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# XENOBIOTIC - INDUCED PEROXISOMES IN THE RABBIT LIVER

*HOLOVSKÁ K., ALMÁŠIOVÁ V., CIGÁNKOVÁ V.*

Depatrment of Histology and Embryology, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic

**ABSTRACT**

The study investigated changes in ultrastructure of the liver in the rabbit (*Oryctolagus cuniculus*) which was administered bendiocarb for 3 days. Electron microscopy was used to observe changes in ultrastructure of hepatocytes. The changes were focal and the effect on the liver was not uniform. Some hepatocytes showed no obvious changes in their ultrastructure. In the changed hepatocytes, we observed dilatation of bile capillaries but no visible alterations in the intercellular contacts. Many hepatocytes showed considerable increase in the number of peroxisomes. We observed, that bendiocarb induced ultrastructural changes of hepatocytes but only to a moderate extent.

## INTRODUCTION

The current agricultural practice involves the use of many protective chemical substances which contribute to considerable environmental contamination. These environmental contaminants include also insect control preparations. Carbamate insecticides are widely used for agricultural and indoor purposes. They act as acetylcholinesterase (AChE) inhibitors which affect many organs, such as peripheral and central nervous system, muscles, liver, brain and heart. Bendiocarb is a toxic insecticide. In the body it undergoes hydrolysis to organic metabolites which can also affect the total toxicity of this insecticide. Bendiocarb is rapidly detoxified and excreted (Bendiocarb, 1982). Despite that many scientists observed various morphological changes in different organs. Immunosuppressive effects of bendiocarb were observed after its long-term administration (Mojžišová, 2004). It also induced morphological changes in lymphatic organs. Flešárová et al. (2007) performed morphometric analysis of the thymus exposed to bendiocarb and observed decreased number of cells and reduced volume of its medullar portion. An adverse effect of bendiocarb on the structure of lymph nodes was confirmed by Petrovová et al. (2010) and Špaleková et al. (2010). Almašiová et al. (2012) observed a subchronic influence of bendiocarbamate on histological structure of kidneys which exhibited changes of varying extent in both the cortex and medulla. Bendiocarb caused damage not only in renal corpuscles, but also in individual parts of renal tubules.

Carbamate insecticides are metabolised particularly in the liver due to various chemical reactions aimed at conversion of lipophilic compounds to hydrophilic ones. Because of that our study focused on the influence of bendiocarbamate on the structure and ultrastructure of liver in the common rabbit, the non-target species.

## MATERIAL AND METHODS

### Animals and diets

The experiment was carried out on 12 rabbits (*Oryctolagus cuniculus*), 54-day old, hybrid Hyla-27, mean weight 2.0±0.20 kg. The clinically healthy rabbits were kept in a well ventilated environment and received a standard diet Norm-type-0-10 (BIOFER, 7313/A) and water *ad libitum*. The animals were divided into two groups, six animals in each (control and day 3). Rabbits in experimental group were administrated bendiocarb (96 % Bendiocarb, Bayer) perorally at a dose of 5 mg/kg per day in capsule (Petrovová et al., 2010). The condition of the animals, their manipulation and use corresponded with the requirements of the ethical commission.

**Ultrastructural study**

The samples intended for transmission electron microscopy (TEM) were fixed in 3 % glutaraldehyde, postfixed in 1 % $OsO_4$ (both in a phosphate buffer pH 7.2-7.4), dehydrated in acetone and embedded in Durcupan ACM (Fluka). The ultrathin sections were cut on an ultramicrotome Tesla BS 490, stained with uranyl acetate and lead citrate and evaluated under a transmission electron microscope Tesla BS 500.

**RESULTS**

In the control group we observed hepatocytes of polyhedric shape with a centrally located, round, euchromatic nucleus and pronounced nucleolus. Plasma membranes of neighbouring hepatocytes contacted by their lateral surfaces where one could observe canalicular forms, the bile capillaries. Microvilli protruded into their lumen. In these places the plasma membranes were connected by desmosomes and tight intercellular junctions. The plasma membrane of the apical portion of hepatocytes contained microvilli which extended to the space of Disse.

Mitochondria prevailed in the cytoplasm of hepatocytes. They had oval or round shape with cristae mitochondriales. Their matrix contained electron-dense granules. The hepatocytes had well developed endoplasmic reticulum (ER) and Golgi system. The granulated endoplasmic reticulum consisted of dense, parallel canals with numerous ribosomes. In the cytoplasm, there were also free ribozomes arranged into spirals or rosettes.

The space of Disse was separated from sinusoids by fenestrated endothelial cells. Their cytoplasmic membrane formed short interconnected projections. The cytoplasm of endothelial cells contained compact, oval nucleus, mitochondria and pinocytal vesicles. Kuppfer cells could be observed in blood sinusoids. They had large, oval, euchromatic nucleus of irregular shape. The cytoplasm was light, contained mitochondria, endoplasmic reticulum and lysosomes of various shape and size. Ito cells, the so-called lipocytes, could be observed sporadically in the space of Disse. Their nucleus was large and irregular. A characteristic feature of these cells was the presence of fat droplets of varying size (Fig. 1).

In the experimental group**,** which were administered bendiocarb for 3 days**,** we observed ultrastructural changes of varying extent in the liver. The organ was not affected uniformly. The ultrastructure of some hepatocytes was comparable with that in the control. On the other hand, the number of peroxisomes was markedly increased in many hepatocytes. These organelles had considerably electron-dense matrix and occurred on the periphery of hepatocytes, or were uniformly distributed in the cytoplasm. Small fat droplets appeared sporadically in the hepatocytes (Fig. 2). Pronounced changes were observed also in bile capillaries. They were dilated and the microvilli were reduced. However, there were no visible changes in intercellular contacts of adjacent hepatocytes. These cells showed changes in both bile capillaries and the nucleus. The nucleus had an irregular shape but retained its nucleal membrane (Fig. 3).

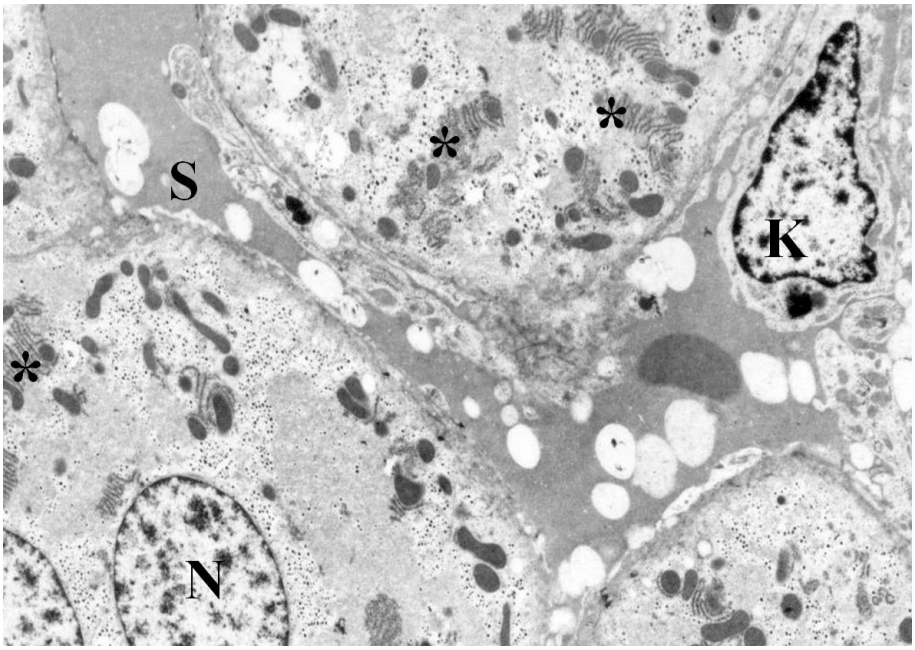


**Figure 1** Ultrastructure of liver of control rabbit. N - nucleus; S - sinusoid; K - Kupffer cell; asterisk - rough endoplasmic reticulum
Magn. 4 250 x

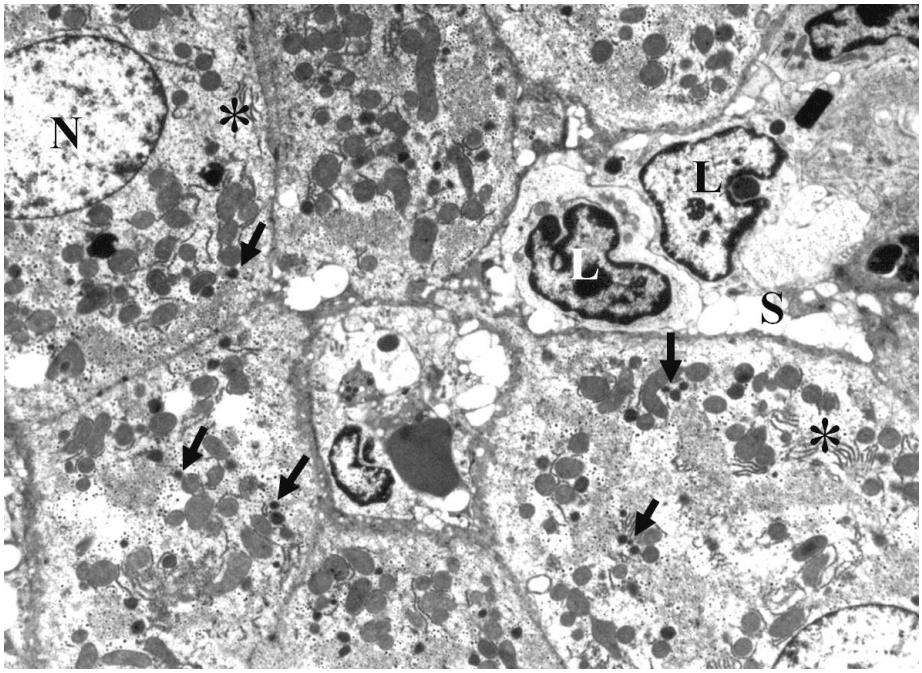


**Figure 2** Ultrastructure of liver on day 3 of the experiment. N - nucleus; S, sinusoid; L - lymphocyte; asterisk - rough endoplasmic reticulum, arrow - peroxisomes
Magn. 4 612 x

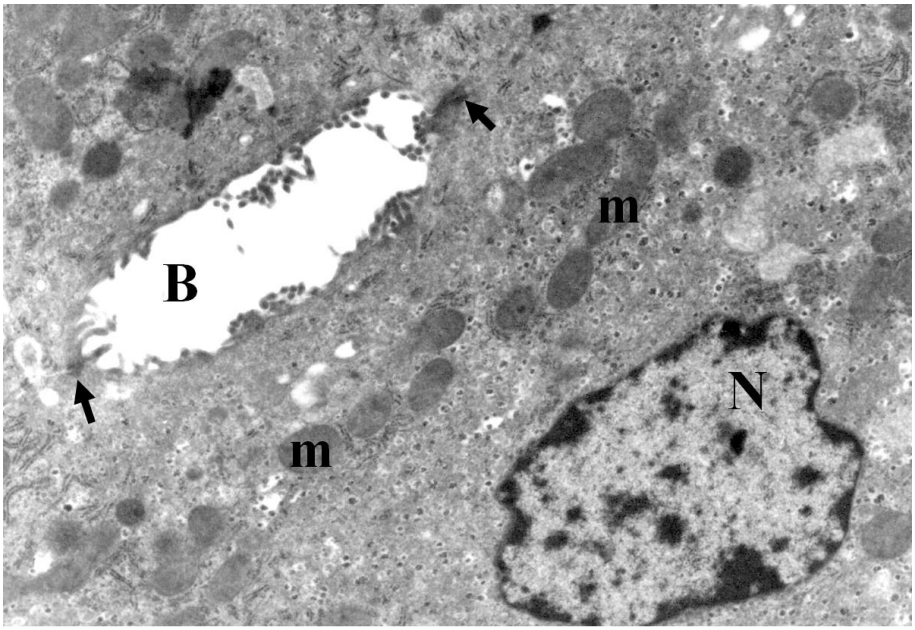


**Figure 3** Ultrastructure of liver on day 3 of the experiment. N - nucleus; B - bile canaliculus; m - mitochondrion; arrow - intercellular contacts
Magn. 6 517 x

**DISCUSSION**

The liver plays an important role in many essential functions of base metabolism. It is the principal organ of accumulation, biotransformation and excretion of contaminants, for example pesticides (Matos et al., 2007). The study of ultrastructure of the rabbit liver after administration of bendiocarb showed changes of different extent. The most pronounced changes involved bile capillaries. They were dilated and their microvilli were reduced. In many hepatocytes we observed considerably increased number of peroxisomes. The peroxisomes are highly dynamic organelles. Depending on cell type, the number of peroxisomes per cell as well as their shape may vary greatly (Singh, 1997). Certain chemicals are capable of increasing the size and number of peroxisomes, and are designated as peroxisome proliferators (PP). Peroxisome proliferators include hypolipidemic drugs, plasticizers and organic solvents used in chemical industry, pesticides and many toxic environmental pollutants. The liver is a target organ of many toxic compounds including PP and these chemicals regulate gene transcription through a receptor, PPARα, which is expressed in hepatocytes (Dansen et al., 2001). As the number of peroxisomes and their enzymatic equipment depend not only on the cell type but also on various other factors, one can assume that bendiocarbamate or some of its metabolites induced increase in their number in hepatocytes.

Also the inflammation that was observed in our previous experiments (Holovská et al., 2011), could induce changes in the function of peroxisomes. Inflammatory cytokines, such as TNF-alpha, suppress catalase and enzymes of β-oxidation of fatty acids (Beier et al., 1992, 1997). Changes in the activity of these enzymes in peroxisomes may explain both the disorders of metabolism of fats and increased production of reactive oxygen species (ROS) (Schnitzky, 1987). The metabolism of ROS is an important function because ROS are considered important mediators in a variety of cellular pathological processes. Alterations in the structure of peroxisomes that were observed following ischemia-reperfusion were reflected in a decrease in peroxisomal activities (e.g. catalase, beta-oxidation of lignoceric acid and others).

Investigations of the influence of bendiocarbamate on the activity of antioxidant enzymes in the rabbit showed no changes in the activity of catalase in experimental groups. On day three of the experiment, there were detected pronounced changes in the activity of glutathione peroxidase ($GP_{H2O2}$) and increased content of TBARS, an important indicator of oxidative stress (Sobeková et al., 2009).

**CONCLUSION**

Although bendiocarb does not accumulate in the body, it induced inflammatory changes in the liver of rabbits. They were observed under a light microscope, particularly on day 3 of the experiment. Inflammatory changes affected not only the portobiliary space but spread along the periphery of lobules. Electron microscopy confirmed changes in the ultrastructure of hepatocytes. Bendiocarb induced dilatation of bile capillaries and caused marked increase in the number of peroxisomes. The changes observed in our study were of moderate extent. Our observations allowed us to assume that due to its detoxication abilities the liver was capable of coping with the harmful effects of the investigated pesticide.

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