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Alcohol-induced injury of mitochondria in hepatocytes of mini-pig fetuses

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Summary. The present study describes the occurrence of abnormal mitochondria in the cytoplasm of hepatocytes from half-term mini-pig fetuses, whose mothers in addition to their ordinary fodder have received alcohol (ethanol) in daily amounts comparable to those consumed by human alcoholics. The abnormal mitochondria exhibit elongation and distortion, disorientation of cristae and the appearance of paracrystalline and electron-dense material in the matrix. These ultrastructural changes show striking similarities to the mitochondrial injuries seen initially in the hepatocytes of human alcoholics and probably reflect damage to the mitochondria caused by alcohol itself.

Key words: Alcohol – Mitochondria – Hepatocyte – Fetus – Mini-pig

A number of studies have demonstrated that alcohol (ethanol) exposure may cause changes in hepatocyte ultrastructure, both in human and in animal material (Rubin and Lieber 1972; Schaff and Lapis 1979; Petersen 1980). Some of the most conspicuous changes concern the mitochondria, which exhibit alterations of size, shape, cristae and matrix (Svoboda and Manning 1964; Porta et al. 1965; Oudea et al. 1970; Kiessling and Pilström 1971; Ma 1972; Beskid et al. 1975; Petersen 1977). In the matrix, the occurrence of alcohol-induced paracrystalline material has consistently been described in human material, but in animals only in a study on the rhesus monkey (Voelz 1968). The present study on hepatocytes from mini-pig fetuses demonstrates alcohol-induced alterations of mitochondria, which show striking similarities to those found in alcohol-exposed human hepatocytes, including the appearance of paracrystalline material.

Material and Methods

The material comprised liver tissue from 18 half-term fetuses taken from 3 alcohol-treated mothers, and liver tissue from 6 half-term fetuses taken from 3 non-treated mothers. The fetuses were removed 60 days after copulation. The crown-rump lengths varied between 11 cm and

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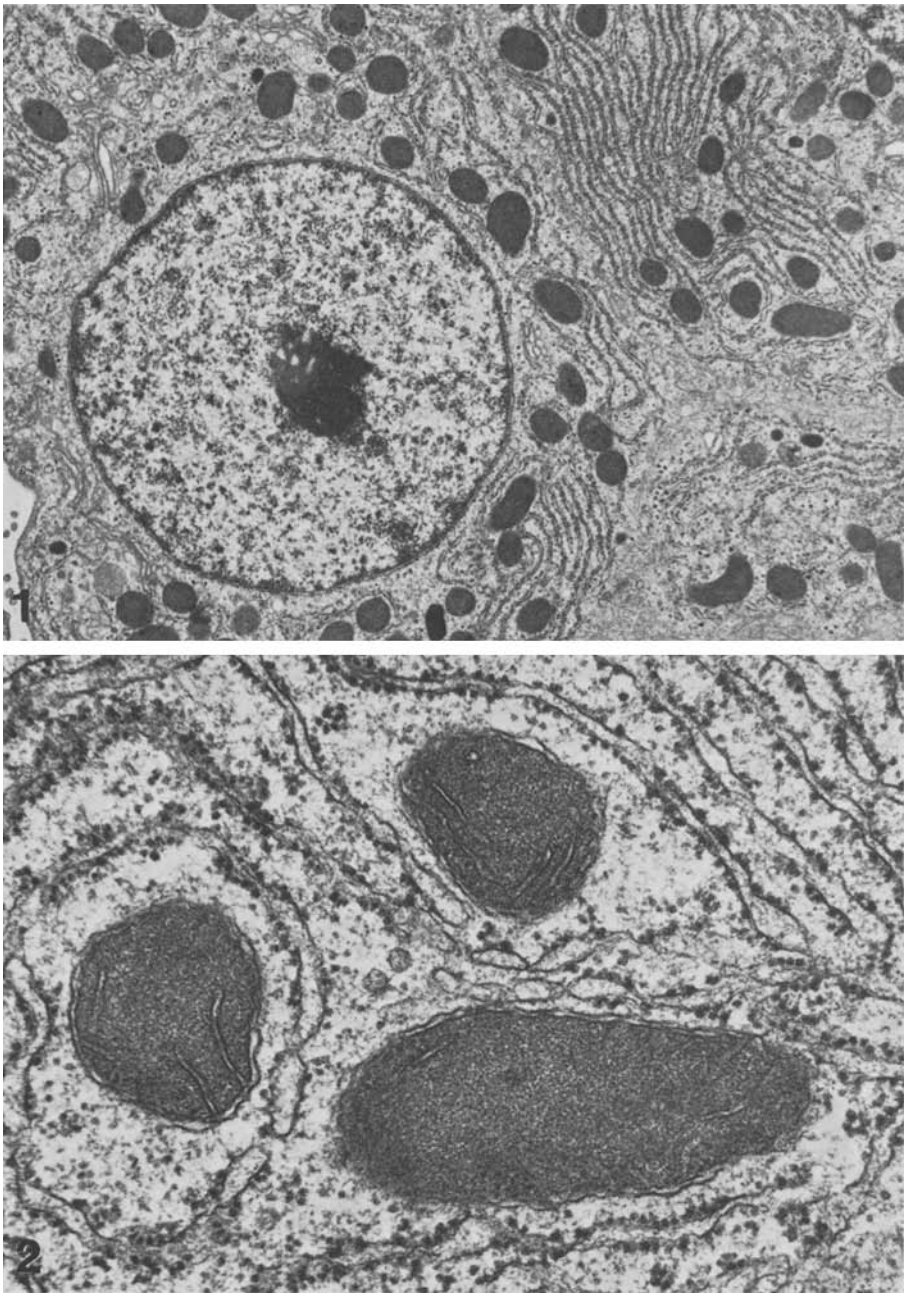

Fig. 1. Electron micrograph of a hepatocyte from an untreated mini-pig fetus, showing a homogeneous population of ovoid mitochondria. ×8100

Fig. 2. Higher magnification of Fig. 1 showing mitochondria with short lamellar cristae projecting into an electron-dense finely granular matrix. ×48500

12 cm both for alcohol-exposed and for non-exposed fetuses. The pregnant mini-pigs were given Anco® fodder, which contained 13% digestive protein and an addition of vitamins and minerals. Water was given ad libitum. The alcohol-treated sows received the following daily additions of ethanol: one mini-pig (90 kg) received 100 g ethanol for 20 days before death, one mini-pig (120 kg) received 100 g ethanol for 10 days and 200 g ethanol for the last 10 days before death, and one mini-pig (80 kg) received 300 g ethanol for 17 days before death. The ethanol was given as a 40% aqueous solution in two equal parts mixed with the fodder in the morning and in the evening. The mini-pigs consumed fodder mixed with ethanol like ordinary fodder, immediately and completely.

After the mother had been sacrificed by shooting, the uterus was taken out in toto. The fetuses were removed one by one, and an incision made through the anterior abdominal wall. Cutting perpendicular to the inferior margin of the liver 1–1.5 mm thick triangular slices were obtained.

From the sows, tissue slices of the same dimensions were cut near the inferior margin of the liver. Within 10 min after death of the sow all liver slices were immersed in 2% formaldehyde + 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0, 20° C). The slices were fixed for 1.5 h and postfixed in 2% OsO_4 in 0.1 M cacodylate buffer for 2 h. The slices were dehydrated through increasing concentrations of ethanol, transferred to propylene oxide and embedded in Epon 812.

Ultrathin sections were cut from a zone situated 30–130 µm below the mesothelial surface (approximately 500 µm below the cut surfaces). The sections were contrasted with uranyl acetate and lead citrate and examined in a Philips EM 300 and a Jeol 100 CX electron microscope.[1]

Results

In the half-term mini-pig fetus no definite structuralization into liver lobules is found. The investigated zone exhibits hepatocytes arranged in irregular anastomosing rows.

In the hepatocyte of the control fetus the dominant organelles are mitochondria and granular endoplasmic cisternae (Fig. 1). The mitochondria are rod-shaped or ovoid with lamellar cristae projecting for a varying distance into the electron-dense finely granular matrix. The mitochondria are approximately 0.5 µm in diameter and 1.5–2 µm long (Fig. 2). A few are up to 4 µm long. The cytoplasm also contains many microbodies, dense bodies, well-developed Golgi complexes and a smooth endoplasmic reticulum. Areas of glycogen and occasional lipid droplets are also found.

After alcohol treatment a prominent feature is a change in the mitochondria. In contrast to the mitochondria of control fetuses, which comprise a very homogeneous population of equally sized and shaped mitochondria, those after alcohol treatment vary with regard to shape, size, arrangement of cristae and structure of matrix (Figs. 3–5). The shape varies from a straight rod-shape – via a crescent – to a circular or spherical form. Some mitochondria show saccular and narrow parts. Some are very long, up to 7 µm with a normal diameter, and some are spherical with a diameter of up to 2 µm. The long mitochondria exhibit two types of arrangement of cristae: one with longitudinally oriented tubular cristae, often stretching

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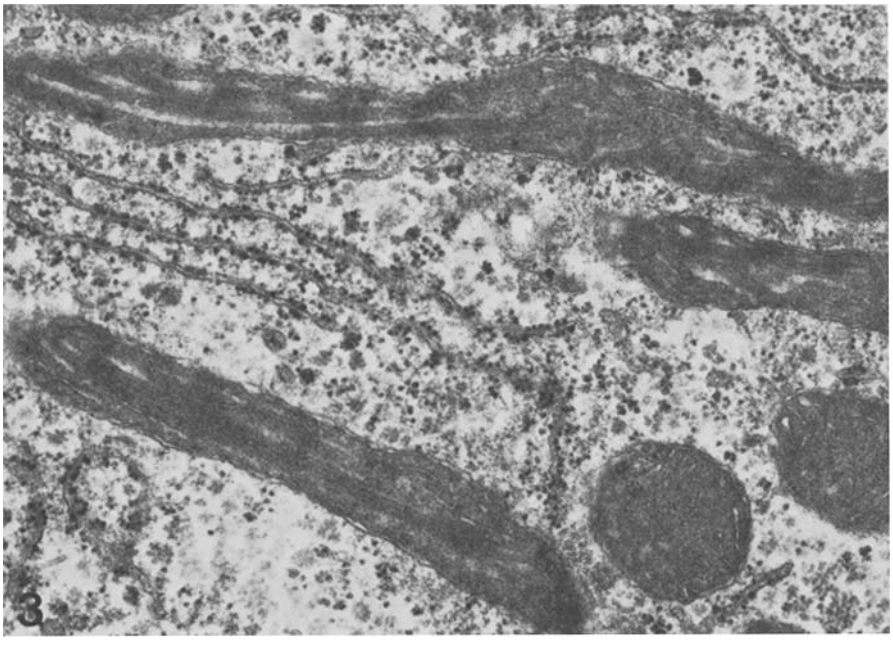

Fig. 3. Electron micrograph of a hepatocyte from an alcohol-exposed mini-pig fetus. Note elongated mitochondria with longitudinally oriented cristae. × 31300

through the whole length of the mitochondrion (Fig. 3) and one with numerous short cristae, arranged as parallel, orderly stacked folds extending from the inner membrane for a short distance into the matrix (Figs. 4, 5). In the type with stacked cristae, paracrystalline material is regularly found in the matrix. This material forms a lattice with transversal and longitudinal bars. The width of both bars is approximately 4 nm and the distance between the bars is approximately 7 nm (Fig. 5). In many mitochondria a homogeneous dense material is found between cristae in the periphery of the matrix (Fig. 5). On rare occasions long mitochondria with tubular cristae and interposed paracrystalline material are found.

After ethanol treatment a greater number of autophagic vacuoles are seen. These are often found in small groups mixed with mitochondria. Most of the vacuoles are spherical, often with an irregular outline. Most have a size of 0.5–1 μm, while some are larger, up to 3 μm. The vacuoles contain electron-dense material, fat droplets and myelin bodies. Sometimes remnants of mitochondrial cristae are seen.

Discussion

The hepatocytes of the alcohol-exposed mini-pig fetus show profound changes of mitochondria and an increased number of autophagic vacuoles. Elongation and distortion of mitochondria, disorientation of cristae and appearance of electron-dense material in matrix after alcohol exposure have pre-

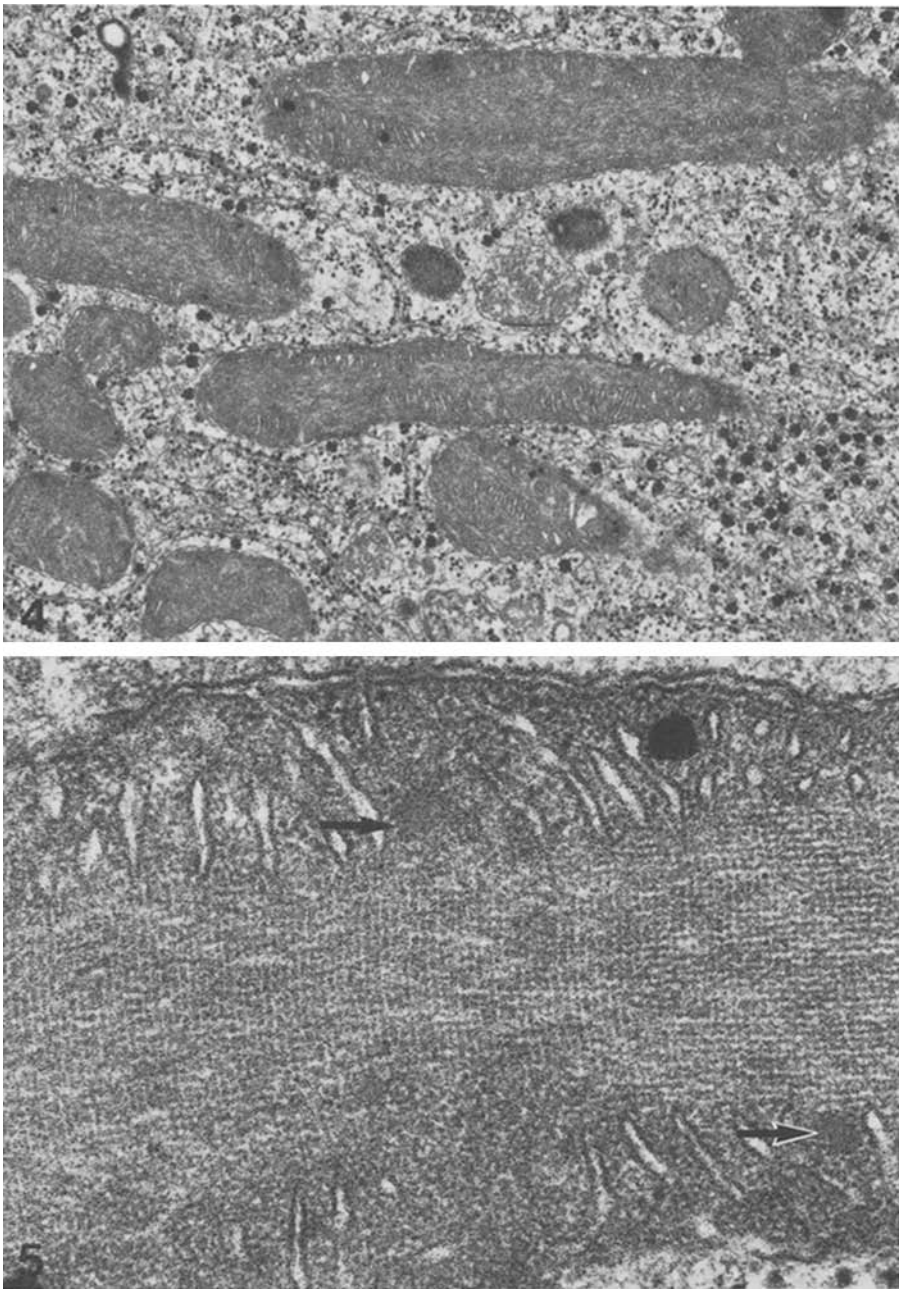

Fig. 4. Electron micrograph of a hepatocyte from an alcohol-exposed mini-pig fetus. Note elongated mitochondria with numerous short stacked cristae and paracrystalline material. ×25800

Fig. 5. High magnification of a mitochondrion with numerous short cristae. The matrix contains paracrystalline material, which forms a regular lattice. Note also the homogeneous material between cristae (*arrows*). ×105500

viously been demonstrated in the rat (Porta et al. 1965; Oudea et al. 1970; Rubin et al. 1970), in the dog (Beskid et al. 1972), in pups (Beskid et al. 1975), in primates (Voelz 1968; Rubin and Lieber 1972) and in man (Mugnaini 1964; Svoboda and Manning 1964; Lane and Lieber 1966; Kiessling and Pilström 1971; Ma 1972; Petersen 1977). In all the human studies paracrystalline material has been demonstrated in the matrix of the mitochondria. Apart from one study on primates (Voelz 1968), this paper is the first to describe similar alcohol-induced inclusions in the mitochondrial matrix of an experimental animal. However, paracrystalline material is also found in liver mitochondria from dog and pig treated with other agents (Ghadially 1975). The structural changes of the mitochondria have been shown to be associated with changes in mitochondrial enzyme content and activity (Rubin et al. 1970; Beskid et al. 1972; Koch et al. 1977). The profound morphological changes found in the present investigation probably indicate mitochondrial injury rather than adaptation as emphasized by Rubin et al. (1970). This is also confirmed by the find of an increased number of autophagic vacuoles containing remnants of mitochondria. The mitochondrial changes are not found in the alcohol-treated mothers or in control animals. In view of the debate as to the role of ethanol or acetaldehyde in the production of liver cell damage in alcoholics (Rubin et al. 1970; Isselbacher 1977) this study may indicate that the mitochondrial injury in fetuses is caused by alcohol per se rather than acetaldehyde since 1) transfer of alcohol through the placenta occurs unhindered and results in almost equal concentrations in mother and fetus (sheep: Dilts 1970; hamster and monkey: Ho et al. 1972; rat: Kesäniemi and Sippel 1975), 2) placenta prevents acetaldehyde generated from ethanol in the maternal rat liver from entering the fetal circulation (Kesäniemi and Sippel 1975), 3) ethanol metabolism is absent in the human fetus in the first trimester (Pikkarainen 1971) or low throughout gestation as shown in the hamster and monkey fetus (Ho et al. 1972), 4) alcohol dehydrogenase activity is low in fetal liver tissue (human: Pikkarainen and Räihä 1967; rat: Räihä et al. 1967) and not increased by ethanol administration (Räihä et al. 1967). In the present investigation an inadequate diet can probably be excluded as a cause, since the pregnant mini-pigs received the alcohol as an addition to their ordinary fodder and probably secured a sufficient supply of nutrients for the fetus.

Thus it may be concluded that the mitochondrial injury which is found in the fetal hepatocytes and which shows striking similarities with that seen in human alcoholics is caused by alcohol per se.

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