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Hepatocyte Ultrastructure in the Bovine Paraplegic Syndrome

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ABSTRACT

An ultrastructural study was made in liver biopsies from two control cows and one cow suffering from bovine paraplegic syndrome **(BPS)**. Alterations observed included a decrease of glycogen particles, changes in mitochondrial shape and electron-density, and the association of multiple elements of smooth endoplasmic reticulum with mitochondria. Peroxisomes surrounded by several layers of concentric membranes were observed. This study supports the point of view that BPS could be produced by the combined effects of different specific nutritional deficiencies.

KEY WORDS

Bovine paraplegic syndrome, hepatocyte, ultrastructure

INTRODUCTION

Skeletal muscle from animals with bovine paraplegic syndrome **(BPS)** shows different alterations recently described and related to several mineral deficiencies which produce myopathies (1). These myopathies have been produced experimentally by phosphorous (2,3,4), magnesium (5) and potassium[6] deficiencies. BPS is a pathological condition which affects Zebu-bos taurus hybrids, specially cows with high physiological exigencies including pregnancy and lactation.

It has been shown that partial starvation provokes ultrastructural changes of rough endoplasmic reticulum and mitochondria (7), and the disappearance of glycogen particles (8) in rat liver parenchymal cells. Additionally, hepatomegaly has been associated with Cu deficiency (9). However, hepatocyte ulrastructure has not been described in experimental models related to mineral deficiencies. In this work the hepatocyte ultrastructure alterations in BPS are related.

MATERIAL AND METHODS

Liver biopsies were obtained from two normal cows and one with BPS. Blocks of approximately 3 mm x 1 mm were fixed in 3 % glutaraldehyde in phosphate buffer at pH 7.4 and 320 mOsm, postfixed in 1 % OsO_4 and embedded in LX-112 resin (Ladd Research Inc, Burlington). Ultrathin sections were stained with uranyl acetate

and lead citrate and observed in a Hitachi H-500 transmission electron microscope. Thick sections (1-2 μm) were stained with toluidine blue for light microscopy.

RESULTS

Similarly to the observed in different mammals [10], hepatocytes from normal adult bovines present large mitochondria, peroxisomes, rough endoplasmic reticulum (**RER**), and glycogen particles associated with smooth endoplasmic reticulum (**SER**) (**Fig. 1**).

Hepatocytes from paraplegic animals exhibited mitochondria with an almost electron transparent matrix, and scarce or absent glycogen particles (Fig. 2, 3, 4, 5, 6, 7). Some mitochondria showed irregular contours (**Fig. 2, 3**) but most of them were rounded (**Fig. 4**), and some partially covered by concentric membranes of **SER** (**Fig. 3, 4, 5**). In **Fig. 3** and **4** it is possible to observe that such membranes are connected with **RER** cisternae. **RER** was particularly abundant close to the nucleus (**Fig. 3**). Concentric membranes were located surrounding peroxisomes (**Fig. 5**), forming myelin-like figures (**Fig. 4**), and complex associations with cisternae of **RER** (**Fig. 6**). Additionally, concentric membranes were found limiting cytoplasmic zones containing free polysomes (**Fig. 7**).

DISCUSSION

Most of evidences supports the point of view that **BPS** is a nutritional problem. The comparison of **BPS** skeletal muscle alterations with those found in some specific deficiencies reinforces this opinion [1]. Because of these reasons we compared the hepatic abnormalities we found with those previously reported in nutritional problems.

The hepatocytes in **BPS** showed a decrease of glycogen particles, the same change has been reported in experimentally starved animals [7, 8]. Nevertheless, hepatic glycogen is a highly unstable molecule and its decrease is an unspecified finding. We could observe that hepatic mitochondria in **BPS** were surrounded by several layers of **SER** which were connected with **RER**. On the contrary, hepatic mitochondria from partially starved rats were outlined by **RER** [7]. Hepatocytes from fasted rats showed a proliferation of **SER** dispersed in the cytoplasm [8]. We did not find this change. In starved rats large and pleomorphic mitochondria have been observed [7]. In **BPS** irregular and rounded mitochondria were seen. These altered mitochondria exhibited a background matrix with a very low electron-density. Such matrix modifications could be related to nutritional deficiencies. In fact, two different ultrastructural

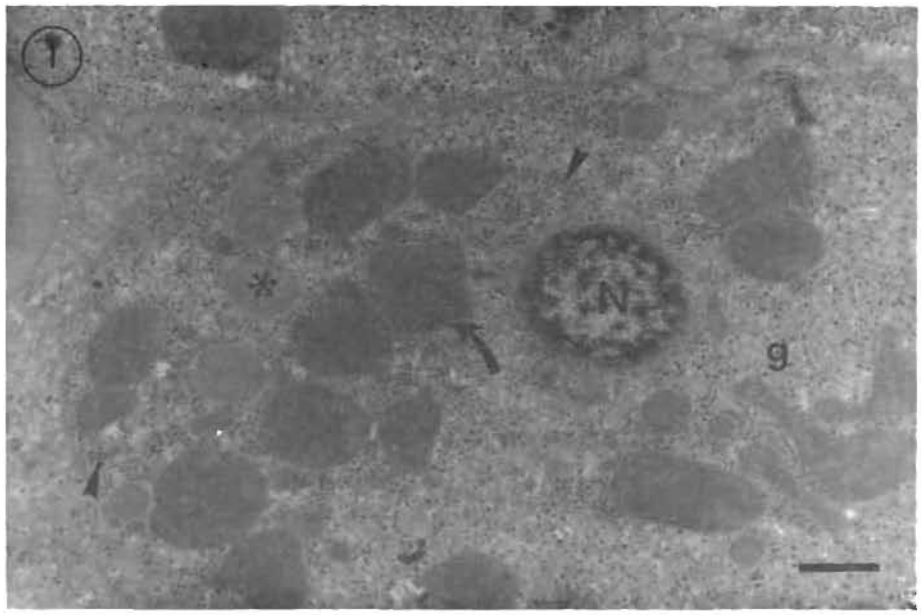

Fig. 1. Section from bovine normal hepatocyte. Abundant mitochondria (arrow), peroxisomes (asterisk), rough endoplasmic reticulum (arrowheads) and glycogen particles (g) are shown. Note that nucleus (N) was cut at the periphery. Bar = 1μm.

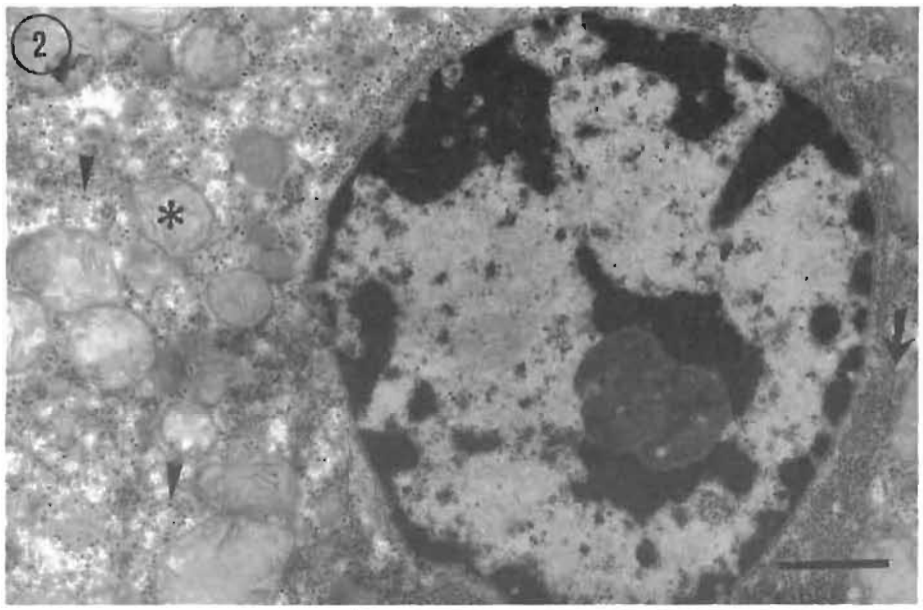

Fig. 2. All the following figures are hepatocyte sections from animals with **BPS**. Mitochondria (asterisk), **RER** (arrow) and glycogen particles (arrowheads) are located around the nucleus. Bar = 1 μm.

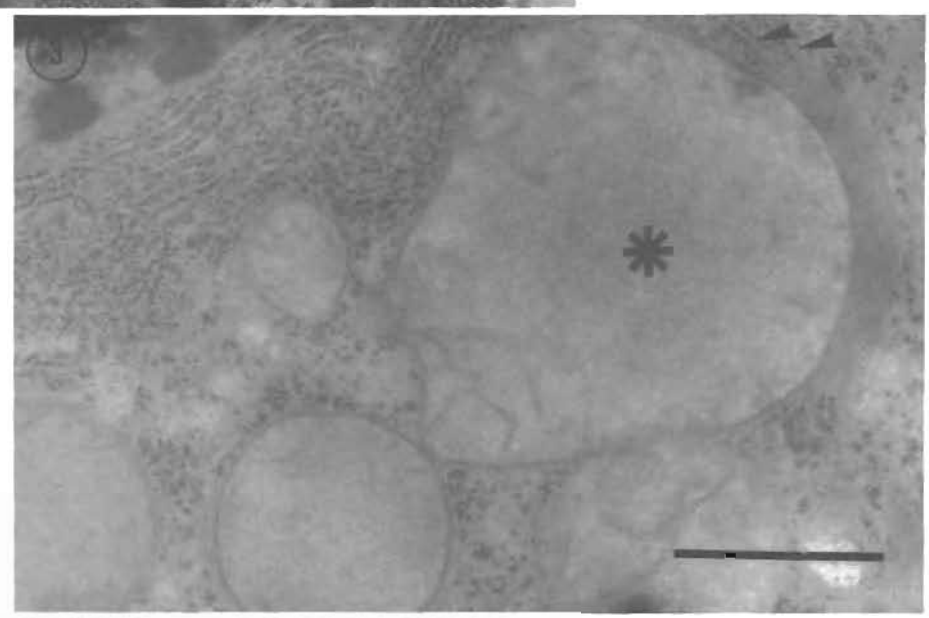

Fig. 3. A mitochondrion (asterisk) is partially covered by concentric membranes of **SER** which are connected with **RER** (arrowheads). Bar = 1 μm.

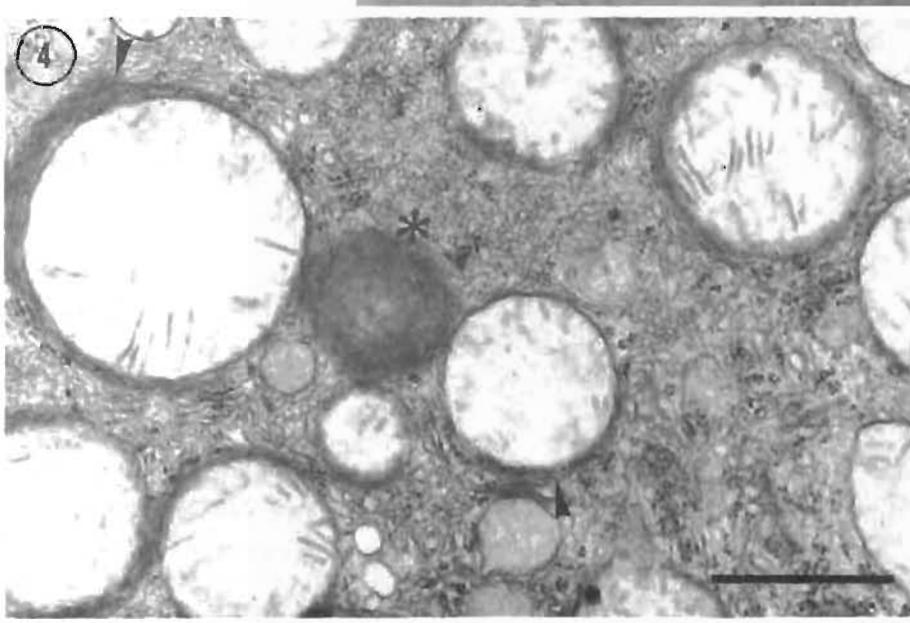

Fig. 4 .Some rounded mitochondria are covered by SER membranes (arrowheads). Note a myelin-like figure and circular profiles of SER (asterik). Bar = 1 μm.

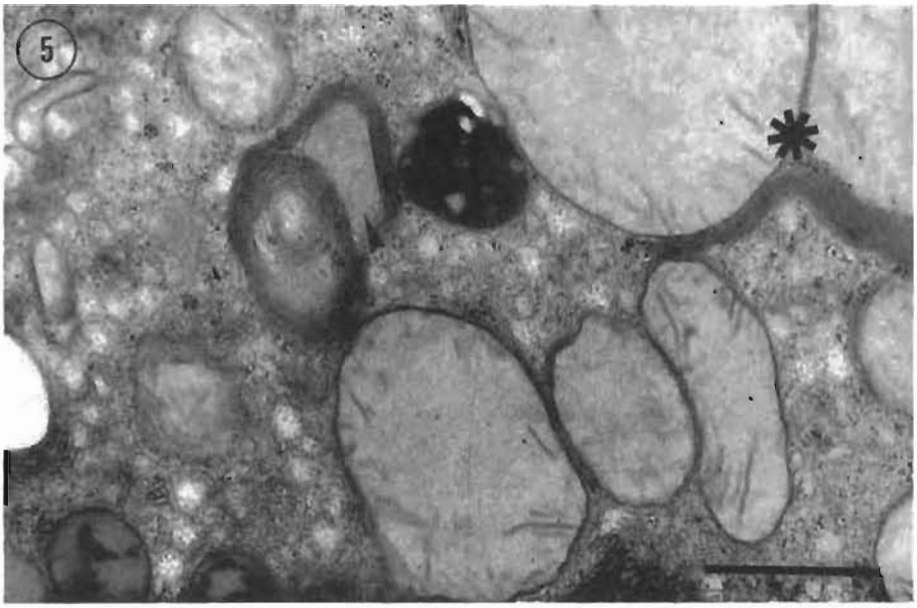

Fig. 5. SER concentric membranes are found in contact with mitochondria (asterisk) and a peroxisome (arrowhead).
Bar = 1 μm.

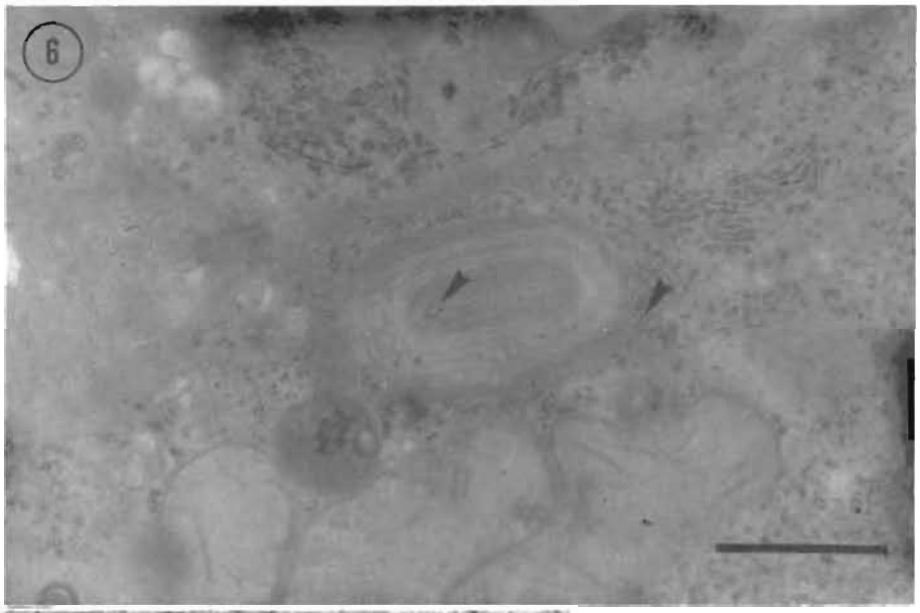

Fig. 6. A myelin-like figure is associated with **RER** (arrowheads). Bar = 1 μm.

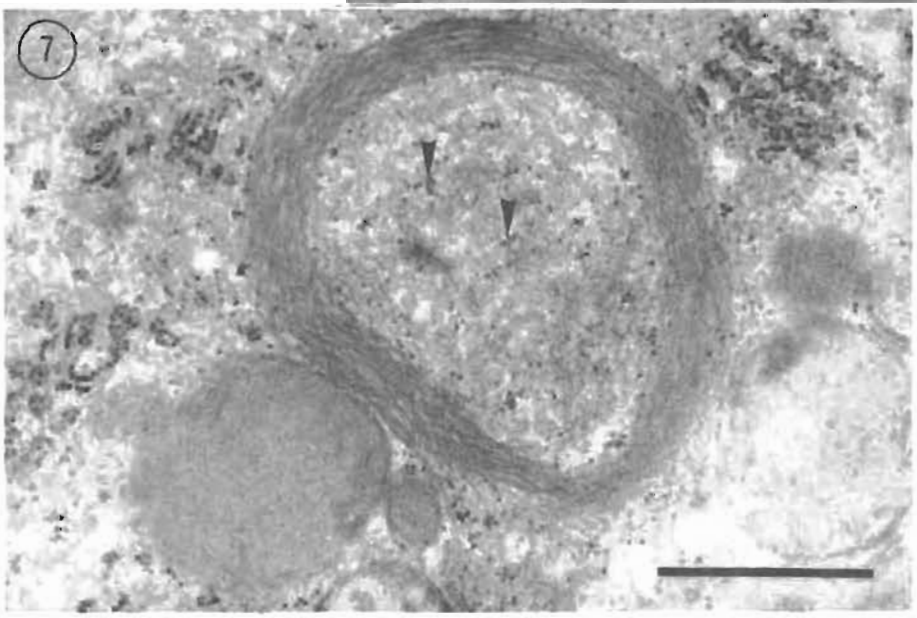

Fig. 7. SER concentric membranes limit a zone with polysomes(arrowheads). Bar = 1 μm.

states have been observed in mitochondria depending on whether they are respiring without ADP or actively phosphorilating [11]. It is noteworthy that skeletal muscle mitochondria have been found altered and undergoing autophagia in experimental hypophosphatemia [12, 13]. In these works mitochondria were not surrounded by concentric membranes. In cooper deficiency mitochondrial hypertrophy and hyperplasia have been found in brain and heart. A mild hepatomegaly was also observed [9].

In **BPS** peroxisomes are surrounded by several layers of concentric membranes. In normal hepatocytes it is common to see a single **SER** element around peroxisomes [14]. Abnormalities of peroxisomes were observed neither in partially starved [7] or in fasted rats [8]. The association of mitochondria and peroxisomes with concentric membranes is an interesting finding because both are respiratory organelles. Peroxisomes are engaged in H_2O_2-based respiration reactions [14] and capable of degrading fatty acids via beta-oxidation [15]. This relationship could be a connection between **BPS** and energy metabolism disturbances as caused by nutritional deficiencies.

Ours results show that **BPS** exhibits a distinct histopathological picture. This finding indicates that , as a nutritional problem, **BPS** seems to be different from starvation caused by food restriction. It is possible that **BPS** is caused by the combined effects of different specific nutritional deficiencies.

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RESUMEN

Se realizó un estudio ultraestructural de biopsias provenientes de dos vacas controles normales y de una afectada por el síndrome parapléjico del bovino (**SPB**). Las alteraciones observadas incluyeron una disminución de las partículas de glucógeno, cambios en la forma mitocondrial y en la densidad electrónica de su matriz, así como la asociación de elementos múltiples del retículo endoplasmático liso con ese organelo. Asimismo, se localizaron peroxisomas rodeados por varias capas de membranas dispuestas concéntricamente. El presente estudio apoya el punto de vista según el cual el SPB es producido por los efectos combinados de varias deficiencias nutricionales específicas.

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