Effects of Alcohol on the Ultrastructures of Rat Liver

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To investigate the effect of alcohol on the ultrastructural changes of liver the experiment was undertaken by dividing in 3 groups (control, alcohol and alcohol-carnitine groups). Chronic administration of ethanol to adult rats led to striking fat accumulation and ultrastructural changes in the liver. Mitochondrial abnormalities, dilation of endoplasmic reticulum, focal cytoplasmic degradation, dilation of bile canalicli and swelling of nucleus were observed. In alcohol-carnitine group, there were less fat accumulation and ultrastructural changes than alcohol group. These alterations suggest that ethanol is very toxic to the liver and carnitine prevents fat accumulation induced by alcohol.

KEY WORDS: Ethanol, Carnitine, Ultrastructure, Rat liver

Among the organs, liver is the primary system attacked by alcohol abuse since ethanol is obligatorily metabolized in the liver. Many biochemical evidence indicates that ethanol has a direct as well as an indirect effect on hepatic metabolism (Lieber, 1980; Mezey, 1978; David et al., 1983). One of the principal manifestations of these effects is the accumulation of lipid in liver cells. During prolonged ethanol feeding triacylglycerol accumulates in hepatocytes as cytoplasmic lipid droplets (Iseri et al., 1966; Kondrup et al., 1979). The ultrastructure of the fatty liver associated with chronic alcoholism has well documented (Porta et al., 1965; Schaffner et al., 1963). Electron microscopically, the most striking changes have been reported in mitochondria (Svoboda and Manning, 1964; Kiessling et al., 1964). These changes included enlargement and disfigurement of mitoehondria and their cristae, lobate forms, and intramitochondrial crystalline inclusion.

Some other reports demonstrated that alcohol itself, independent of nutritional factors, rapidly produces fatty liver and hepatic ultrastructural changes (Rubin and Lieber, 1967; Lane and Lieber, 1966). Ethanol affects almost every organ system in the body. A noteworthy change in the endoplasmic reticulum reported by Porta and

co-workers (1965) was the appearence of parallel tubules of smooth endoplasmic reticulum in straight or concentric arrays, which were associated with glycogen particles. Cell damage was also evidenced by focal cytoplasmic degradation in the form in increased autophagic vacvoles and residual vacuolated bodies (Svoboda and Manning, 1964).

Carnitine (3-hydroxy-4-trimethylaminobutyric acid) was isolated from meat in 1905. Carnitine is crucial to fatty acid oxidation, as evidenced by the serious consequence of its deficiency in humans (Firits and Yue, 1964; Rebouche and Engel, 1983). Since carnitine normally facilitates fatty acid transport and oxidation into mitochondria (Bremer, 1962), perhaps a very high liver acyl carnitine level in vivo was needed to enable oxidation of part of the ethanol-induced increase in hepatic triglycerides to take place, thereby simultaneously decreasing the liver lipid content and improving the energetics of the cell (Hosein and Bexton, 1975). Gross and Henderson have demonstrated that carnitine can be absorbed from the small intestine into the systematic circulation, so that it seems evident that exogenous carnitine derived from the diet as well as that derived from endogenos synthesis contributes to the body's car-

nitine pool (Gross and Henderson, 1984).

This study was undertaken to investigate the effect of carnitine on the ultrastructural changes of fatty liver induced by ethanol ingestion.

Materials and Methods

Male Sprague-Dawley rats weighing approximately 200 to 250 gm were placed in separate cages with wire-meshed floor in an air-conditioned room. Rats were treated as follows.

(1) Control group: These rats were received adequate food and water before being sacrificed.

(2) Alcohol group: These rats received 35 % ethanol (6 g/kg) in solution (w/v) in saline by gastric intubation.

(3) Alcohol-carnitine group: These rats were treated as in alcohol group, except that carnitine (0.1 mg/body weight) was added to the solution,

Five rats from each group were killed at the end of 3 weeks. Abdominl cavity was opened under light ether anesthesia and liver were removed. For light microscopy, liver was removed and fixed in buffered neutral formalin. The liver was then embedded in paraffin and stained with hematoxylin and eosin. For electron microscopy, small blocks of liver were fixed in 2 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) solution for 2 hrs at 4°C. After fixation the blocks of tissue were washed over night in three changes of phosphate buffer at 4°C and post fixed in 1 % OsO₄ for 1 hr. Then dehydrated in graded series of alcohol and embedded in Epon 812. Sections cut with an LKB ultratome were stained with uranyl acetate and lead citrate. The preparations were then examined with an JEM 100 SX transmission electron microscope at 80 KV.

Results

At the light microscopic level, all liver specimens appeared essentially the same with relatively well organized lobular structure consisting of normal appearing hepatic parenchymal cells. Control biopsy specimens appeared essentially normal.

Most control specimens showed no fats or rare cells containing small fat droplets.

The biopsy specimens taken from alcohol group showed increased fat droplets. No alcoholic hyalin of mallory was seen. The biopsy specimens taken from alcohol-carnitine group appeared as same as control group. Most specimens showed no fat or less fat than those of alcohol group.

The control biopsy specimens appeared normal or displayed only minor deviations by electron microscopy. The mitochondria were usually within the normal range of size and shape, and only occasional aberrnat forms were present. Parallel arreys of rough profiled endoplasmic reticulum were conspicuous. The smooth endoplasmic reticulum was generally not unusual, but in several cases a mild increase in the vesiculation of smooth endoplasmic reticulum was present. The other cytoplasmic organells, such as free ribosomes, nuclei, Golgi complex, microbodies, and bile canaliculi appeared normal. No cell degradation and numerous vaculated bodies were seen (Fig. 1).

All biopsy specimens obtained after alcohol administration showed prominent cytoplasmic changes. Cytoplasmic lipid droplets were present (Fig. 2), and the amount of cytoplasmic fat generally correlated with that seen with the light microscope. In all of the specimen obtained from alcohol group, there was striking variability in mitochondrial size and shape. Many were distorted, enlarged and assumed bizarre shapes (Fig. 3). Some mitochondria were extremely elongated (Fig. 4), and they are usually larger and more distorted than those in the control biopsies. Other mitochondria showed degenerative changes, characterized by rare fraction of the matrix and formation of intramitochondrial "myelin" figures (Fig. 5). The another feature was the presence of intramitochondrial inclusions (Fig. 6).

In most specimens in alcohol group, the endoplasmic reticulum was abnormal. Rough endoplasmic reticulum was generally scarce. Smooth endoplasmic reticulum was increased, and they showed often dilated and vesicular appearances (Fig. 7). A noteworthy feature in most specimens obtained after alcohol administration was the presence of focal cytoplasmic degradation (Fig. 8). Autophagic vacuoles were also prominent. Lysosomes were
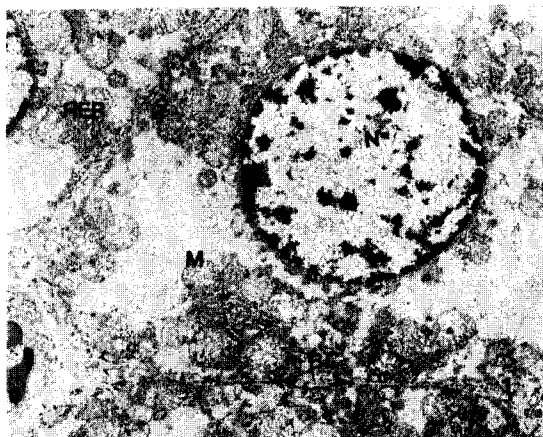
Fig. 1. Electron micrograph of liver control group. Mitochondria (M) and rough endoplasmic reticulum (RER) appear normal. N, nucleus; M. mitochondria; BC, bile canaliculi. Original magnification, × 3,000
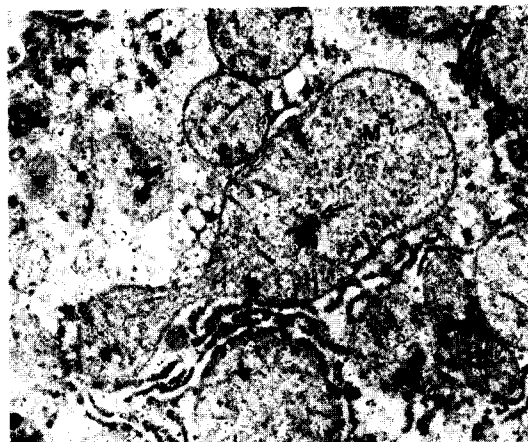

Fig. 3. Bizarre shaped mitochondria of alcohol group. Note the cytoplasmic inclusion in mitochondria (M) (arrow). Original magnification, × 15,000

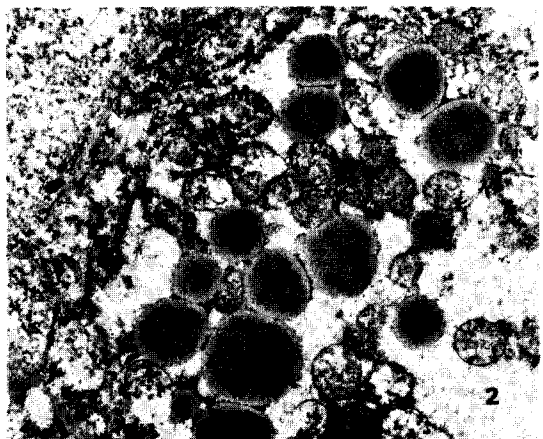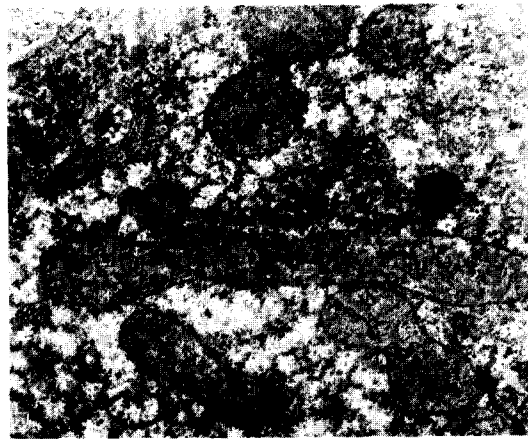**Fig. 2.** Many ctyoplasmic fat droplets (F) of alcohol group. Original magnification, × 6,000

Fig. 4. Extremely elongated mitochondria of alcohol group. Original magnification, × 15,000

only moderately increased. focal cytoplasmic degradation was more severe than control and alcohol-carnitine groups. After alcohol administration the vesicles and cisternae of the Golgi complex were frequently dilated (Fig. 9). Many nuclei were swollen (Fig. 10), and dilation of some bile canaliculi with loss of microvilli was seen in most specimens (Fig. 11).

In most specimens obtained from alcohol-carnitine group showed as same feature as alcohol group, except some aspects. There were no cytoplasmic degradation and swollen nucleus (Fig. 12).

Dilation of some bile canaliculi with loss of microvilli was not present or rarely seen. Some mitochondria were elongated and distorted, but less than those of alcohol group. Fat droplets were less present than alcohol group.

Discussion

The metabolic effects of ethanol and carnitine have been well summalized in the previous studies (Sachan and Berger, 1987; Rodgers and O'Brien,
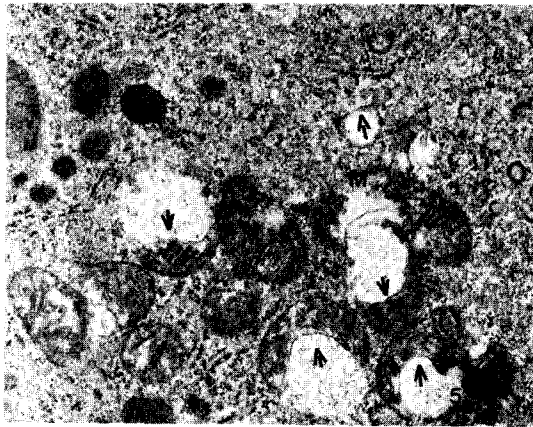
Fig. 5. There are many intramitochondrial "myelin" fi-
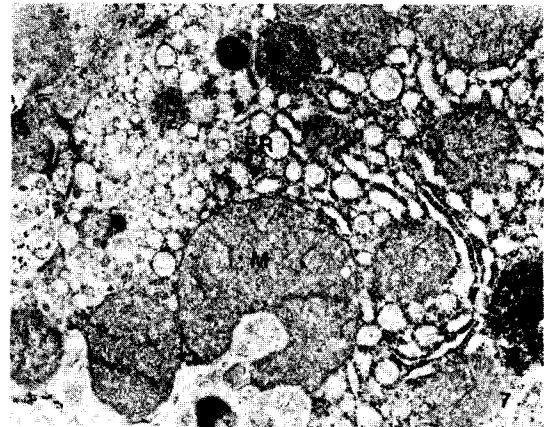gures (arrow). Original magnification, × 15,000

Fig. 7. Endoplasmic reticulum (ER) of alcohol group is
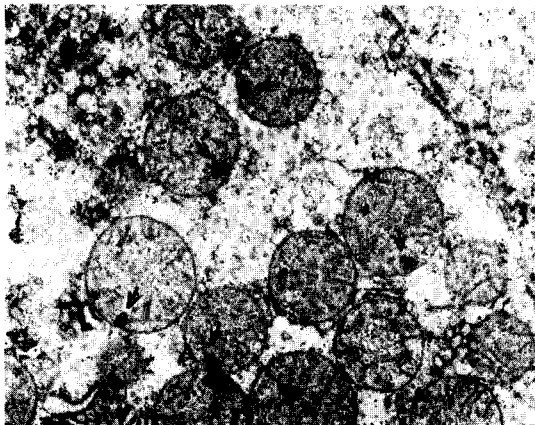dilated and vesicular. M, mitochondria. Original magni-
fication, × 10,000

Fig. 6. Several mitochondria (H) in a single field contain
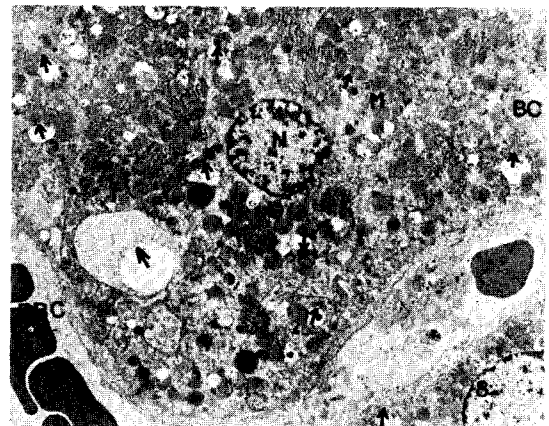inclusions (arrows). Original magnification, × 10,000

Fig. 8. Cytoplasmic degradation is present in alcohol
group of liver (arrows). N, nucleus; rer, rough endoplas-
mic reticulum RBC, red blood cell; M, mitochondria;
BC, bile canaliculi. Original magnification, × 2,000

1974; Lin, 1976).

Results indicated that alcohol produced a fat
accumulation and ultrasturctural changes of liver.
The fat deposited in the liver after administration
of ethanol. It was assumed to be an accumulation
of triglycerol in hepatocytes. But in the alcohol-
carnitine group, fat accumulation was less than
alcohol group. Carnitine, a cofactor of long-chain
fatty acid oxidation, plays an important role in
lipid metabolism. It is noteworthy that supple-
ments of carnitine would prevent the accumula-
tion of fat induced by ethanol. It must be a protec-
tive action of carnitine. Hosein and Bexton (1975)
have also shown that protective action of carnitine

on liver lipid metabolism after ethanol administra-
tion and it is similar to our results.

The most striking hepatic ultrastructural effects
after ethanol ingestion are alteration of
mitochondrial shape and of endoplasmic reticu-
lum, and increased focal cytoplasmic degradation.
None of these changes alone are specific for alco-
hol intoxication, but they are identical with results
from similar studies (Rubin and Lieber, 1968; Lie-
ber and Rubin, 1986). the observed mitochondrial
alterations my be the morphologic counterpart of
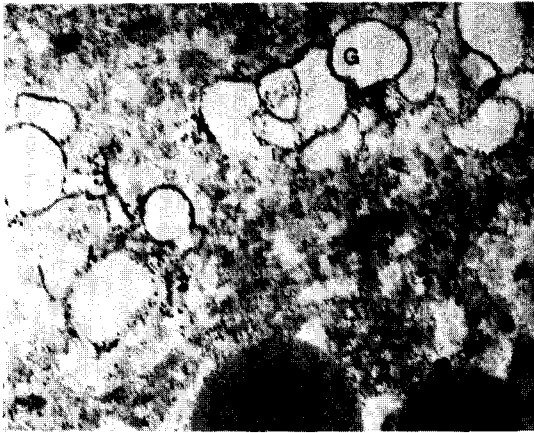impaired mitochondrial function, reflected in de-

Fig. 9. Cisternae of the Golgi complex (G) are dilated in alcohol group of liver. F, fat droplet. Original magnification, × 15,000
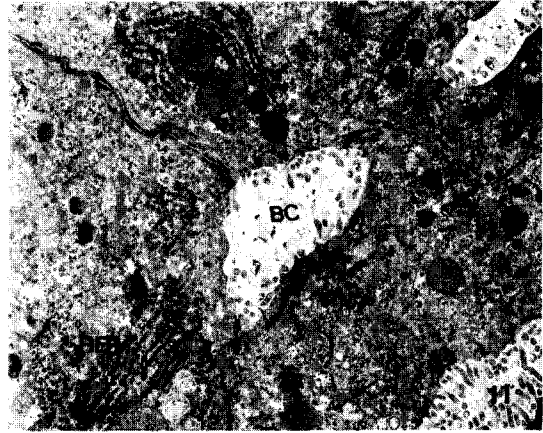

Fig. 11. Bile canaliculi (BC) of alcohol group are dilated with loss of microvilli. M, mitochondria; RER, rough endoplasmic reticulum; Ly, lysosome. Original magnification, × 6,000
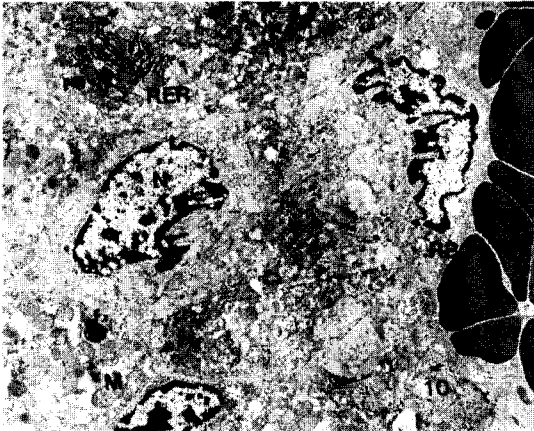

Fig. 10. Nucleus (N) of sinusoidal endothelial cells are swollen, P, peroxisome; M, mitochondria; RER, rough endoplasmic reticulum; RBC, red blood cell. Original magnification, × 2,500
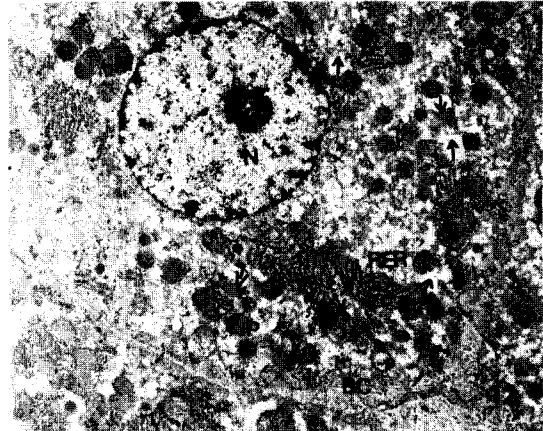

Fig. 12. In alcohol-carnitine group, cytoplasmic degradation (arrows) is less present than alcohol group. N. nucleus; RER, rough endoplasmic reticulum; BC, bile canaliculi; P, peroxisome; M, mitochondria. Original magnification, × 2,500

creased hepatic oxidation of fatty acids and acetate, after alcohol administration (Lieber et al., 1967).

In view of the fact that crystalline structures are formed upon hydration of phospholipids in vitro, the mitochondrial crystalline inclusion may reflect hydration of liberated phospholipids in damaged mitochondria (Lieber and Schmid, 1961). On the other hand they may results from increased synthesis of mitochondrial phospholipids (Kuyper, 1962). It would appear that mitochondrial altera-

tions in fatty metamorphosis of the liver are part of the spectrum of mitochondrial response to injury and possibly represent fundamental pathologic responses of these organelles to an altered chemical or physical milieu. In the alcohol-carnitine group, mitochondrial alterations were less than alcohol group. Carnitine may be act indirectly by somehow stimulating mitochondrial metabolism. There is evidence that, after alcohol treatment, carnitine-specific entry sites on the liver

mitochondrial membrane may be altered (Hosein and Bexton, 1975). By facilitating the transfer of substrates across such altered membranes, carnitine may be stimulating oxidative processes within such mitochondria.

Smooth-surfaced endoplasmic reticulum is a primary site for drug metabolism and lipoprotein synthesis (Conney, 1967) and also part of the microsomal fraction that is involved (Hamilton et al., 1967). It is therefore not surprising to find increased smooth endoplasmic reticulum in hepatocytes, since alcohol administration leads to increase in activities of hepatic drug metabolizing enzyme, increased circulating lipoproteins and enhanced cholesterol biosynthesis (Rubin et al., 1968). Thus, whereas the mitochondrial changes appear to reflect injury that leads to decreased functional capacity, the alterations in smooth endoplasmic reticulum may be "adaptive" and result in increased functional capacity.

The increased in hepatic facal cytoplasmic degradation may be due to an alcohol intoxication and produce liver injury. while alcohol-carnitine group, cytoplasmic degradation was not appeared. Carnitine appeared to protective action on liver injury induced by ethanol.

Dilation of bile canaliculi with loss of microvilli and swelling of nucleus are due to dehydration of tissue induced by ethanol. But alcohol-carnitine group, it was not observed.

The results of this study suggest that increased fatty acid oxidation induced by ethanol was adequately supported by supplementing carnitine. The effects of carnitine on the liver is protective action of liver cell damage including mitochondrial alterations, cell degradation, wideing of bile canaliculi and swelling of nucleus.

This study shows that alcohol has a profound effect on ultrastructural changes on the liver and carnitine may act by preventing fat accumulation and cell damage in the liver induced by alcohol.

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흰쥐 간장의 미세구조에 미치는 Alcohol의 영향

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알콜이 흰쥐 간장의 미세구조에 미치는 영향을 관찰하기위해 대조군, 알콜 처리군, 알콜-carnitine처리군으로 나누어 실험하였다. 만성적인 알콜 투여는 간에서의 지방축적을 유발하였으며 미토콘드리아의 이상, 소포체의 팽대, 세포질의 손상 Bile canaliculi의 확장 및 핵의 수축 등의 미세구조적 변화를 가져왔다. 알콜-carnitine으로 처리한 군에서는 지방의 축적과 미세구조의 변화가 알콜 처리군에서 보다는 약하였다. 이러한 구조적 변화는 알콜이 간에 매우 유독하며 carnitine은 알콜에 의해 유발된 지방의 축적을 방지해 보여주는 것으로 사료된다.