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Ultrastructure of liver from piglets fed Tower rapeseed oil

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Summary. Effect of Tower rapeseed oil (RO) containing lower (0.4%) erucic acid on the piglet liver was studied by electron microscopy. The animals were placed in two groups of four and were fed the diets for eight weeks. Animals in the treated group were given a basal diet comprising corn-soybean plus Tower RO at a 10% concentration; animals that were given the basal diet with no added oil served as the controls. Architecture of the liver from animals of the control group appeared normal. Ultrastructural changes in the liver of RO-fed animals included dilation of lumens and loss of microvilli in bile canaliculi. In addition, closely aligned mitochondrial cristae, elevated number of peroxisomes, and an unusual increase in the size and number of membrane bound spaces in zone 3 of liver acini were noticed. These alterations may reflect a disturbance in piglet liver functions following Tower RO feeding.

Key words: Piglet liver, Electron microscopy, Tower rapeseed oil, Liver ultrastructure

Introduction

Rapeseed oil (RO) and meal are important sources of protein and energy. Tower variety RO has been widely used as an energy supplement in animal feed and in many food products for humans. Historically, rapeseed is the fifth most important oilseed crop in the world, and Canada was a major producer and exporter of the oil (Fenwick and Curtis, 1980). Fatty acid composition of rapeseed and other dietary oils has been investigated by many workers (Downey, 1971; Appelquist, 1972; Banfield, 1974; Hulan et al., 1976; Daun and Hougan, 1977). RO lipids are known to contain variable amounts of the mono-unsaturated fatty acid, cis-13-docosenoic acid, commonly referred to as erucic acid (EA) (Vles, 1975). In canola oils, the modern rapeseed varieties known as «double low» (Ward, 1977), EA constitutes less than 5% of the total fatty acid present in the oils. Other long chain fatty acids with 20, 22 and 24 carbon atoms have also been detected in RO (Haeffner, 1970; Conacher and Page, 1972).

Pathological effects following excessive feeding of the rapeseed products to animals have been reported. Earlier investigations point to EA as the possible causative factor in the cardiotoxicity (Abdellatif and Vles, 1970; Beare-Rogers et al., 1972), and in growth retardation in the rat (Thomsson and Boldingh, 1955). Kramer et al. (1973) state that feeding RO containing high EA causes lipid accumulation in the heart, adrenal glands and skeletal muscle of rats within the first week of feeding. Short term myocardial alteration was reported in rats fed diets containing as little as 2% EA (Engfeldt and Brunius, 1975). Several reviews of the early studies on RO implied that EA was a cause of adverse nutritional effects (Johnson, 1977) but others considered the low ratio of saturated/monounsaturated fatty acids in the oil to be a contributory cause (Borg, 1975).

Singh et al. (1976) reported ultrastructural alterations in the liver of pigs fed diets containing RO with high EA content. Even a diet containing RO with low levels of EA resulted in a heightened number of peroxisomes, an observation that was later corroborated by Christiansen et al. (1979), and reduced number of mitochondria with irregularly arranged cristae. Conversely, Beare-Rogers (1977) reported absence of histopathological alterations in the liver of pigs fed various types of RO. Thus, the effects of Tower RO on growing pigs remain poorly understood. The ultrastructural alterations in liver of piglets after feeding Tower RO with low (0.4%) EA content are described in this report.

Materials and methods

Animals

Eight Yorkshire piglets were weaned at four weeks of age and equal number of animals were placed in the two groups. Animals in the control group received corn-soybean meal with no added fat. Animals in the treated group received the control diet comprising the meal plus 10% Tower RO (*Brassica napus*, 0.4% EA content). The 10% fat when added to the control diet replaced the same amount of corn meal. The animals were kept in

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individual cages for eight weeks. The piglets were approximately 20 kg at the time of termination of the experiment. The animals were initially stunned by electric shock and subsequently were euthanized by severance of the jugular vein.

Electron microscopy

Approximately 1 mm^3 specimens of liver were obtained, and fixed in 2% buffered (0.1M phosphate) glutaraldehyde, and postfixed in 2% osmium tetroxide prepared in the same buffer. The fixed samples were dehydrated in ascending concentrations of ethanol, cleared in propanol, and infiltrated with and embedded in Epon resin by conventional methods (Singh et al., 1981) for transmission electron microscopy. Semithin sections of the selected areas in the specimens were cut with an ultramicrotome. Thin sections were prepared with an ultramicrotome, mounted on carbon-coated grids, contrasted with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a Jeol (JEM-100S) electron microscope at 80 kV.

Results

Control group animals

The liver from piglets of the control group were unaltered in morphology (Fig. 1). Infrequently binucleated hepatocytes were observed. The smooth endoplasmic reticulum (SER) comprised close-meshed networks of tubular profiles adjacent to glycogen particles. The rough endoplasmic reticulum (RER) consisted of membranes arranged as stacks of flattened cisternae (Fig. 1). In many instances, a stack of RER cisternae could be seen in close proximity to a bile canaliculus. The pleomorphic mitochondria had round-to-elongated profiles (Fig. 1). Typically the mitochondrial cristae were perpendicular to long axis of the organelle. Peroxisomes were revealed infrequently in these images. Glycogen granules were apparent as electron-dense particles (Fig. 1). The particles occurred both in α and ß forms randomly distributed in close association with SER throughout the cytoplasm, and were seen more frequently in zone 2 and 3 of liver acini. Lipid droplets that occurred randomly in a classic lobule were round. Short and slender microvilli from biliary surfaces of the hepatocytes projected into the canalicular lumen. Cytoplasm lacunae or membrane bound spaces (MBSs), a feature in the pig, were electron-lucent regions of variable shape that were bound by a single membrane in the hepatocyte cytoplasm. Number of these spaces was higher in zone 1 than in the other two zones.

Treated animals

Hepatocytes from the animals fed Tower RO were altered. Dilation of bile canaliculus lumen accompanied by a loss of microvilli (Figs. 2, 3) was a striking feature in the cells. A reduction in the number of microvilli was variable ranging from a total loss in a canaliculus to the disappearance of only a few microvilli in other canaliculi. Some abnormally dilated lumens contained debris (Fig. 3). The mitochondrial morphology in the hepatocytes was similar to that in the corresponding cells from the animals of the control group; nevertheless, a few mitochondria possessed condensed cristae as illustrated in Figs. 3 and 4. Orientation of the cristae was altered in that these were arranged parallel to long axis of the organelle. The SER profiles had increased (Fig. 3) in comparison with those in the parenchymal cells from animals in the control group. MBSs were abundant, particularly in zone 3 hepatocytes. In some instances, MBSs deformed the nucleus (Fig. 2). Lysosomal elements (Fig. 2) and peroxisomes (Figs. 3, 4) were more abundant than in the cells from animals of the previous group. The above is a generalized description of the alterations that occurred in all the RO-fed animals.

The nuclei, RER and Golgi complex were unaltered, and lipid droplets distribution in the treated hepatocytes was similar to that in the cells of animals from the control group.

Discussion

Ultrastructural alterations were revealed in the liver parenchymal cells of piglets fed diets containing 10% Tower RO for eight weeks. The changes comprised abnormal bile canaliculi and mitochondria, and augmentation of SER profiles and peroxisomes numbers. In addition, size and number of MBSs had elevated. Microvilli of bile canaliculi are the seat of bile formation initiation, and bile composition is determined by both canalicular and ductular function (Chen and Chen, 1977). Thus a loss of microvilli, as depicted in Figs. 2 and 3 of the present study, may affect the active secretion of bile and the diffusion of water and small inorganic ions that may cause cholestasis (Chen and Chen, 1977). A slow flow of bile which is caused by partial blockage of the canaliculi by debris (Fig. 3) may be compensated for by dilation of canaliculi. Either partial blockage or dilation of bile canaliculi coupled with the loss of microvilli that were widespread in the liver of treated animals in our study may affect normal production and flow of the bile which, in turn, may lead to cholestasis and/or disturbance of lipid metabolism.

The number of peroxisomes had elevated in treated piglets in the present study. The porcine peroxisome size, as described by Singh et al. (1978), was not changed. Recently, De Craemer et al. (1994) have shown mouse hepatic peroxisomes remained unaltered in size but were heightened in number after administration of a high-fat diet. Furthermore, they pointed out an increase in size of the organelle following treatment with xenobiotic peroxisome proliferators. The ß-oxidation of fatty acids catalyzed by peroxisomes is well documented (Lazarow, 1994). However, most of the data were obtained by studies on the rat liver. For instance, in rat

liver the organelle contains three acyl-CoA oxidases including palmitoyl-CoA oxidase (Van Veldhoven et al., 1992) that oxidises CoA esters of long-chain fatty acids; rapeseed oils contain such type of fatty acids (Appelquist, 1972). This is tempting to speculate that ß-oxidation mechanism in hepatic peroxisomes of pigs is similar to that in the rat which produced abnormal number of the organelle following Tower RO feeding to the growing pig in the present study. In addition, Ishii et al. (1980) and Neat et al. (1980) have reported heightening of hepatic ß-oxidation in rats fed high-fat diet.

Mitochondria contain cristae that arranged generally perpendicular to long axis of the organelle (Ghadially, 1988). Longitudinally and closely arranged mitochondrial cristae may indicate an interference with the normal function of the liver. Because of the critical role played by the mitochondrial inner membrane, its perturbation may lead to impairment in their function which may affect the cell viability (Sternlieb, 1979). Rohr and Riede (1973) suggest that the condensed configuration of mitochondria is due probably to hypermetabolism in the cell.

Lysosomes are capable of digesting and degrading

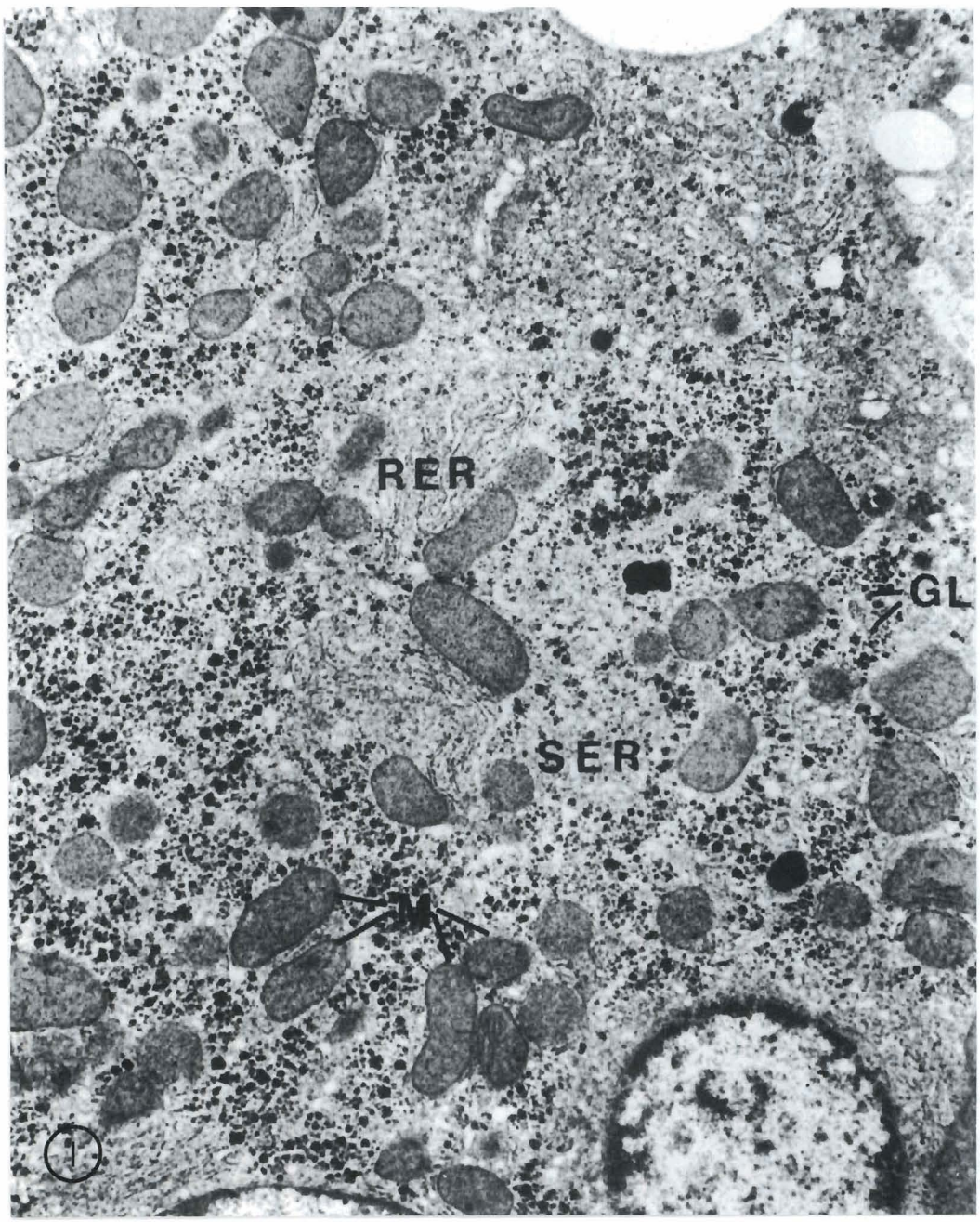


Fig. 1. Electron micrographs of portions of two hepatocytes from a piglet of the control group. A normal distribution and morphology of organelles including rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and mitochondria (M) is depicted. GL: Glycogen particles. x 9,000

cell matter since they contain a large complement of hydrolytic enzymes (Seglen and Bohley, 1992). In our study an apparent augmentation of lysosomal elements and residual bodies (Fig. 2) is considered a direct response to the RO feeding because the comparable cells in animals from the control group did not reveal this lesion. Probably a large amount of hydrolytic enzymes was required for autophagy of abnormal number of organelles and inclusions in the treated cells.

The MBSs, a normal feature of porcine hepatocytes (Flaks, 1971), were present particularly in zone 1 (periportal area) of the liver acini in animals of the control group. Shahidi (1983), and Singh and Shahidi (1986) have described the MBSs and other features (also Gilroy et al. 1995) of the growing pig liver. The spaces were more commonly seen in the cells of the treated liver in zone 3 (perivenous area). Although functions of the MBSs are obscure (Svendsen, 1974), these spaces have been reported to augment the physiologically important surface of hepatocytes, and thus elevate the level of exchange of nutrients and metabolic products between blood and the cytoplasm (Soares et al., 1980).

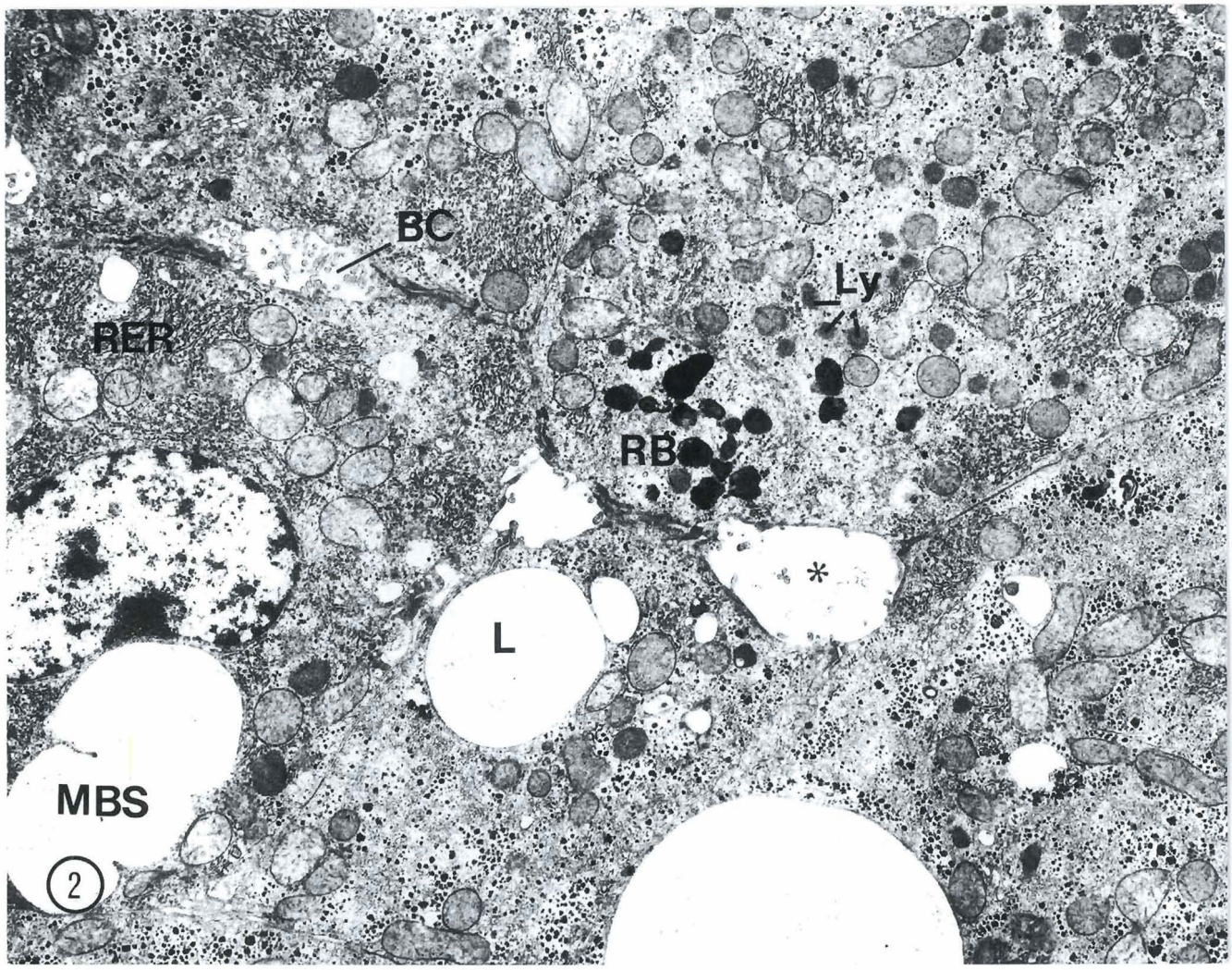
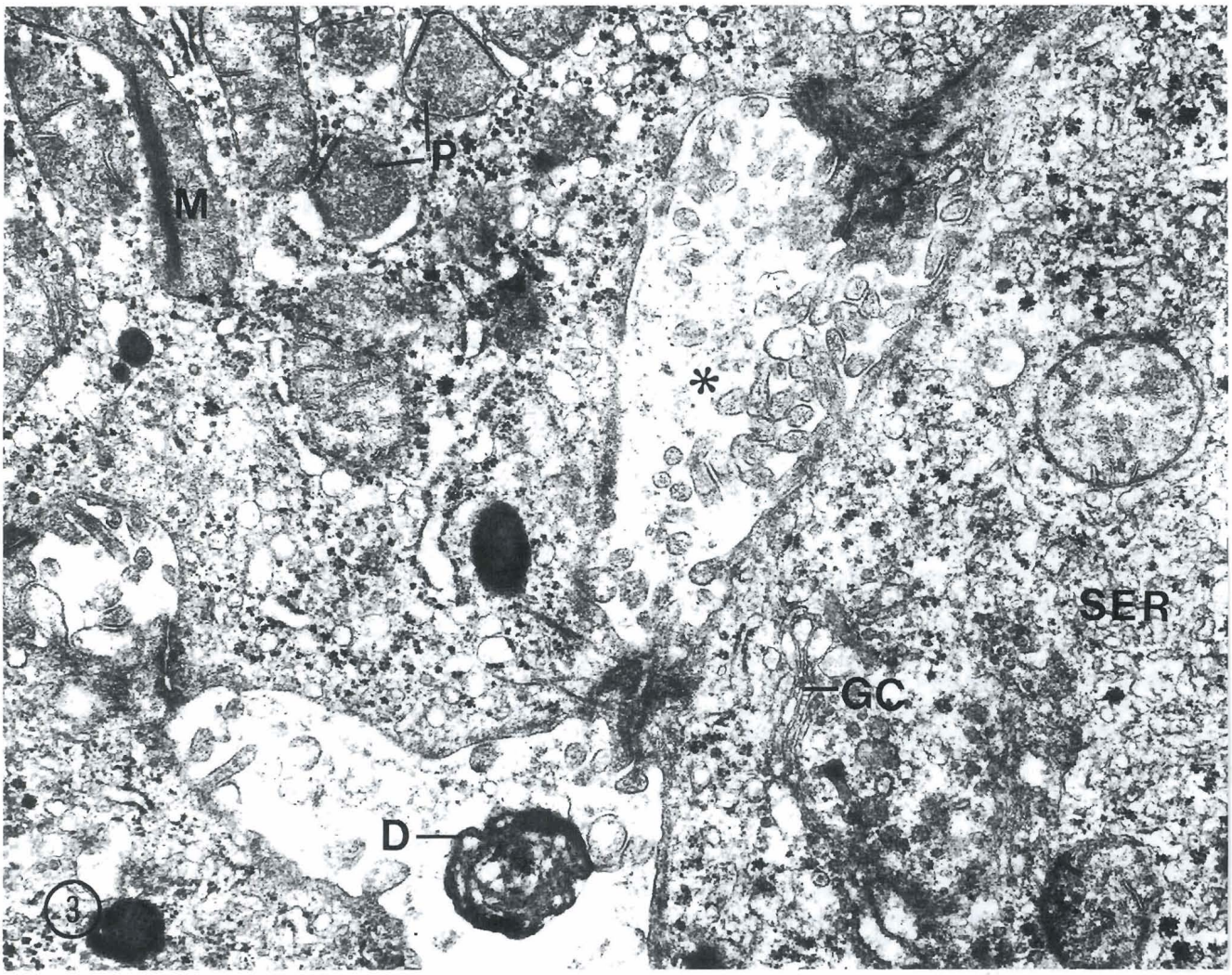
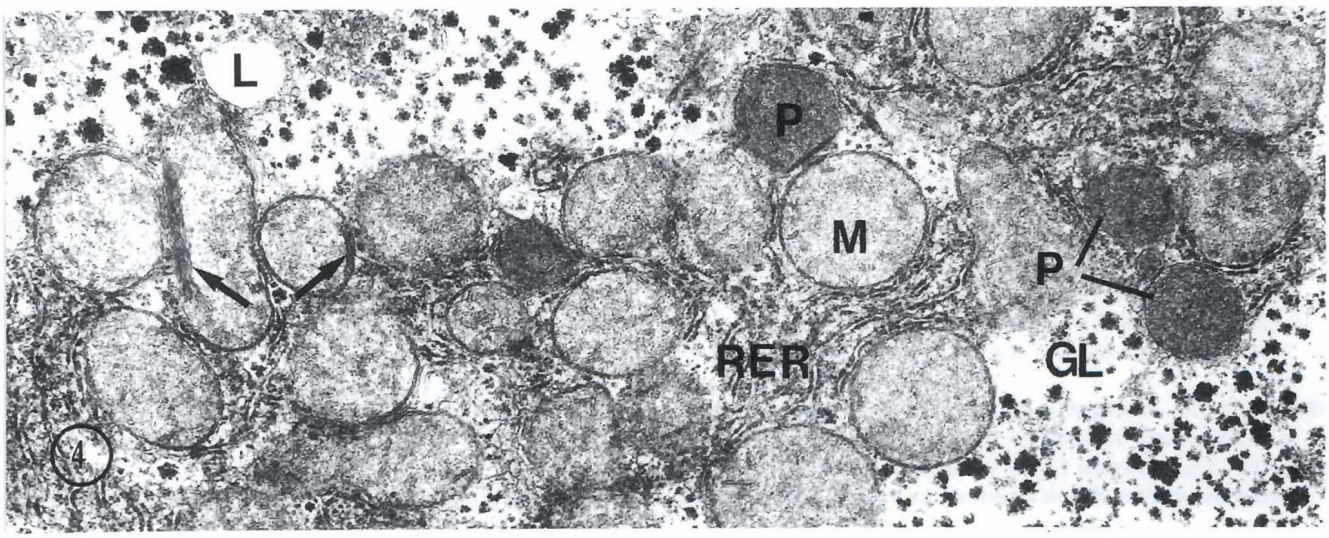


Fig. 2. Micrograph of portions of several hepatocytes from a Tower RO-fed piglet. A bile canaliculus (BC) contains a few microvilli that are missing from another canaliculus (*). Lysosomal elements (Ly) including residual bodies (RB) are common. A membrane bound space (MBS) has deformed a nucleus. L: Lipid droplet; RER: Rough reticulum. x 7,000

Fig. 3. Micrograph of portions of hepatocytes from an oil-fed animal. An altered bile canaliculus (*) and another containing debris (D) is shown. Note the proliferated smooth reticulum (SER) and an altered mitochondrion (M). GC: Golgi complex; P: Peroxisomes. x 25,000

Fig. 4. Micrograph of a small field of hepatocyte from oil-fed animal. Mitochondria that contains cristae arranged parallel (arrows) to long axis of the organelle are demonstrated. Many peroxisomes (P) are revealed in the image. GL: Glycogen particles; L: Lipid droplets; M: Mitochondrion; RER: Rough endoplasmic reticulum. x 20,000


P
M
*
SER
GC
D
3


L
P
M
P
RER
GL
4

the abundance of MBSs in perivenous hepatocytes of a classic lobule raises the question that they may have a significant role to play in the cell functions in relation to the geographic location of a hepatocyte in the liver acinus. The unusual number and size of the spaces in this zone may be a result of Tower RO feeding. Presence of the spaces in the hepatocytes has been documented in animals which were subjected to experimental conditions (Bianchi et al., 1972; Borowicz et al., 1973), and in human liver (Soares et al., 1980). The oil or its metabolites may have damaged the cells in zone 3 more effectively than in zones 1 and 2 resulting in formation of the large size and number of the spaces in the cells. MBS is formed where there is a localized or limited loss of cell constituents to save the remaining cytoplasm from further loss. Hence, the large size and number of the spaces may be indicative of a reaction of the cells to cytoplasmic loss caused by feeding the oil to the animals since in more advanced alterations, MBSs coalesced with each other to form large spaces.

Ultrastructural alterations revealed in the present study lead us to the conclusion that feeding piglets with Tower RO (containing 0.4% EA) at a 10% level of the diet may cause liver dysfunction. Further work to elucidate the EA metabolism coupled with quantitation of the liver lesions would provide more information on the mechanism of Tower RO toxicity.

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