TRYPANOSOMA EVANSI: ANALYSIS OF THE ULTRASTRUCTURAL CHANGE IN HEPATIC CELLS DURING MURINE EXPERIMENTAL INFECTIONS

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ABSTRACT
The ultrastructural study of the liver in mice experimentally infected with a local isolate of *Trypanosoma evansi* reveals changes in the dimensions of the mitochondria and lipid droplets, as well as alterations in some cytoplasmic organelles of the hepatocyte. Likewise, demonstrates modifications in the sinusoid endothelial cell cytoplasm and fenestrae. The quantitative analysis of the qualitative observed changes provides statistical significance to the described changes. The presence of trypomastigotes in sinusoids together with hepatocytes lipid droplets and other observed changes could suggest that liver is a cryptic target in the life cycle of *T. evansi.*

Key words: *Trypanosoma evansi,* liver, ultrastructure.

TRYPANOSOMA EVANSI: ANALISIS DEL CAMBIO ULTRAESTRUCTURAL EN CELULAS HEPATICAS DURANTES INFECCIONES MURINAS EXPERIMENTALES.

RESUMEN
El estudio ultraestructural del hígado de ratones infectados experimentalmente con un aislado local de *Trypanosoma evansi* revela cambios en las dimensiones de las mitocondrias y las gotas lipídicas, así como alteraciones en otros organelos de los hepatocitos. Igualmente, demuestra modificaciones en el citoplasma de la célula endotelial del sinusoide y de las fenestras. El análisis cuantitativo de las observaciones cualitativas proporciona significancia estadística a los cambios descritos. La presencia de tripomastigotes en los sinusoides, conjuntamente con gotas lipídicas provenientes de hepatocitos, así como los cambios observados sugerirían al hígado como blanco críptico en el ciclo de vida de *T. evansi*.

Palabras clave: *Trypanosoma evansi,* hígado, ultraestructura.

INTRODUCTION

Venezuelan equine *Trypanosoma evansi* trypanosomosis constitutes a significant detriment for the extensive bovine farming, since infected and/or potentially infected equines are used in livestock management [1].

However, papers describing ultrastructural alterations brought about by *T. evansi* isolates derived from Venezuelan hosts are scanty [2-4], and focus on ultrastructural qualitative aspects.

This study explores the qualitative ultrastructural change in the hepatocytes of mice experimentally infected with *T. evansi* obtained from an equine naturally infected in the Venezuelan savanna. In addition, a quantitative approach is included in order to understand the progressive and irreversible change observed in the hepatocyte.

MATERIALS AND METHODS

Groups of *Mus musculus* (NMRI; ♀; 20 gr body weight) were intra-dermal inoculated (20 trypomastigotes/mouse) with parasites obtained from a naturally infected *Equus asinus* at the "Terecay" cattle ranch (Guarico State, Venezuela). Starting on day 3 post-infection and every

other day until mice's dead, one mouse/group was randomly selected and killed in ether atmosphere. Through surgical ablation the liver was removed, the quadrate lobe was moved apart and systematically sectioned in 2 mm^3 fragments.

The Karnovsky fixative solution (2.5% glutaraldehyde, 37% formaldehyde in Millonig buffer; pH 7.4, 320 mOsm) was employed as the first fixative. Afterward, the sample was washed in Millonig buffer and post-fixed in 1% osmium tetroxide (same pH and osmolarity). Subsequently, was submerged in distilled water and dehydrated in an ethanol series of increasing concentrations (50-100%). Later, the fragment was infiltrated with propylene oxide and included in epoxy resin. The contrasted ultrathin sections (60-80 nm) [5,6] were observed with a Philips CM-10 electron microscope (80 kV). The micrographic record was done on 8.3×10.2 cm negatives.

Digitalized serial micrographs were examined, and the hepatocytes area of the mitochondrial profiles, as well as the lipid droplets was recorded. The derived numerical sets were quantitatively explored by means of Analysis of the Variance (ANOVA).

RESULTS AND DISCUSSION

The liver is an essential internal organ being affected by *T. evansi* [7-10] and *T. brucei* [11,12]. Common lesions include congestion, hemorrhage, polyhedral shape loss, vacuolization, and fatty degeneration that could culminate with major liver failure.

The research dealing with the ultrastructure of local *T. evansi* is restricted to three papers [2-4], and does not cover liver alterations. This work describes the hepatocyte's change with special reference to the comparison between the area of mitochondria and lipid droplets in infected and control animals.

The ultrastructure of the hepatocytes in control animals is normal and does not shows any sign of aberrant change (Fig. 1).

Our results show alterations mainly represented by conclusive signals of necrosis and a significant increase in size and number of mitochondria and lipids droplets.

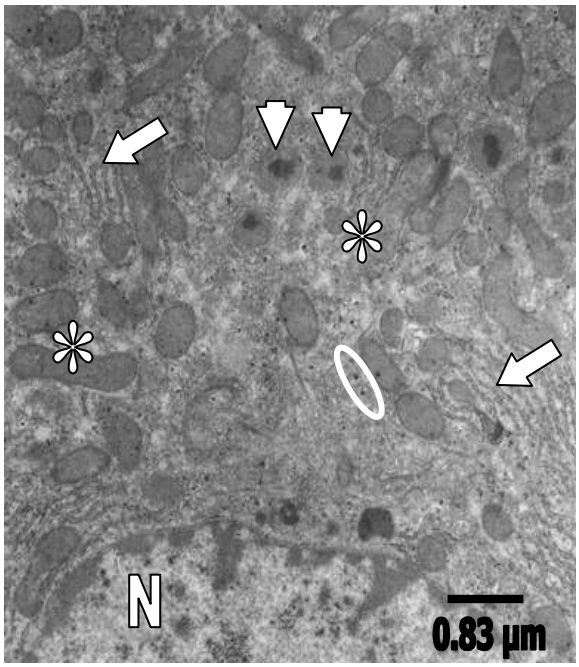

Fig. 1. Section of a normal hepatocyte. See peroxisomes (arrowheads), mitochondria (asterisks), rough endoplasmic reticulum (arrows), glycogen particles (oval) and a nucleus (N).

In addition, a drop in the number of rough endoplasmic reticulum cisternae and peroxisomes is accompanied by modifications in the hepatic sinusoids and the vascular endothelium. Nevertheless apoptosis was not observed.

The area of the mitochondrial profiles increases in time as a swelling consequence (Fig. 2; Fig. 5). Changes in their polymorphism became progressively greater (Fig. 2). Mitochondria were seen in close association with lipid droplets and rough endoplasmic reticulum. In the Figure 2 it is possible to see an increase of empty areas because of loss of glycogen particles.

Significant increasing in the number and dimensions of the lipid droplets were evident which was not only seen in hepatocyte cytoplasm (Fig. 3) but also inside sinusoids, Fig. 4 and Fig. 6.

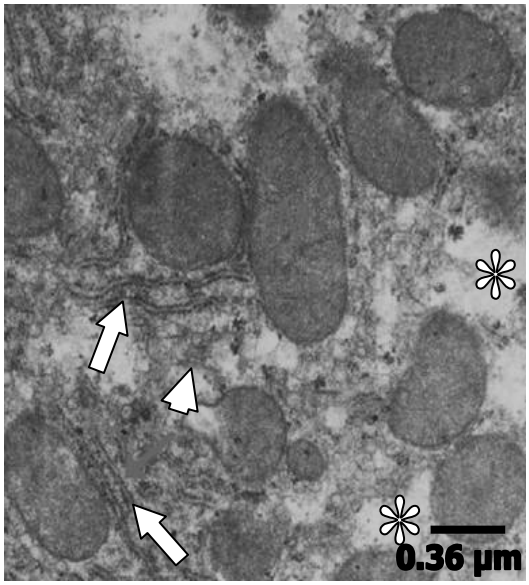

Fig. 2. Close association between mitochondria and rough endoplasmic reticulum (arrows), swollen mitochondrion (arrowhead), and empty cytoplasmic areas (asterisks).

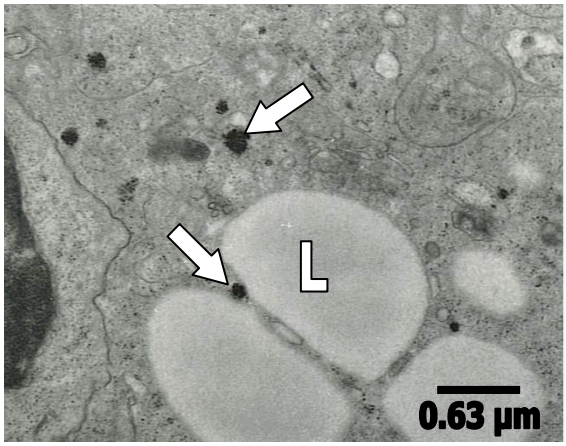

Fig. 3. Degenerated hepatocyte showing abundant lipid droplets (L), and few α-glycogen particles (arrows).

Lipid accumulations in the liver parenchymatous cells and sinusoids match with pathologic conditions associated to fatty degeneration [12].

The number of lysosomes showed an increment. Such variation is associated to increasing electron density [12]. The increment in the number of lysosomes in hepatocytes has been observed in Wilson`s disease [13] liver alteration in mice envenomed with uracoan rattlesnake venom [14] and perimetastasic syndromes [15]. On the contrary, the number of peroxisomes exhibited a diminution, and its content was progressively changing to deep electron-dense.

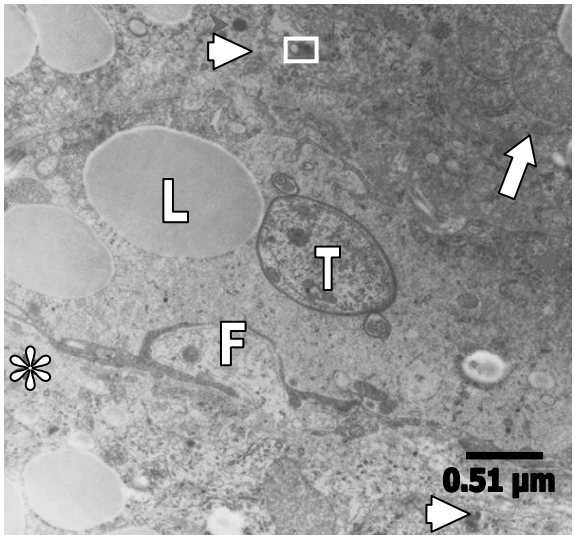

Fig. 4. Lipid droplets (L) and a trypanosome (T) are seen inside a sinusoid. Enlarged fenestrae (F). Hepatocytes exhibit swollen mitochondria (arrow), lysosomes (arrowhead), lipofuscin granule (rectangle). Note the lack of microvilli in Disse space (asterisk).

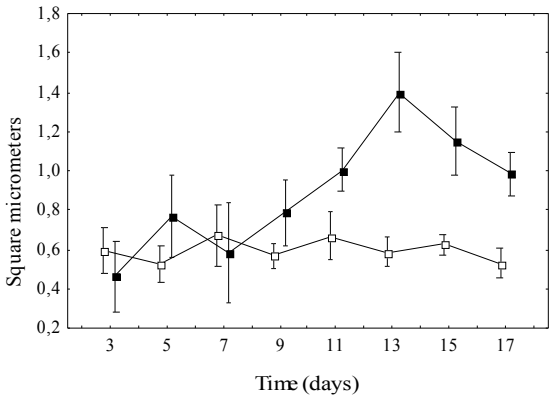

Fig. 5. Area variation in the mitochondria, showing sequence of change in the mitochondria´s area of the hepatocyte throughout the time. ■: Infected animals; □: Control animals. The intervals represent the mean´s standard error under $\alpha \leq 0.05$

Such findings have been associated with disorders autoimmune compromise as paraneoplasic [2] and perimetastasic effects [15]. Lipofuscin granules were characterized by marked alterations and form heterogeneity. Similar changes were previously reported [14], however associated to aging [16].

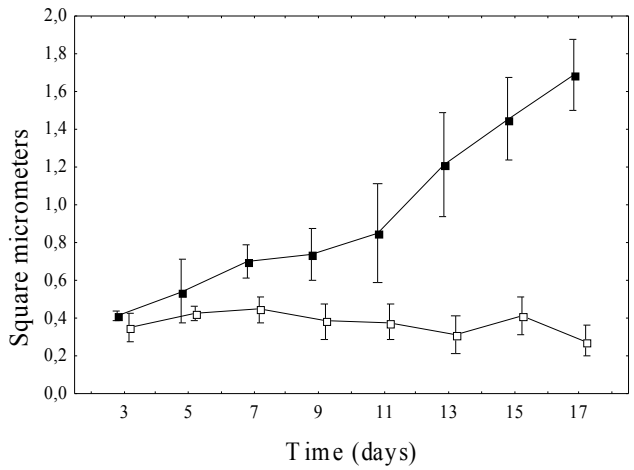

Fig. 6. Area variation in the lipid droplets. The figure shows the sequence of change in the lipid droplets area contained in the hepatocyte throughout the time. ■: Infected animals; □: Control animals. The intervals represent the mean´s standard error ($\alpha \leq 0.05$)

Trypanosomes were seen inside sinusoids (Fig. 3). In this figure it is possible to observe that fenestrae are enlarged and endothelial cell cytoplasm shows areas with different width. The presence of trypomastigotes in the hepatocytes is an important finding, since it indicates extravasation of a classical monomorphic blood trypanosome.

The irreversible and progressive hepatocyte damage leads to the loss of any cytoarchitectural pattern. The micrographs show evident secluded particles of α-glycogen (Figure 4).

The numerical approach to the change was quantitatively scanned by means of ANOVA. The technique points out significant changes ($p < 0.05$) in the area of the mitochondria and the lipid droplets (Table I; Table II).

Table I. ANOVA results on the michondrial area. Time: Experimental days; condition: The condition of being infected or not being infected; condition × time: Action of both variables upon the change process; F: Fischer´ F; p: p-level; *: Significant values. The values with an asterisk implicate differences between the control and the experimental groups.

	F	p
Time	2,1858	0,003 *
Condition	16,2752	0,002 *
Condition × Time	2,2520	0,004 *

Table II. ANOVA result on the lipid droplets area. Time: Experimental days; condition: The condition of being infected or not being infected; condition × time: Action of both variables upon the change process; F: Fischer´ F; p: p-level; *: Significant values. The values with an asterisk implicate differences between the control and the experimental groups.

	F	p
Time	4,2953	0,001 *
Condition	63,1930	0,000 *
Condition × Time	5,5902	0,000 *

CONCLUSION

Sequential changes in the mitochondria and lipid droplets of the hepatocyte were evident both qualitatively and quantitatively. Several cytoplasmic organelles changed its submicroscopic attributes. The coexistence of the described qualitative and quantitative hepatic changes with sinusoidal trypanosomes could imply the hepatocyte as a cryptic target cell in the life cycle of *T. evansi* suggesting steatosis as the main pathogenic process.

ACKNOWLEDGEMENTS

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