Electron Microscopic Study of the Liver after Prolonged Use of Alcohol

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SUMMARY
The effect of prolonged use of alcohol on the liver was experimentally studied by electron microscopy. In addition, the protective action of Liv.52 on the liver was experimentally assessed in 35 young albino rats.

Experimental findings show that lysosomal release of proteolytic enzymes is increased in chronic alcoholics. It also displaces the mitochondria, E.R., and ribosome and this could be the cause of decreased protein synthesis. The administration of Liv.52 could protect the cells of the hepatic parenchyma from this sequence of alcoholic damage to a considerable extent and assist in protein synthesis by activating the ribosomal enzymes.

INTRODUCTION
The consumption of alcohol is increasingly evident in modern society. So much so that, after heart disease and cancer, alcoholism ranks as the world's biggest health problem today. Even occasional, moderate drinking impairs liver function. Whereas, it has been established beyond doubt that prolonged ingestion of alcohol has graver effects of damaging the hepatocytes a potential precursor to cirrhosis of the liver.

Liver is the key organ responsible for metabolising hormones, vitamins and any drugs taken orally. Many scientists have studied the pivotal role of the liver in the metabolism of minerals, hormones and vitamins. Similarly, the processes involved in the degeneration of the liver tissue have been studied by other workers, both biochemically and under light microscopy. Some reports are available where attempts have been made to stimulate depressed liver function by the administration of various drugs including gluco-corticoids. However, very few reports are available where the effect of chronic alcoholism on the liver cells has been studied under electron microscopy.

In many studies, an indigenous compound, Liv.52 has been cited as one of the remedies for the correction of many liver disorders. The author's own in vitro studies have shown that the addition of Liv.52 in the culture medium could prevent the degeneration of the cirrhotic liver. The beneficial effect of Liv.52 has been confirmed by electron microscopy in the liver of mice treated with carbon tetrachloride.

Here the sequence of degenerative changes in the liver following prolonged use of alcohol, have been studied under electron microscopy. In addition, the effect of Liv.52 on the liver subjected to alcoholic conditions was also studied.
METHODS
Thirty five young albino rats of both sexes, weighing 100-120 gms were selected for this experimental study. These animals were divided into four groups. Group I containing ten animals was given 0.5 ml of absolute alcohol per day orally for 30 days, whereas the ten animals in Group II were given 0.5 ml absolute alcohol orally along with Liv.52 2-3 drops every day for 30 days. Animals in Group III received 0.5 ml absolute alcohol orally every day for 30 days and were then given Liv.52 2-3 drops for the 30 days thereafter. The remaining five animals served as the Control group and received only water up to 30 days. The animals were sacrificed by decapitation at the end of 15, 30 and 60 days for experimental study in different groups.

After sacrificing the animals, their livers were dissected and fixed in Bouins fixative solution for histological study. The tissue was also fixed in glutaraldehyde solution for electron microscopic study. It was then embedded in araldite and sections were cut with the Porter Blum ultramicrotome, stained with double stains i.e. uranyl acetate and lead nitrate. Copper grids containing the sections were then examined under Philips Electron Microscope, EM-300.

RESULTS
Macroscopic observation of the liver in those animals treated with alcohol alone (Group I) showed discoloration with roughness of surface. The liver had become brittle as compared to the normal liver in the Control Group. The liver of those animals concomitantly administered Liv.52 along with alcohol (Group II) showed a little firmness to touch and patchy normalization in the colour of the liver. Nearly normal liver condition was observed in the animals who received Liv.52 for 30 days after the administration of alcohol (Group III).

Electron microscopic study of the alcoholic liver In Group I revealed serrated and elongated nucleus with many activated lysosomes in the cytoplasm (Fig.1). There was absence of mitochondria and the whole cytoplasm was full of homogenous material (Fig.2). It was devoid of both rough and smooth endoplasmic reticulum; some dilated endoplasmic reticulum; some dilated endoplasmic sacs could be seen. As the administration of alcohol continued, collagen fibrils gradually appeared replacing a maximum area (Fig.3). Besides, no sign of glycogenesis could be seen. In comparison, the normal liver (Control Group) showed an oval nucleus with nucleolus and a fair amount of chromatine materials. A number of mitochondria, smooth and rough endoplasmic reticulum studded with ribosomes could also be seen. Glycogen granules were more marked (Fig.4).

Those animals treated with Liv.52 simultaneously with alcohol (Group II) showed dilated endoplasmic sacs and a significant increase in the smooth endoplasmic reticulum, studded with ribosomal granules (Fig.5). The serration of the nucleus was still evident. No mitochondria could be seen. At the same time, thee was a marked reduction in lysosomes (Fig.6).

Those animals treated with Liv.52 after discontinuing the administration of alcohol (Group III) showed a normal shaped nucleus with nucleolus. There was a fair amount of reappearance of mitochondria, surrounded by rough endoplasmic reticulum and ribosomes (Figs.7 and 8). The dilation of endoplasmic sacs was almost absent. Besides, no collagen fibrils could be seen; on the other hand, there was an increase in the glycogen granules.
Fig. 1: Electron microscopic picture of liver treated with alcohol. It shows elongated hypertrophied nucleus (N) with serrated nuclear membrane marked with arrow. Cytoplasm containing a fair number of different sizes of lysosomes (L). Very few ribosomes could be seen (R) x 13000.

Fig. 2: Similar picture showing a number of cells having nucleus (N) of different shape and serrated appearance of nuclear membrane (arrows), scattered ribosomes without endoplasmic reticulum could be seen. Rest of the cytoplasm is devoid of any fine structure but contains homogenous substance. Some patchy area of collagen fibril formation could be seen (CF) x 6300.

Fig. 3: Showing dilated rough endoplasmic reticulum (RER) with extensive collagen fibril formation (CF) x 13000

Fig. 4: Electron microscopic picture of normal liver, showing an oval nucleus (N) and a nucleolus (NL), with fair number of mitochondria (M) scattered smooth endoplasmic (SER) reticulum, and deposition of glycogen granules (R) x 13000.

DISCUSSION

Earlier in vitro studies have indicated that glycogenesis is increased by utilization of more glucose in the presence of Liv.52. This was further corroborated by studying the fine structure of the liver cells, where the parenchymal cells from Liv.52-treated rats were shown to contain a fair glycogen accumulation in comparison with experimentally damaged liver cells.

Results of this electron microscopic study showed that the chronic use of alcohol could disperse the mitochondria, S.R., R.E.R along with ribosomes. On the other hand, it induced the activation of lysosomes, which plays an important part in the resultant inflammation of fibrosis. The administration of Liv.52 to these rats inhibited the activity of lysosomes, probably by stabilizing the
lysosomal membrane. Liv.52 also stimulated the accumulation of mitochondria, glycogen and increased the S.E. activity. At the same time, a fibrosis could also be seen.

**Fig. 5:** Electron microscopic picture of liver treated with alcohol along with Liv.52, shows well developed rough endoplasmic reticulum (RER) and dilated to form endoplasmic sacs (E). A significant increase in the accumulation of smooth endoplasmic reticulum could be seen (SER) x 21500.

**Fig. 6:** Similar liver showing nucleus (N) with a nucleolus (NL) and decrease in the serration of the nucleus. The cytoplasm shows rough endoplasmic reticulum (RER) arranged in parallel direction and studded with ribosomes x 17000.

**Fig. 7:** Electron microscopic picture of liver treated with Liv.52 after administering alcohol. It shows normal shaped nucleus (N) and a nucleolus (NL). Cytoplasm shows a fair number of mitochondrial (M) and reappearance of well-arranged rough endoplasmic reticulum (RER) without any dilatation x 6000.

**Fig. 8:** Similar picture in higher magnification x 13000.

A similar observation on hepatic glycogen deposition by glucocorticoid was earlier reported by Baxter and Forsham. Besides, Rancourt and Litwack and Mahby et al. had reported increased glycogen deposition and its relation to the S.E.R. It has also been demonstrated that the administration of corticoids could protect the endoplasmic reticulum from damage by carcinogenic drugs. De Man and Block described the proliferation of S.E.R. in response to cortisone and noted an association of S.E.R. with glycogen particles. On the other hand, contrary findings have also been reported by some workers.
The available observations could well be correlated with our findings that chronic alcoholism induces lysosomes to release more proteolytic enzymes. This in turn displaces the mitochondria, E.R. and ribosomes, which are unable to synthesize nucleic acid. The release of proteolytic enzymes from the lysosomes leads to inflammation, resulting into fibrosis.

The protective action of Liv.52 as seen in this study suggests that Liv.52 inhibits the production of proteolytic enzymes and at the same time stimulates the accumulation of mitochondria, E. R. and ribosomes. This action is apparently similar to the effects of hydrocortisone. However, Liv.52 even in large doses has not been reported to cause the typical side effects of steroids like sodium retention, susceptibility to infection and delayed wound healing. On the other hand, it has been reported to cause positive nitrogen balance\textsuperscript{12,20}.

It is therefore unlikely that the drug acts by releasing endogenous steroids or through a direct hydrocortisone-like action. Liv.52 appears to have a different mode of action. The activation of nucleic acid synthesis by affecting an increase in the proliferation of E. R. and ribosomes (its related enzymes, glucose-6-phosphatase) was observed after the administration of certain minerals\textsuperscript{4}. This corroborates the present findings. It suggests that the damage to the hepatic parenchymal cells could be averted by the use of Liv.52 in chronic alcoholism. Furthermore, it also indicates that the interaction and metabolism of Liv.52 in the liver, takes place at the level of the rough endoplasmic reticulum and ribosomes. At the same time, the cortisone-like activity needs to be examined by further study.

REFERENCES


