ADRENAL GLAND ULTRASTRUCTURAL CHANGES IN MICE INOCULATED WITH SCORPION *Tityus discrepans* (BUTHIDAE) VENOM

Cambios Ultrastructurales de la Glándula Adrenal en Ratones Inoculados con Veneno de Escorpión *Tityus discrepans* (Buthidae)

***Alexis Rodríguez-Acosta*[1], *Miriam Strauss*[2], *Magdalena Pulido-Méndez*[3] *and Héctor José Finol*[4]**

[1]*Sección de Inmunoquímica, Apartado 47423.* [2]*Sección de Biología Celular, Instituto de Medicina Tropical "Dr. Félix Pifano"*
[3]*Instituto de Medicina Experimental.* [4]*Centro de Microscopía Electrónica.*
Facultad de Ciencias, Universidad Central de Venezuela. Caracas 1041, Venezuela. E-mail: rodriguf@camelot.rect.ucv.ve

ABSTRACT

Clinical signs that appear in mice as a consequence of the toxic action by *Tityus discrepans* venom, such as hypotension, sweating, coldness, tachycardia, pulmonary acute oedema are accompanied by or dependant upon changes in the ultrastructural organisation of adrenal glands cellular and subcellular components. These changes may resemble the damage found in envenomed humans. To evaluate adrenal gland subcellular response to *Tityus discrepans* venom, ten male C57/Bl adult mice were at random divided into two groups: the experimental group was intraperitoneally injected with scorpion venom at a lethal dose of 0.5 mg/Kg of body weight and controls received saline solution by the same route. Samples from adrenal glands were taken and prepared for electronic transmission microscopic study. The samples were observed in a Hitachi- 300 electron transmission microscopy. The most relevant adrenal ultrastructural findings in this model were a great number of cortex cells that showed mitochondria and smooth endoplasmic reticulum generalised swelling. Autophagic vacuoles containing mitochondrial residues were also seen. Extensive microvasculature changes were also observed. Capillary wall changes were frequently characterised by endothelial cell cytoplasm thickening with degenerated swollen mitochondria. In some illustrations medullar capillaries wall disruption and necrosis were observed.It is suggested that adrenal gland ultrastructural damages are produced by toxins present in this scorpion venom and are responsible for some of the clinical signs observed in envenomed animals.

Key words: Adrenal gland, *Tityus discrepans*, ultrastructural changes, venom.

RESUMEN

Se plantea la hipót'esis, que los signos clínicos aparecidos en ratones, como consecuencia del efecto tóxico del veneno de escorpión (*Tityus discrepans*), tales como hipotensión, sudoración, enfriamiento, taquicardia y edema agudo pulmonar, se acompañan o son dependientes de los cambios ocurridos en la organización de los componentes celulares y subcelulares de las glándulas adrenales. Estas variaciones pudieran ser similares al daño encontrado en humanos accidentalmente envenenados o con insuficiencia adrenal. Para evaluar la respuesta subcelular de la glándula suprarrenal al veneno de *Tityus discrepans*, fueron estudiados diez ratones machos adultos C57/Bl en dos grupos: el grupo experimental de 5 ratones fue inyectado intraperitonealmente a una dosis de 0.5 mg/Kg de peso y los 5 ratones control, que solo recibieron solución salina, por la misma vía. Se tomaron muestras de las glándulas adrenales para el estudio ultraestructural por microscopía electrónica de transmisión. Las muestras fueron observadas en un microscopio de transmisión Hitachi H-300. Las alteraciones ultrastructurales halladas en las adrenales fueron: se evidenció un gran número de células de la corteza que exhibian amplio edema de las mitocondrias y del retículo endoplásmico liso. Se observaron vacuolas autofágicas contentivas de residuos de mitocondrias. También fueron evidenciadas extensas alteraciones de la microvasculatura, caracterizados por engrosamiento del citoplasma de las células endoteliales, con degeneración edematosa mitocondrial. En algunas áreas medulares los capilares tenían necrosis e interrupción de la pared. El daño ultraestructural de las glándulas adrenales, demostrado en este trabajo y producido por toxinas presentes en el veneno de escorpión, es responsable de algunas de las manifestaciones clínicas presentadas por los animales experi-

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mentalmente inoculados y son similares al envenenamiento accidental en humanos.

Palabras clave: Glándula adrenal, *Tityus discrepans* , cambios ultraestructurales, veneno.

INTRODUCTION

Scorpion envenomation is common in Venezuela. Probably scorpions cause more serious injuries than any other venomous animal is this country. Some species pose no more threat than a bee, but others are highly dangerous, especially to children [3, 6, 12, 17]. Studies of the American species of venom scorpions have revealed a variety of proteins, some with neurotoxic activities [2, 7, 19].

So far, in Venezuela only the genus *Tityus* of scorpions from the eastern and central western areas of the country has been studied for its venom composition. The clinical manifestations of scorpion envenoming result from the effects of its toxins that cause severe, burning and immediate local pain [13, 15]. In children under 6 years old, stings of certain species (*Tityus discrepans*) may cause serious symptoms including nausea, excessive salivation, profuse sweating, vomiting, convulsions, arterial hypotension or hypertension, cardiac arrhythmia, pulmonary oedema, intense abdominal pain and death (fatalities rarely occur in adults) [16]. Laboratory findings such as hyperglycaemia and increased amylase enzyme activity on *Tityus* envenomed patients have been found [12]. However, as far as we known non adrenal gland ultrastructural studies from scorpion envenomed animals have been performed anywhere. This is the first report that shows the devastating effects of scorpion venom in the ultrastructure of this gland. This work focuses, in particular, on the morphologic events caused by venom activity at subcellular level.

MATERIALS AND METHODS

Venom

Tityus discrepans venom was a donation from the Biotechnology Laboratory of the Pharmacy Faculty at the Universidad Central de Venezuela.

Animals

Ten C57/Bl adult male mice 18-22 g of body weight originating from the Animal House of the Tropical Medicine Institute of the Universidad Central de Venezuela (Caracas, Venezuela) were used. Mice were randomised divided into two groups: 5 experimental mice were intraperitoneally injected with a Lethal Dose 50 (LD_{50}) of 0.5 mg/kg (0.1 mL) body weight of scorpion venom. 5 control mice were injected by the same route with saline solution (0.1 mL). All animals were allowed free access to a standard diet and water *ad libitum*. The investigation complies with the norms get out in the guide for the care and use of laboratory animals, published by the US National Institute of Health [4].

Preparation of specimens for electron microscopy

After 48 h the control and experimental animals were sacrificed by neck dislocation and samples of adrenal gland were immediately obtained after killing the dissected adrenal gland tissues were immediately in *situ* fixed with 3% glutaraldehyde and 1% OsO_4, both fixatives diluted in pH 7.4, 320 mM phosphate buffer, dehydrated in ethanol and embedded in LX-112 resin (Ladd Research Inc.) [24]. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a Hitachi H-300 transmission electron microscope with an accelerating voltage of 70 KV [20].

RESULTS

Mice from the control group showed no clinical signs. Experimental mice showed signs of shock (coldness, collapse, tachycardia, sweating) such as it has been described in human adrenal gland insufficiency. The macroscopic aspects of adrenal glands were as follows: controls showed glands of pink normal aspect and weight. Glands from envenomed mice were increased in weight and yellowish (data not shown).

Adrenal gland from mice controls did not show any ultrastructural changes. Envenomed mice showed profound changes in both cortex and medulla of adrenal gland. A great number of cortex cells mitochondria and smooth endoplasmic reticulum showed generalised swelling, FIG. 1. Autophagic vacuoles containing mitochondrial residues were also seen, FIG. 1. Medullary chromaffin cells damage also was extensive and characterised by organelle swelling, including granule containing vesicles and Golgi, FIG. 2. Medullary cells appear to have diminished granular content in several areas. The results showed ample microvasculature alterations. Capillary wall changes were frequently characterised by endothelial cell cytoplasm thickening with abundance of free polisomes and degenerated swollen mitochondria, FIG. 3. Myelin-like forms and cytoplasmic membranous remains also were found, FIG. 4. In some fields, medullary capillaries wall disruption and necrosis were observed, FIGS. 5 and 6.

DISCUSSION

As far as it is known, clinical signs of shock characterised by hypotension or hypertension, tachycardia or bradycardia and profuse sweating [10] have not been related anywhere to the ultrastructural damages in the adrenal gland of scorpion envenomed victims. The changes at cellular or subcellular levels in the adrenal glands from this murine model probably are similar to what it is happening in human adrenal gland of envenomed patients. Some investigators proposed that toxins induces proteases, nucleases and lipases enzymatic activation, producing a cytoskeleton alteration leading to an actin and tu-

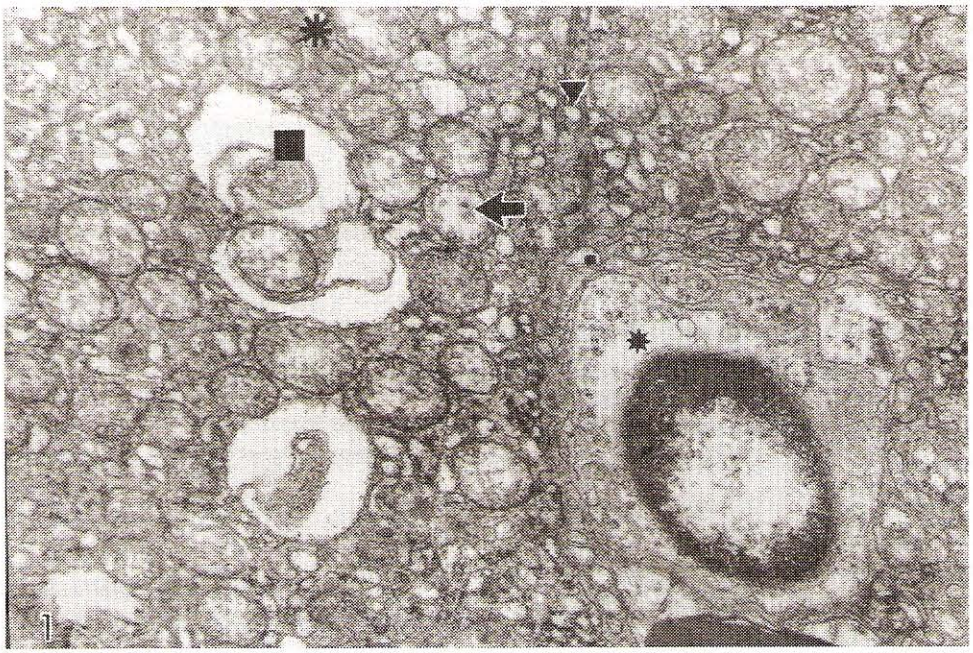

FIGURE 1. ELECTRONMICROPHOTOGRAPH OF CORTEX CELLS. SWOLLEN MITOCHONDRION (ARROW), SWOLLEN SMOOTH ENDOPLASMIC RETICULUM (ARROWHEAD), AUTOPHAGIC VACUOLE (SQUARE). THE CAPILLARY ENDOTHELIAL CYTOPLASM IS ALTERED (NO LUMEN IS SEEN) (STAR). X 24,000.

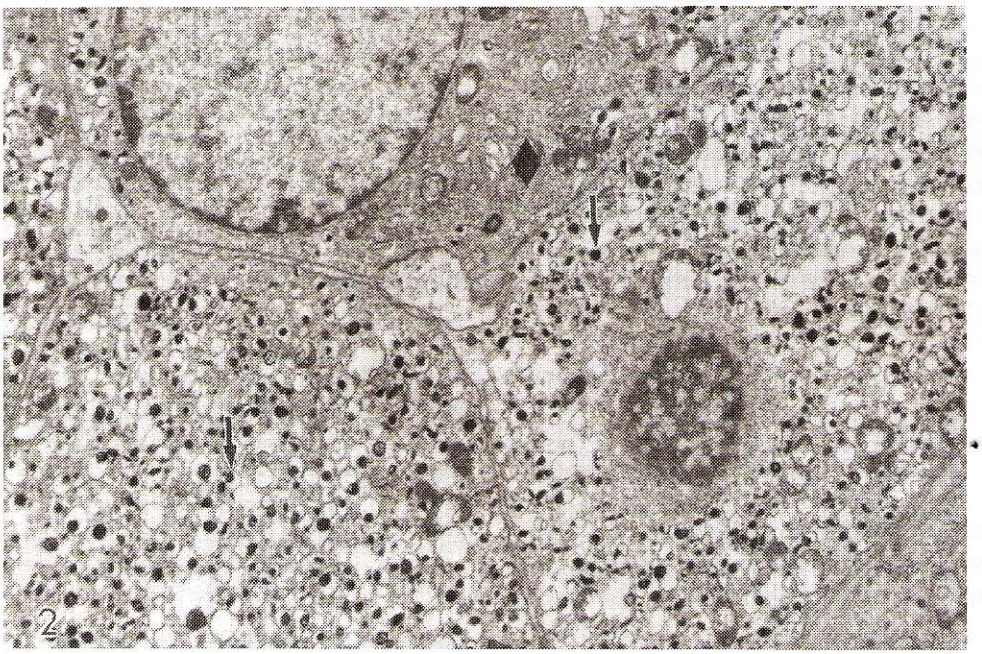

FIGURE 2. IN CHROMAFFIN CELLS. SWOLLEN GRANULE CONTAINING VESICLES (ARROWS), SWOLLEN GOLGI (ROMBUS). X 10,500.

bulin disruption [23]. These results are coincident with the pathophysiologic phenomena described by different authors [5, 10, 21] in other species of *Tityus*. They refer that the scorpion toxins probably act in the ionic channels at cellular membrane level. In several kind of cells, mainly excitable cells, Na^+/Ca^{2+} exchange represents a major participant in intracellular Ca^{2+} regulation [21] and consequently toxins that interfere with the Na^+/K^+- ATPase or that differently modify Na^+ concentration will have fast secondary effects on Ca^{2+}. Alterations of the plasmalemmal Ca^{2+} regulation consequently constitutes a major site of toxic effects on cell injury. Decreased concentrations of Ca^{++} also affect cell-cell communication and, if extended, may result in detachment of desmosomes, intermediate junctions, and tight junctions [23], as it was observed in endothelial cells. Other author [5] outlined that the effects produced by *Tityus serrulatus* toxins depend of the action at sodium channels

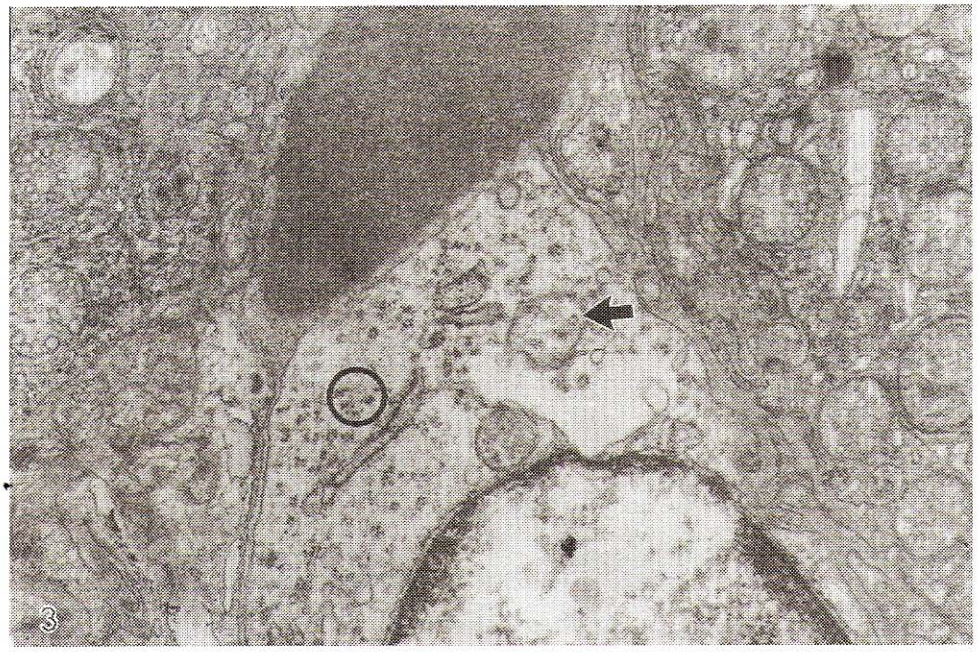

FIGURE 3. CORTICAL CAPILLARY. ALTERED ENDOTHELIAL CELL MITOCHONDRION (ARROW). CYTOPLASMIC AREA WITH FREE POLISOMES (CIRCLE). X 30,000.

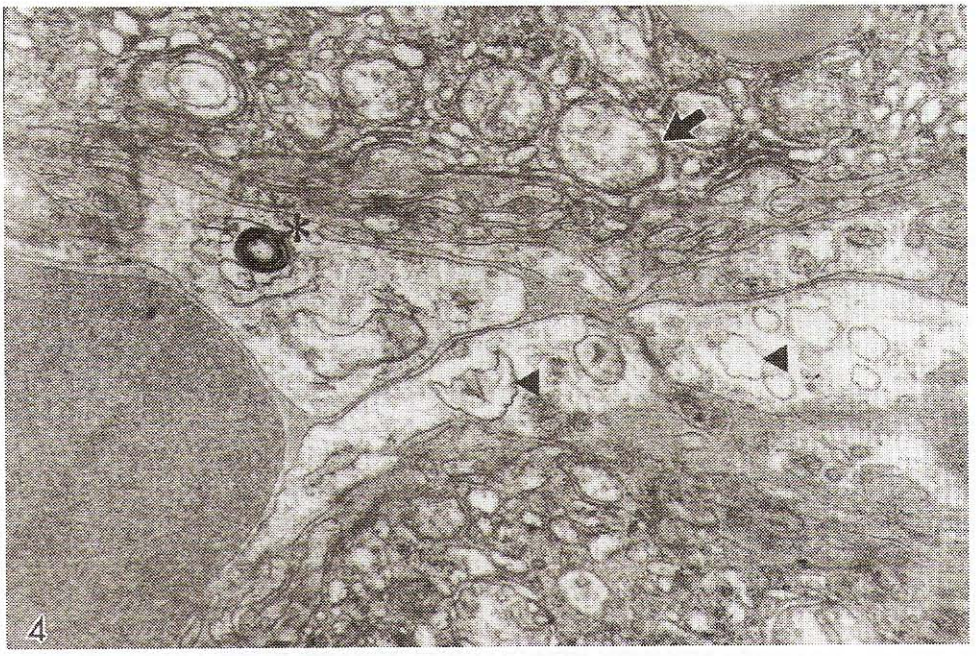

FIGURE 4. CORTICAL CAPILLARY. CYTOPLASMIC MEMBRANOUS RESIDUES (ARROWHEADS), MYELIN-LIKE FORMS (ASTERIK). NOTE SWOLLEN CORTICAL CELL MITOCHONDRIA (ARROW). X 30,000.

specific sites with the subsequent membrane depolarisation and acetylcholine and catecholamin release from the terminal nervous and adrenal gland. Across the progression from sublethal to lethal injury, marked changes in membrane integrity occur involving all membranes in the cell. In mitochondria, for instance, we have found changes from normal to swollen conformation that may be a result of an inner membrane phospholipids modification. Mitochondria damages characteristically occur under conditions where respiration and/or oxidative phosphorylation are altered [18].

In conclusion the results suggests that the scorpion venom toxins induce proteases, nucleases and lipases enzymatic activation, producing a cytoskeleton change. Moreover, the scorpion toxins probably act in the ionic channels at cellular membrane level. Various investigators [1, 8, 14] have reported haemorrhagic effects of scorpion venom in different tissues. Our findings at the microvasculature may produce this haemorrhagic activity. Another mechanism contributing to the genesis of endothelial damage, following the toxin injection, would be the release of substances which act on that cells, such as kinins, his-

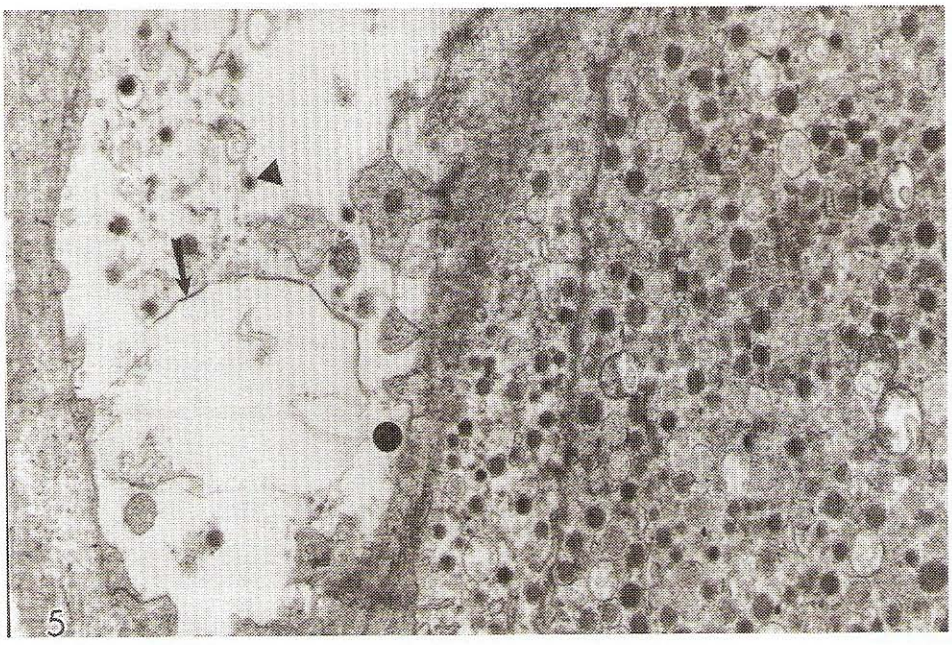

FIGURE 5. MEDULLAR CAPILLARY. WALL DISRUPTION (BLACK CIRCLE), GRANULES (ARROWHEAD) AND MEMBRANOUS RESIDUES (ARROW) IN THE LUMEN. X 18,000.

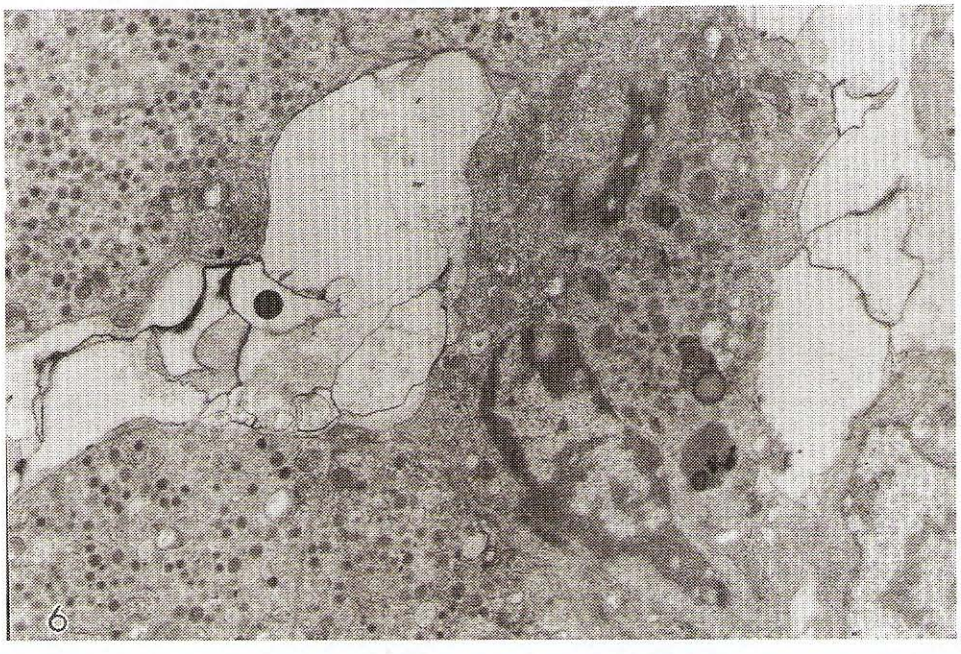

FIGURE 6. NECROTIC MEDULLAR CAPILLARY WITH MEMBRANOUS REMAINS IN THE LUMEN (BLACK CIRCLE). X 10,500.

tamine and prostaglandins [9, 22]. These whole ultrastructural changes induce to an adrenal hormonal dysfunction that contribute to the clinical signs of envenomed human and animals.

CONCLUSIONS

This study reveals that *Tityus discrepans* venom would be involve in the aetiology of ultrastructural adrenal gland damages causing lesions that induce the signs of shock, collapse, tachycardia and sweating such as it is described in human adrenal gland insufficiency.

The wide toxic activity of scorpion *Tityus discrepans* is ratified in this work and it is included the description of a new target organ for the venom action of this dangerous species.

RECOMMENDATIONS

It is suggested to carry out post-mortem studies in human cases died by scorpionic accident, in order to evidence similar lesions found in these experimental envenomed mice.

It will be necessary in the future to work with very defined scorpion (*Tityus discrepans*) venom fractions to identify the molecule (s) causing the subcellular damage described in this work.

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