Ultrastructure of Acute Tetracycline Induced Liver Change

Tetracycline induces fatty metamorphosis of the liver[1,2]. This can be achieved by prolonged administration or by a single large dose[3,4]. Inhibition of protein synthesis[5,6], and blockade of oxydative phosphorilation[7] are considered to be the basic metabolic derangements which cause impaired secretion of lipoproteins and accumulation of triglycerides in liver cells. Ultrastructural changes induced by tetracycline in liver cells have so far not been described, except a suggestion of mitochondrial swelling in a liver biopsy from a patient with presumptive tetracycline intoxication[8].

Twenty-one female Fisher rats, weighing 100–150 g each, were given a LD_{50} 250 mg/kg tetracycline dissolved in 1 ml of isotonic saline in a single i.p. injection. During the experiment the animals were on standard laboratory animal food and had free access to water. Animals were sacrificed by decapitation in groups of 3 at the following intervals: 15 and 30 min, 1.5, 3, 6, 12 and 24 h after the injection. 3 control animals were given 1 ml of saline i.p. They were killed 1.5, 6 and 12 h after the injection. Small pieces of liver were immediately fixed in cold 4% glutaraldehyde, phosphate buffered to pH 7.3 for 24 h at 4 °C, postfixed in cold 1% osmium tetroxyde, phosphate buffered to pH 7.3 for 2 h and subsequently dehydrated in graded solutions of aceton, and finally embedded in Durcopan 'Fluka'. Ultrathin sections were cut on a LKB ultramicrotome, contrasted with lead citrate and uranyl acetate and examined in an Opton 9A electron microscope.

The ultrastructural appearance of normal hepatocytes of rat[9] has been adequately described and our observations do not differ significantly from these previous reports. 15 min following the injection, the liver cells show no ultrastructural changes, and have the same appearance as in the control animals. At 30 min, cytoplasmic architecture did not change, except for disappearance of glycogen granules and slight swelling of mitochondria. This phenomenon was observed throughout the entire lobule. Swell ling of mitochondria became even more prominent in the subsequent 2 experimental groups, reached its maximum at 3 h and decreased thereafter. At 3 h, moderate dilatation of endoplasmic reticulum and disappearance of ribosomes from the membranes became evident. Small membrane-bound lipid droplets (liposomes) started to accumulate in the cytoplasm, but larger fat droplets without limiting membranes were also seen. An increased number of membrane bound dense bodies and cytosegresomes was noticed. All these changes were more prominent in the centrolobular hepatocytes. At 6 h the difference between the centrolobular hepatocytes and the cells from other parts of the lobule became more evident. The centrolobular liver cells exhibited a marked dilatation and vesiculation of endoplasmic reticulum with abundant accumulation of fat droplets. In the cells from the periphery of the lobule, endoplasmic reticulum was dilated and to some extent devoid of ribosomes, but the fat droplets were sparse and predominantly in the form liposomes. Some of the centrolobular cells have undergone necrosis, with pycnosis of their nuclei and complete disorganization of the cytoplasmic architecture and disruption of organelles. Foci of necrosis comprised usually 3–4 cells and were most irregularly scattered and were found in but a small number of all the hepatic lobules. All over the lobules, but especially in the centrolobular zones, the Kupffer cells became prominent: their cytoplasm enlarged and was filled with large phagosomes stuffed with cellular detritus. At 12 h the overall picture did not basically change. At 24 h almost all cells, except the few necrotic ones, regained the

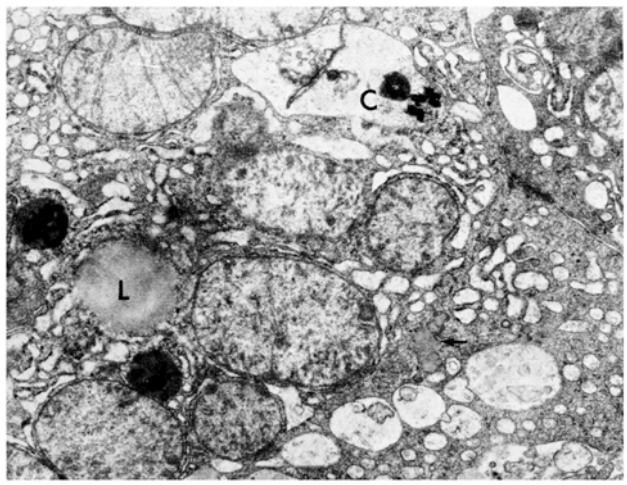

Fig. 1. Centrolobular liver cell at 3 h. Swelling of mitochondria and dilatation of endoplasmic reticulum which is mostly devoid of ribosomes. Lipid droplets without membrane (L) and with membrane (a cluster at arrow). 2 dense bodies and a cytosegresome (C). ×12,923.

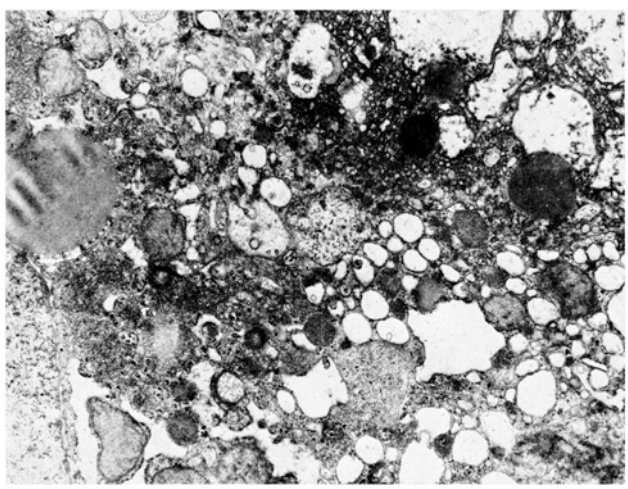

Fig. 2. Centrolobular liver cell at 6 h. Lipid droplets are more abundant. Pronounced vesiculation and dilatation of endoplasmic reticulum. ×11,846.

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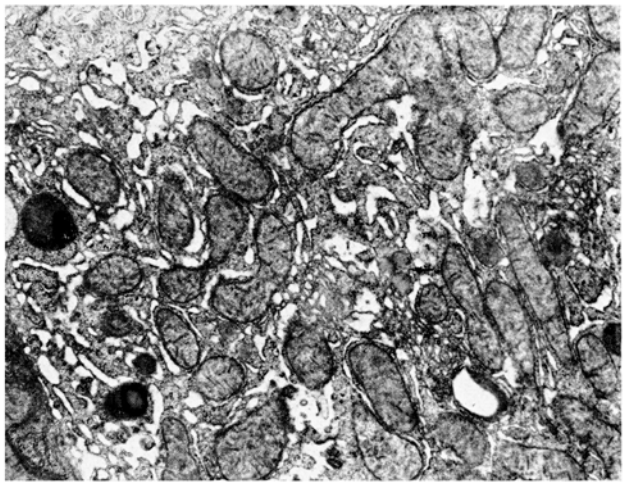

Fig. 3. Hepatocyte, periphery of the lobule, at 6 h. Note dilatation of endoplasmic reticulum. Liposomes at arrow. ×11,846.

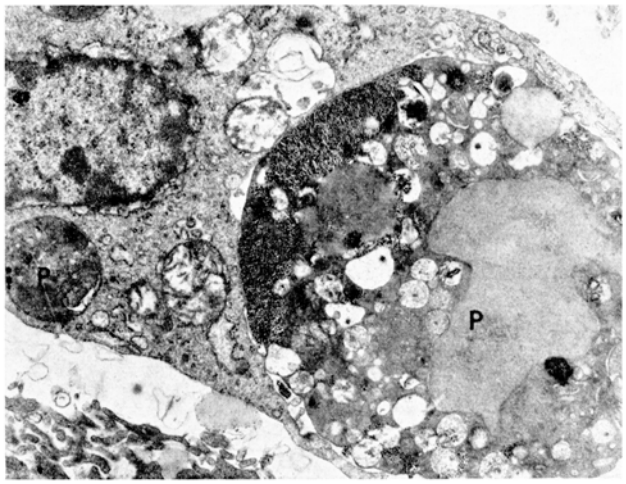

Fig. 4. Kupffer cell at 6 h. Large phagosomes (P) in the cytoplasm. ×12,923.

normal appearance, the increase of fat droplets notwithstanding.

It should be noted that no significant changes in the appearance of hepatocellular nuclei were seen, the necrotic cells notwithstanding. Intercellular bile canaliculli and sinusoids were occasionally dilated. Swelling of microvilli on both sides of liver cells was frequently observed, but was considered due to glutaraldehyde fixation and was encountered in control animals as well. Bile ductular cells did not undergo any significant changes.

The main target organelle affected by tetracycline is the endoplasmic reticulum. Tetracycline inhibits the binding of aminoacyl-sRNA to ribosomes and the protein synthesis[6]. This is in part reflected in the dilatation of endoplasmic reticulum and the disaggregation of the ribosomes from the membranes. Blockade of acceptor protein (apoprotein) synthesis leads to inhibition of formation and excretion of lipoproteins and acounts for the accumulation of fat in the hepatocytes. The ultrastructural changes induced by tetracycline are in this respect similar to those induced by other inhibitors of protein synthesis, such as orotic acid[10] or ethionine[11].

The affinity of tetracycline for mitochondria was demonstrated previously[7,12]. WRUBLE et al.[8] have considered the swelling of mitochondria as the main ultrastructural change in the liver biopsy in a clinically suspected case of tetracycline toxicity. Our study indicates that binding of tetracycline to mitochondria is accompanied by changes which could be interpreted as the morphological equivalent of biochemical derangements.

As tetracycline accumulates predominantly in centrolobular hepatocytes[13], one can assume that only these cells are exposed to excessive amounts of the drug and therefore undergo irreversible changes. Disruption of liver cells and extrusion of damaged cytoplasmic organelles is accompanied by increased scavanger activity of Kupffer cells. High concentrations of tetracycline observed in Kupffer cells[13] probably reflect in part their participation in evacuating the cellular dentritus containing large amounts of the antibiotic.

Summarizing our findings, we should like to point out that the ultrastructural findings induced by tetracycline correlate and are in full accord with the biochemical data reflecting the action of this antibiotic on the liver cells. These changes are not specific and are mostly reversible within the first 24 h after injection. Focal centrolobular necrosis was sparsely encountered but represents the only irreversible change induced by a single large dose of tetracycline.

Zusammenfassung. Einmalige i.p. Gabe von Tetrazyclin in sublethaler Dose führt zur Verfettung der Rattenleber. Die Dilatation und Vesikulation des Endoplasmatischen Retikulums und die Quellung der Mitochondrien sind die auffälligsten, Veränderungen die der Verfettung der Leberzellen vorausgehen. Diese Veränderungen sind unspezifisch und reversibel.

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[14] The generous help of Dr. Hans Popper, The Mount Sinai School of Medicine, in revizing the original manuscript is gratefully aknowledged.

Fluoride-Resistant Acid Phosphatase System of Nociceptive Dorsal Root Afferents

Acid phosphatase activity in the nervous system is mainly, but not exclusively, associated with the lysosomes of nerve cells. It has been shown by GEREBTZOFF et al.[1-3] that in Lamina II of the posterior column (Rolando substance) in the rat spinal cord, there exists a peculiar, conspicuously strong acid phosphatase activity differing from the trivial lysosomal localization. This exceptionally intense acid phosphatase activity of the Rolando substance