial wall at 5 hours.

In this experimental model under light microscopy, there was not demonstrable lesions in kidney. But, electronic microscopy ultrastructural studies carried out from 5 hours evidenced the first subcellular processes caused by bee venom toxic activity in the examined tissues. At 5 hours, changes at the tubular and glomerular levels of renal tissue could be observed. These findings allow to propose that, for the early renal lesion diagnosis, the routine technique alone, based on microscopy of light, is not sufficient, but rather it is necessary to incorporate ultrastructural studies to evidence the first processes of cellular damage that would allow the initiation of a precocious therapy of patients.

CONCLUSIONS

In finale, as in the case of other authors [15], these results suggest that phospholipases and mellitin induce muscle damage upon intraperitoneal injection in mice as a consequence of either a direct mechanism in the case of phospholipases or an indirect mechanism, probably hypoxia/ischemia that supervenes in skeletal muscle and the kidney as result of microvasculature injury caused by histamine, mellitin and phospholipases. The severe distant effects we observed in muscle fibres and the kidney suggest that substantial amounts of these toxins can be present in the venom, the components of which need to be characterised.

In this work it has extensively demonstrated the bee venom activity on different tissue structures of envenomed mice, which could be extrapolated to the human envenomation, where since clinical and physiopathological point of view it has been described similar findings.

ACKNOWLEDGEMENTS

Authors thanks an anonymous referee for his excellent correction of the manuscript.

BIBLIOGRAPHIC REFERENCES

[1] ANDERSON, S.G.; Ownby C.L. Systemic hemorrhage induced by proteinase H from *Crotalus adamanteus* (eastern diamondback rattlesnake) venom. **Toxicon.** 35:1301-1313. 1997.

[2] ANONYMOUS. Principles of laboratory animal care. In: National Institute of Health of USA (Ed.): **Guide for the care and use of laboratory animals**. Maryland, USA. Mimeo: 1-86. 1985.

[3] AZEVEDO-MARQUES, M.M.; Ferreira, D.B.; Costa, R.S. Rhabdomyonecrosis experimentally induced in Wistar rats by africanized bee venom. **Toxicon**. 30: 344-348. 1992.

[4] BECK, F.; THURAU, K.; Gestraunthaler, G. Pathophysiology and pathobiochemistry of acute renal failure. **The kidney: Physiology and pathophysiology**. In: Academic Press New York. 2**:** 3157. 199

[5] BOURGAIN, C.; PAUTI, M.; FILLASTRE, J.; et al. Envenimation massive aprés piqures de ábeilles africaines. **La Presse Medicale**. 22: 1099-101. 1998

[6] DOS REIS, M.; COSTA, R.; COIMBRA, T.; et al. Renal changes induced by envenomation with africanized bee venom in female wistar rats. **Kidney. Blood. Press. Res**. 20:271-277. 1997.

[7] DOS REIS, M.; COSTA, R.; COIMBRA, T.; et al. Acute renal failure in experimental envenomation with africanized bee venom. **Renal Failure.** 20: 39-51. 1998.

[8] Gopalakrishnakone, P.; Dempste, D.W.; Hawgood, B.J.; et al. Cellular and mitochondrial changes induced in the structure of murine skeletal muscle of crotoxin, a neurotoxic phospholipase A2 complex. **Toxicon**.22**:** 85-92. 1984.

[9] GUTIERREZ, J.M.; OWNBY, C.L.; ODELL, G.V. Isolation of a myotoxin from *Bothrops asper* Venom: partial characterization and action on skeletal muscle. **Toxicon**. 22: 115-128. 1984

[10] GUTIERREZ, J.M.; ROMERO, M.; NUÑEZ, J.; et al. Skeletal muscle necrosis and regeneration after injection of BaH1, a hemorrhagic metalloproteinase isolated from the venom of the snake *Bothrops asper* (Terciopelo). **Exp. Mol. Pathol.** 62: 28-41.1995.

[11] HABERMAN, E. Bee and wasp venoms. **Science.** 177: 314-322. 1972.

[12] HARRIS, J.B.; MALTIN, C.A. Myotoxic activity of the crude venom and the principal neurotoxin, taipoxin, of the Australian taipan, *Oxyuranus scutellatus*. **Brit. J. Pharm.** 76: 601-608. 1982.

[13] OGURA, M.; URBINA, C.; GONZÁLEZ, M.; et al. **Introducción a la Microscopía Electrónica**. Centro de Microscopía Electrónica. Facultad de Ciencias. Universidad Central de Venezuela (Eds.), Caracas, Venezuela. Mimeo: 1-68. 1997.

[14] OWNBY, C.L.; COLBER, G.T.R. Classification of myonecrosis induced by snake venoms: Venoms from the prairie rattlesnake (*Crotalus viridis viridis*), Western diamondback rattlesnake (*Crotalus atrox*) and the Indian cobra (*Naja naja naja*). **Toxicon.** 26: 459-474. 1988.

[15] OWNBY. CH.; POWELL, J.; JIANG, M.; et al. Mellitin and phospholipase A2 from bee (*Apis mellifera*) venom cause necrosis of murine skeletal muscle *in vivo*. **Toxicon.** 35: 67-80.. 1997.

[16] PULIDO-MENDEZ, M.; RODRIGUEZ-ACOSTA, A.; FINOL, H.J.; et al. Ultrastructural pathology in skeletal muscle of mice envenomed with *Crotalus vegrandis* venom. **J.Sub.Cyt.Pathol**. 31 : 555-61. 1999.

[17] SPEARMAN, A.; KARBER, L. Alternative methods of analysis for quantal responses. In Finney DJ, Griffin Ch, (Ed.). **Statistical method in biological assay**. London. 1-137.1978.

[18] TRUMP, B.F.; BEREZESKY, I.K. Cellular and Molecular Basis of Toxic Cell Injury. In: Acosta, D (Ed.).**Cardiovascular Toxicology**. Raven Press Ltd, New York. 75-111. 1992.

[19] TU, A.T. Local tissue damaging (hemorrhage and myonecrosis) toxins from rattlesnake and other pit viper venoms. **J. Toxicol. Toxin. Rev**. 2: 205-234. 1983.

[20] VETTER, R.; VISSCHER, P. Bites and stings of medically important venomous arthropods. **Int. J. Dermatol.** 37: 481-496. 1998.

[21] ZAK-NEJMAR, T.; MALOLEPSZY, J.; JUTEL, M.; et al. Specific allergen induced motility of peripheral blood mononuclear cells and polymorphonuclear leukocytes in insect sting allergy. **Arch. Immunol.Ther. Exp. (Warz**). 40: 107-111. 1992.