

ULTRASTRUCTURE OF BOVINE LIVER TELANGIECTASIS

Ultraestructura de la telangiectasia hepática bovina.

Jorge A. Soto
Federico Delgado

Unidad de Investigaciones Ultraestructurales
Facultad de Ciencias Veterinarias
Universidad del Zulia
Maracaibo, Estado Zulia, Venezuela.

ABSTRACT

Bovine liver telangiectasis is one of the most common liver lesion found in cattle throughout the world. Liver samples from 49 bovines with telangiectasis and from 29 normal animals were examined by electron microscopy. Ultrastructural changes consisted of dilation of sinusoids and Disse's space, a decrease in the number and size of hepatic cell microvilli, which were swollen or transformed into cytoplasmic blebs of hepatocytes and vacuolar degenerative changes. These changes were regarded as ischemic or hypoxic ones. Endothelial and Kupffer cells were normal. Theories about the pathogenesis of telangiectasis were reviewed and analyzed in relation to our ultrastructural findings. It is concluded that the ischemia of hepatocyte theory is well supported by electron microscopic findings.

Key words: Bovine, liver, telangiectasis, ultrastructure

RESUMEN

La telangiectasia hepática es una de las lesiones más comunes del hígado de los bovinos en todo el mundo. Muestras de 49 hígados bovinos con telangiectasia y de 29 hígados de animales normales fueron estudiadas por microscopía electrónica. Las alteraciones ultraestructurales observadas consistían en dilatación de los sinusoides hepáticos y del espacio de Disse y una disminución en el número y tamaño de las microvellosidades de las células hepáticas, las cuales se mostraban hinchadas o transformadas en ampollas citoplasmáticas de hepatocitos con cambios degenerativos vacuolares. Estos cambios fueron considerados como isquémicos o hipóxicos. Las células endoteliales y de Kupffer eran normales. Se revisaron las teorías acerca de la patogénesis de la telangiectasia y se analizaron en relación a los hallazgos ultraestructurales observados. Se concluye que la teoría de la isquemia del hepatocito es la mejor soportada por las observaciones con el microscopio electrónico.

Palabras claves: Bovino, hígado, telangiectasia, ultraestructura.

INTRODUCTION

Bovine liver telangiectasis, a focal dilation and congestion of a group of hepatic sinusoids, is the most common liver lesion in cattle from Venezuela [5, 19] and other countries of the world [2, 5, 9, 13, 16, 20, 24]. This lesion causes direct economic losses due to liver condemnation by sanitary inspectors and, in severe cases, due to impairment of liver function, indirect losses related to a decrease in milk yield or meat production [15].

The etiology and pathogenesis of bovine liver telangiectasis is still unknown, although several theories have been proposed [1, 2, 3, 6, 8, 9, 10]. There are no previous reports of ultrastructural studies on bovine liver telangiectasis. The purpose of this research was to study the ultrastructure of this lesion in order to get information about its etiology and pathogenesis.

MATERIALS AND METHODS

Liver samples from 49 bovines with hepatic telangiectasis, and from 29 normal bovine livers, were taken between 20 to 35 minutes after death at a local slaughterhouse, fixed by immersion in cold 3% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in alcohol, embedded in PolyBed 812 (Polysciences Inc., Warrington, Penn., USA), cut, stained with lead citrate and uranyl acetate, and observed at the electron microscope.

RESULTS

Hepatocytes from the telangiectatic areas showed clear changes of swelling and degeneration characterized by the formation of electron-lucent, finely granulated, intracytoplasmic vacuoles, swollen and round mitochondria, diminished glycogen granules, slight dilation of the endoplasmic reticulum and lysis of the cytoplasmic matrix; nuclei did not show apparent changes (Fig. 1). Other hepatocytes showed true cytoplasmic blebs which contained cellular organelles, glycogen granules and free ribosomes (Figs. 1, 2, 3, 5). Microvilli were absent or diminished in number and length, becoming broaden at their base; some microvilli persisted as cytoplasmic blebs in the space of Disse, with microtubules and filaments diminished in numbers or absent (Figs. 1, 2, 3, 5, 7). Disruption of intercellular junctions between hepatocytes were observed and free hepatic cells were seen at Disse's space (Fig. 4).

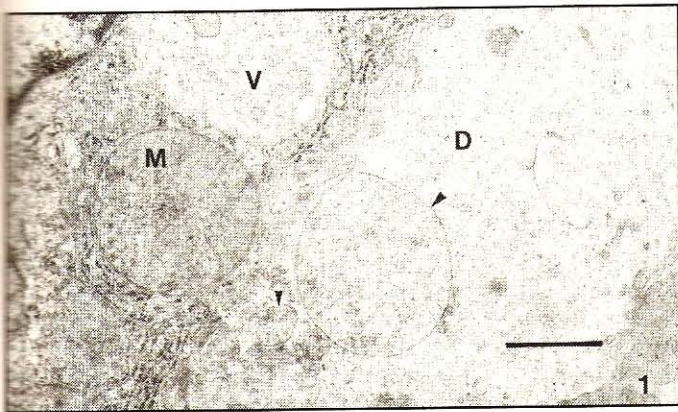


Fig. 1. Hepatocyte showing a round, swollen, mitochondria (M) with diminished cristae and a cytoplasmic vacuole (V). Disse's space (D) is wide, containing swollen microvilli (arrowheads). Bar = 0.5 μ m.

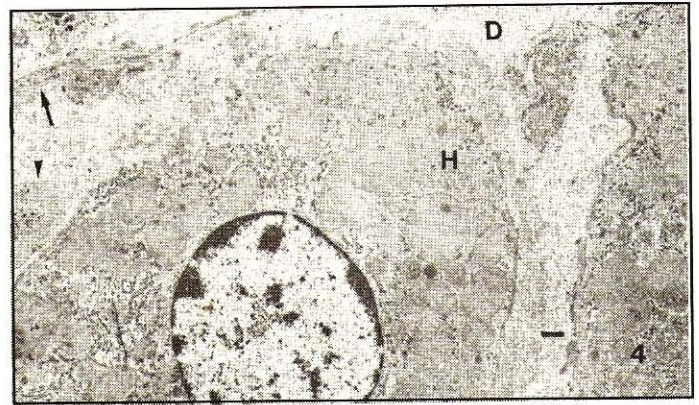


Fig. 4. A free, unattached hepatocyte (H) and a cytoplasmic projection (arrowhead) from another hepatocyte is observed at a wide space of Disse (D). Arrows are pointing out the basal lamina. Bar = 0.5 μ m.

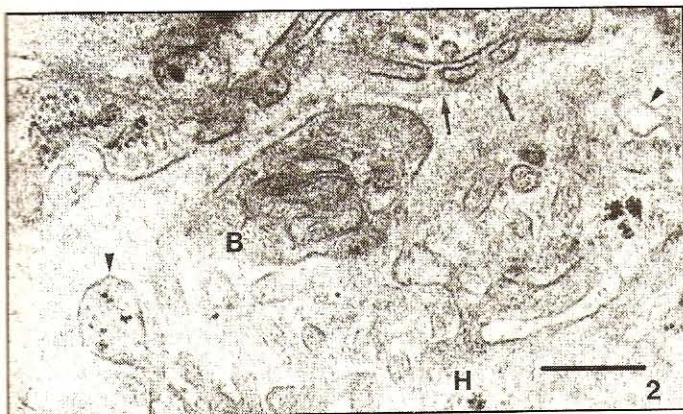


Fig. 2. Disse's space showing scant, swollen, wide microvilli (arrowheads) from a hepatocyte (H) and a cytoplasmic projection or bleb (B) from another hepatocyte. A normal endothelial cell (E) is observed at the sinusoidal wall. Basal lamina is pointed out by arrows. Bar = 0.5 μ m.

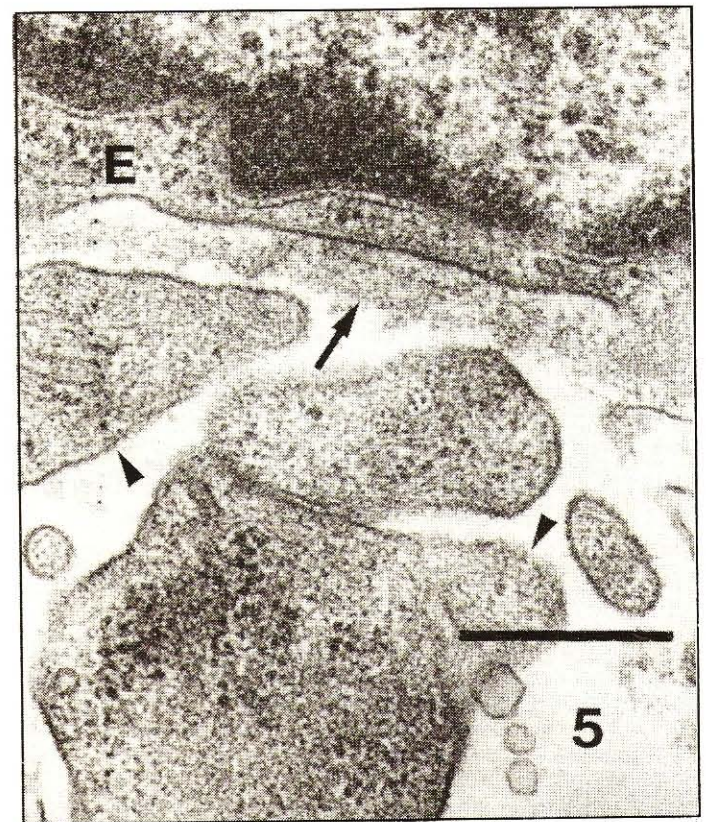


Fig. 5. Disse's space showing several swollen, deformed microvilli (arrowheads). Part of an endothelial cell (E) and an ill-defined basal lamina (arrow), are also observed. Bar = 0.5 μ m.

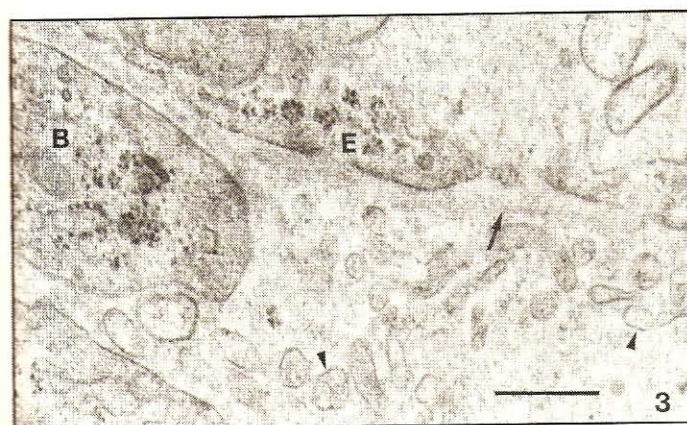


Fig. 3. A cytoplasmic projection or bleb (B) and swollen microvilli (arrowheads) are observed at the space of Disse. Endothelial cell (E); basal lamina (arrow). Bar = 0.5 μ m.

Sinusoids were dilated. The space of Disse was wide, containing scant, broad, microvilli, cytoplasmic blebs of hepatocytes, free hepatocytes, rests of organelles, and, in a great number of cases, erythrocytes and leucocytes (Figs. 1, 2, 3, 4, 6, 7).

The basal lamina was normal or slightly widened ($0.1\mu\text{m}$) and its structure was fuzzier than normal; only in few cases it was lost, and, in these cases, there also was a total destruction or loss of the sinusoidal wall (Fig. 6).

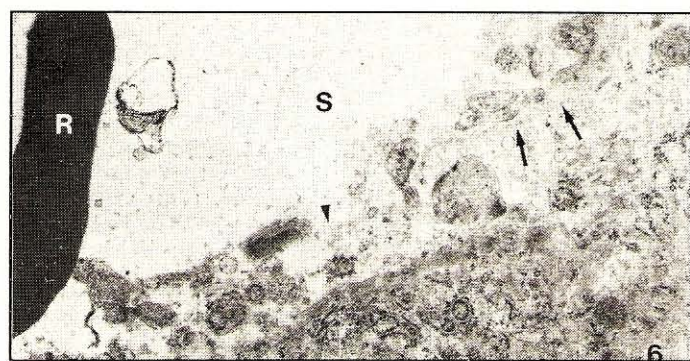


Fig. 6. Sinusoid (S) showing discontinuity of the sinusoidal wall (arrowhead) and an erythrocyte (R) entering the space of Disse. An ill-defined basal lamina (arrows) and part of a hepatocyte (H) are also observed. Bar = $0.5\mu\text{m}$.

The endothelial cells showed a slight increase in the number of their pinocytotic vesicles, otherwise were normal (Fig. 7). Kupffer cells were normal.



Fig. 7. Liver sinusoid (S) showing a normal endothelial cell (E), a widened Disse's space containing swollen microvilli (arrowheads), a cytoplasmic bleb (B) of a hepatocyte. A free mitochondria (M) indicates cell destruction. Bar = $0.5\mu\text{m}$.

The fine structure of hepatocytes of the normal animals, their microvilli, the space of Disse, the basal lamina, the endothelial and Kupffer cells, did not show apparent changes.

DISCUSSION

The basic lesion of liver telangiectasis consists in a focal dilation and congestion of the hepatic sinusoids. Its etiology is unknown. However, in cattle, it has been associated to several factors such as vascular inflammation of the portal system [3], focal hepatic necrosis [8] and toxic plants [21]. In humans, it has been associated to long term therapy with anabolic steroids [14] and in laboratory animals it has experimentally been produced with B1 aflatoxin [7], vinyl chloride [22], Lasiocarpine [18], experimental inoculations with murine leukemia 9H virus [4] or in mice transplanted with granulosa cell tumors or interstitial cell tumors [23].

The pathogenesis of bovine liver telangiectasis is still unknown and several theories have been proposed. Jensen et al. [10], have proposed that telangiectasis may be produced by

occlusion of sinusoids and necrosis of hepatocytes as a consequence of embolism caused by ulcerative lesions of the gastrointestinal tract. They have demonstrated the presence of venopathies associated with liver telangiectasis in animals with spontaneous lesions or experimentally inoculated with *Hemophilus somnus*, and concluded that the ischemia of hepatocytes was the initial cause of the lesion [10]. Getty [8] has proposed that the primary lesion of bovine liver telangiectasis is an inflammatory focus of hepatic necrosis. Andersen and Hart [1] proposed that hepatic telangiectasis is due to glycogen accumulation in the subendothelial region of the sinusoid which distort and destroy the endothelium, allowing the blood to enter the space of Disse, eroding the hepatocytes. Foschi [6] has pointed out that liver compression by tympany or pregnancy can cause vascular compression and telangiectasis.

Our findings have revealed hepatocytic changes characterized by a reduction or loss of microvilli, formation of cytoplasmic blebs into Disse's space and slight dilation of the endoplasmic reticulum, changes that are characteristics of cellular hypoxia. Similar changes have been observed in rats experimentally subjected to hypoxia [11, 12].

Autolysis of hepatocytes can also cause similar changes to those above mentioned [11]. However, in our cases, these changes were disregarded as autolytic because they were not seen in the livers of normal animals subjected to the same method of sacrifice, equal elapsing time between death and fixation and similar electron microscopy technique.

Hepatic cell necrosis and infiltration of leucocytes were not observed in our cases, so our results allow to point out that neither hepatic necrosis nor inflammation were the initial cause of bovine liver telangiectasis. In regard to glycogen, we observed a decrease of it in the hepatocytes, probably due to fasting of animals before sacrifice, and did not see its accumulation in the microvilli, the cytoplasmic blebs, or underneath endothelial cells; consequently, these findings do not support Andersen's theory. Reid et al [15] have demonstrated that fasting is a cause of glycogen depletion in hepatocytes.

Ischemic changes of hepatocytes followed by hydropic or vacuolar degeneration, as seen in our cases, cause a swelling of the affected cell, bringing about a shortening or disappearance of microvilli and can produce a greater tension upon the cell membrane with disruption of intercellular junctions, releasing the hepatocytes into Disse's space or the sinusoidal lumen, if the sinusoidal wall was broken; these changes were observed in few cases.

Dilation of sinusoids and Disse's space is an ultrastructural finding described in cases of liver telangiectasis (or Peliosis) in humans [17]; it was a constant finding in our cases. Dilation of Disse's space is due to shrinkage and reduction of hepatocytic microvilli, retraction of degenerating hepatocytes or to loosening and release of hepatic cells from the liver trabecula. In some cases we observed erythrocytes and leucocytes into Disse's space which indicated breakage of the sinusoidal wall, allowing the blood to enter this space. Destruction of the endothelial lining and the sinusoidal wall have also been observed in human liver telangiectasis [14, 17].

Changes in endothelial or Kupffer cells that could explain the breakage of the sinusoidal wall were not observed; for this reason, we think that sinusoidal wall breakage was just a physical event, probably due to a pressure increase by plasma in the space of Disse.

The changes observed correspond to a lesion initially produced by hypoxia or ischemia of hepatocytes, which brings about, as a consequence, a dilation of Disse's space, shrinkage and release of hepatocytes and breakage of the sinusoidal wall probably due to an increased pressure at the space of Disse.

Our ultrastructural findings support Jensen et al's [10] theory which states that ischemia of hepatocytes is the basic lesion of bovine liver telangiectasis.

ACKNOWLEDGEMENTS

Authors thank Dr. O. Castejón, J. Espinoza and R. Carpersen, Biological Research Institute, College of Medicine, University of Zulia, for technical help, and Mrs. M. Mitchell, for reviewing the manuscript. This research was financed by CONDES-LUZ.

REFERENCES

- [1] Andersen, A.C. and Hart, G.H. Histochemical methods in the study of telangiectasis. *Am. J. Vet. Res.* 13:359-365, 1952.
- [2] Andersen, A.C. The pathogenesis of telangiectasis in the bovine liver. I. The incidence of telangiectasis. *Am. J. Vet. Res.* 16:27-34, 1955.
- [3] Andersen, A.C. The pathogenesis of telangiectasis in the bovine liver. II. Histopathological and microbiological studies. *Am. J. Vet. Res.* 16:217-236, 1955.
- [4] Bergs, V.V. and Scotti, T.M. Virus-induced peliosis hepatitis in rats. *Science* 158:377, 1967.
- [5] Bohórquez, N.J. and Soto, J.A. Prevalencia de Telangiectasia hepática en bovinos de sacrificio. Proceedings X International Congress on Diseases of Cattle, pp. 1008-1021, México, 1978.
- [6] Foschi, M. Su di un caso di telangiectasia maculosa nel fegato di bovino. *Atti della Società Italiana delle Scienze Veterinarie* 24:493-495, 1965.
- [7] Gedek, B. and Hofmann, H. Animal experiments on the hepatotoxic effect of mycotoxins. 2. Chronic changes in the guinea pig. *Zentralbl. Veterinarmed. B* 17:667-676, 1970.
- [8] Getty, R. The histopathology of a focal hepatitis and of its termination (Sawdust and Telang liver) in cattle. *Am. J. Vet. Res.* 7:437-449, 1946.
- [9] Jensen, R.; Frey, P.R.; Cross, F. and Connell, W.E. Telangiectasis, sawdust and abscesses in the livers of beef cattle. *J. Am. Vet. Med. Assoc.* 110: 256-261, 1947.
- [10] Jensen, R.; Johnson, L.W.; Lauerman, L.H.; Tucker, J.O.; Swift, B.L.; Alexander, A.F.; Hancock, H.A.; Flack, D.E.; Braddy, P.M., and Horton, D.P. Ischemia: a cause of hepatic telangiectasis in cattle. *Am. J. Vet. Res.* 43:1436-1439, 1982.
- [11] Lemasters, J.J.; Ji, S. and Thurman, R.G. Centriolobular injury following hypoxia in isolated, perfused rat liver. *Science* 213: 661-663, 1981.
- [12] Lemasters, J.J.; Sternkowski, C.J.; Ji, S. and Thurman, R.G. Cell surface changes and enzyme release during hypoxia and reoxygenation in the isolated, perfused rat liver. *J. Cell. Biol.* 97: 778-786, 1983.
- [13] Malucelli, B.E. Teleangiectasia capilar hepática en bovinos. Estudio histológico e histoquímico. *Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo* 9: 147-158, 1972.
- [14] Olper-Palacci, R. and Aguirre-García, J. Peliosis hepatitis en pacientes tratados con esteroides anabólicos. *Patología* 13:341-353, 1975.
- [15] Reid, I.M.; Harrison, R.D. and Collins, R.A. Fasting and refeeding in the lactating cow. 2. The recovery of liver cell structure and function following a six-day fast. *J. Comp. Pathol.* 87: 253-265, 1977.
- [16] Roberts, J.L. The prevalence and economic significance of liver disorders and contamination in grain-fed and grass-fed cattle. *Aust. Vet. J.* 59: 129-132, 1982.
- [17] Ross, R.C.; Kovacs, K. and Horvath, E. Ultrastructure of peliosis hepatitis in a percutaneous biopsy. *Pathol. Eur.* 7: 273-279, 1972.
- [18] Ruebner, B.H.; Watanabe, K. and Wand, J.S. Lytic necrosis resembling peliosis hepatitis produced by Lasiocarpine in the mouse liver. *Am. J. Pathol.* 60: 247-270, 1970.
- [19] Soto, J.A. and Bohórquez, N.J. Análisis epidemiológico de decomisos sanitarios en bovinos sacrificados en el Estado Zulia. *Rev. Vet. Venezolana* 48: 12-29, 1984.
- [20] Wettimuny, S.G. de S. and Aturaliya D.S. An abattoir study of liver diseases of cattle. *Ceylon Vet. J.* 23: 54-57, 1975.
- [21] Williams, M.C.; Engleke, M.C. and Voigt, P.W. Abstracts 33 Annual Meeting Society for Range Management, pp. 43, 1980.
- [22] Winell, M.; Holmberg, B. and Kronevi, T. Biological effects of vinyl chloride: an experimental study. *Environ. Health Perspect.* 17: 211-216, 1976.
- [23] Wolstenholme, J.T. and Gardner, W.U. Sinusoidal dilatation occurring in livers of mice with a transplanted testicular tumor. *Proc. Soc. Exp. Biol. Med.* 74: 659, 1950.
- [24] Ziaii-Nia, H.; Zakarian, B. and Nowrouzian, I. An abattoir survey on telangiectasis of cattle and its economical losses in Iran. *J. Vet. Faculty University of Thehran* 36:67-84, 1981.