

THE EARLY STAGE OF LIVER INJURY IN THE ALCOHOLIC

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Injury to liver cells and reactive fibrosis of the mesenchyme may occur after only a few years of excessive ethanol intake or after a much longer span, sometimes even decades. We have been particularly interested in the early stages of liver injury and in the progression or repetitive character of the pathologic lesions that lead to recognizable alcoholic liver disease. Innumerable articles have been written on the pathology of liver injury in the alcoholic, but these have been concerned with biopsies taken from patients with symptomatic liver disease or they have been retrospective studies based upon autopsy findings.

MATERIALS AND METHODS

The major portion of this study concerns 100 chronic alcoholic patients who had been drinking heavily just prior to hospitalization. They were chosen at random from those on the admitting ward at the Los Angeles County Hospital. Blood for numerous chemical tests was drawn before any patient received treatment, food or fluids. Biopsies were performed on 45 of the 100 patients within 12 to 72 hours after admission. After paraffin embedding of the sections, slides were stained with hematoxylin and eosin and with Masson's trichrome stains. In addition, at time of biopsy, a small portion of the specimen was immediately fixed in osmium tetroxide and prepared for electron microscopic study. No patient with histologic or other evidence of cirrhosis was included in the final tabulation.

RESULTS

Clinical findings. The study group consisted of 75 men and 25 women. Of the group, 57% were between 40 and 60 years of age. All had been addicted to alcohol for

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many years and each had recently been drinking more than usual. Most of them described this episode as a "spree" or a "binge." Most of them had eaten little or nothing for varying periods, from one day to one month.

Of the symptoms on admission to the hospital, those related to the gastrointestinal tract were by far the most common: 40% complained of vomiting, often of more than 24 hours' duration. Twenty-two patients complained of abdominal pain, most often in the epigastrium. Five of these were later proved to have acute pancreatitis. A history of bleeding from the gastrointestinal tract, consisting of melena or hematemesis, or both, was obtained from 21 patients. The origin of the bleeding was proved in only 12 patients; in these cases, peptic ulcer disease was found to be the cause. Symptoms related to the nervous system, including hallucinations, impending delirium tremens, and neuromuscular disorders, were noted in 29% of the patients.

Nearly all the patients recovered within a week and were able to leave the hospital. However, 6 patients expired, one of hemorrhagic pancreatitis, one of myocardial infarction, one of cardiac arrest, one of mesenteric thrombosis, and one of pneumonia and a fatty liver. The cause of death in the sixth patient was not ascertained even after autopsy.

Laboratory findings. Although many laboratory tests were done, only the electrolyte disturbances and serum bilirubin will be considered briefly in this article. Abnormalities were noted in the serum potassium, phosphorus and calcium levels. Hypokalemia was the most common electrolyte deficiency, being found in 50% of the patients. Whereas in most instances the hypokalemia was mild, in 10 patients the serum potassium was below 3 mEq/liter. In nearly every instance serum potassium levels below 3 mEq/liter were associated with vomiting, or lack of food, or diarrhea. This was invariably true of those with levels below 2.5 mEq/liter. In this latter group, only one patient had a mild alkalosis.

Hypocalcemia occurred in 13 patients and

hypophosphatemia in 20. Although low serum phosphorus or calcium levels might occur alone, in a majority of patients they were associated with hypokalemia. It is not known how a low serum level of phosphorus or calcium might affect the liver cells, but it is of interest that one patient with a calcium level of 3.3 mEq/liter and potassium of 2.2 mEq/liter died of cardiac arrest soon after admission to the hospital. This case brings to mind the many cases of sudden and unexplained deaths among alcoholics that are noted in coroners' offices throughout this country.

Serum bilirubin levels above 2.0 mg/100 ml of blood were noted in 19 patients, but in only 12 of these was jaundice observed on physical examination. Biopsies were obtained on 3 of these patients; each showed a fatty liver but cholestasis was not observed.

Microscopic findings. The most common microscopic abnormality was fatty change; this was noted in 41 of the 45 biopsy specimens, or 91.1%. Included among the livers with fatty change were 5 in which the changes were slight, less than 1% of the liver cells showing any fat vacuoles. The fatty change in 36 patients was classified on the basis of 1+ to 4+, using the classification of 1+ for fatty change that involved a maximum of 25% of the cells; 2+ for 25 to 50%; 3+ for 50 to 75%; and 4+ for more than 75% of the cells. In 22 patients, the fatty change was 1+; in 10 it was 2+; in 4 it was 3+; none was graded 4+. There was good correlation between the lack of food and the severity of fatty change as all patients who gave a history of not eating for two or more weeks had 2+ or 3+ fat in the liver. In contrast, those who said they had been eating at least a small amount of food daily until 24 hours before entry had either no fat or only 1+ change. One patient who claimed he had not eaten for one week had 1+ fat in the liver, and another who had not eaten for three days had 2+ fatty change. In one patient with severe fatty change, a repeat biopsy performed 33 days later showed no stigmata of disease.

Hydropic change was the second most prevalent type of injury to the liver cells; this was seen in 18 patients, or 40%. The excessive, watery cytoplasm either had a pale

granular appearance or it was vesiculated (both types of change are shown in Fig. 1). The vesicles were most often multiple and filled the cytoplasm. Sometimes larger, poorly outlined vacuoles were present that were difficult or impossible to distinguish from fat vacuoles. The lack of a sharp margin around the larger vacuoles indicated that they might be hydropic in origin, but, on examination with the electron microscope, all large vacuoles appeared to be composed of fat. Hydropic swelling was most often centrilobular; rarely were cells at the periphery affected. There was no constant association between the hydropic swelling and either fatty change or early fibrosis. With the electron microscope, the hydropic cytoplasm seen with the light microscope appeared as ill-defined radiolucent areas devoid of any organelles or other structures (Fig. 2). In addition, in some of the specimens the cisternae were greatly dilated (Fig. 3), although the ribosomes showed no significant change. It was considered that the vacuolar type of hydropic change in the liver cells might be related to the hypokalemia in a manner similar to vacuolar nephropathy, but the presence of hydropic cells in 5 patients with normal serum potassium indicates that this is not necessarily true. Nevertheless, hypokalemia was observed in 10 of 17 patients in whom hydropic change was noted on biopsy, whereas in 28 patients without such change only 10 had a low serum potassium. Intracellular potassium levels were not determined.

The mitochondria in some instances appeared normal, and occasionally they had an increased number of cristae. The most prominent change was the presence of swollen mitochondria that contained few or no cristae (Fig. 3). In many of these, dissolution of the mitochondrial membrane was observed (Figs. 3 and 4). In many of the liver cells the mitochondria were concentrated both along the plasma membrane and around the nucleus, the more or less clear space between being devoid of organelles (Fig. 5). The striking appearance of the mitochondria along the cell membranes is easily recognized with the electron microscope.

Hyaline necrosis accompanied by a few neutrophils and moderate sclerosis of the ves-

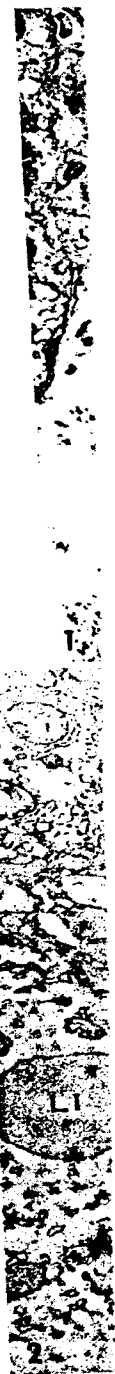


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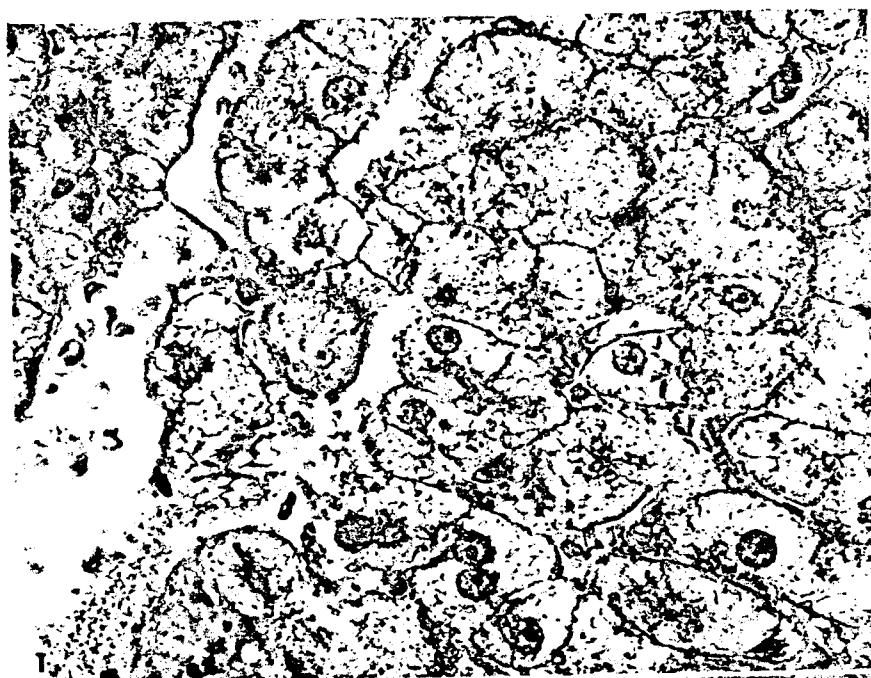


FIG. 1. A vesicular type of hydropic change is seen in many of the enlarged parenchymal cells. Other cells are swollen but are still slightly granular. The patient had a blood alcohol of 274 mg/100 ml on admission to the hospital. He had been on a 10-day "binge" and had not eaten for 3 days. Delirium tremens was the clinical diagnosis. (Hematoxylin and eosin stain; $\times 650$)

FIG. 2. In this cross section of an hepatic sinusoid, the surrounding parenchymal cells show a breakdown of the endoplasmic reticulum in some areas but not in others. The endoplasmic reticulum breakdown may correlate with granular hydropic (HY) swelling. Red blood cells (RBC); lipid droplets (LI); nucleus of liver cell (N). (Lead citrate stain; $\times 9,000$)



FIG. 3. Vesiculation and swelling of endoplasmic reticular cisternae. Ribosomes still line cisternae but the mitochondria show early membrane disruption. Degenerating mitochondria (M); dilated cisternae of rough endoplasmic reticulum (RER). (Lead citrate stain; $\times 32,500$)

FIG. 4. Further vesiculation of endoplasmic reticulum with loss of some ribosomes. An hourglass-shaped mitochondrion (M) shows disrupted membranes. Markedly dilated cisterna of rough endoplasmic reticulum (RER); cisterna of smooth endoplasmic reticulum (SER). (Lead citrate stain; $\times 60,000$)

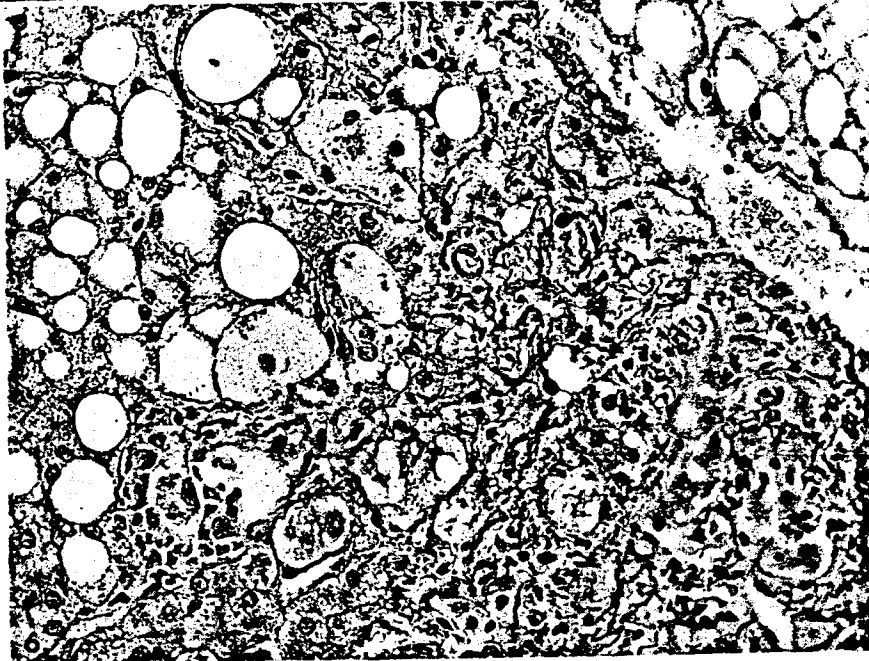
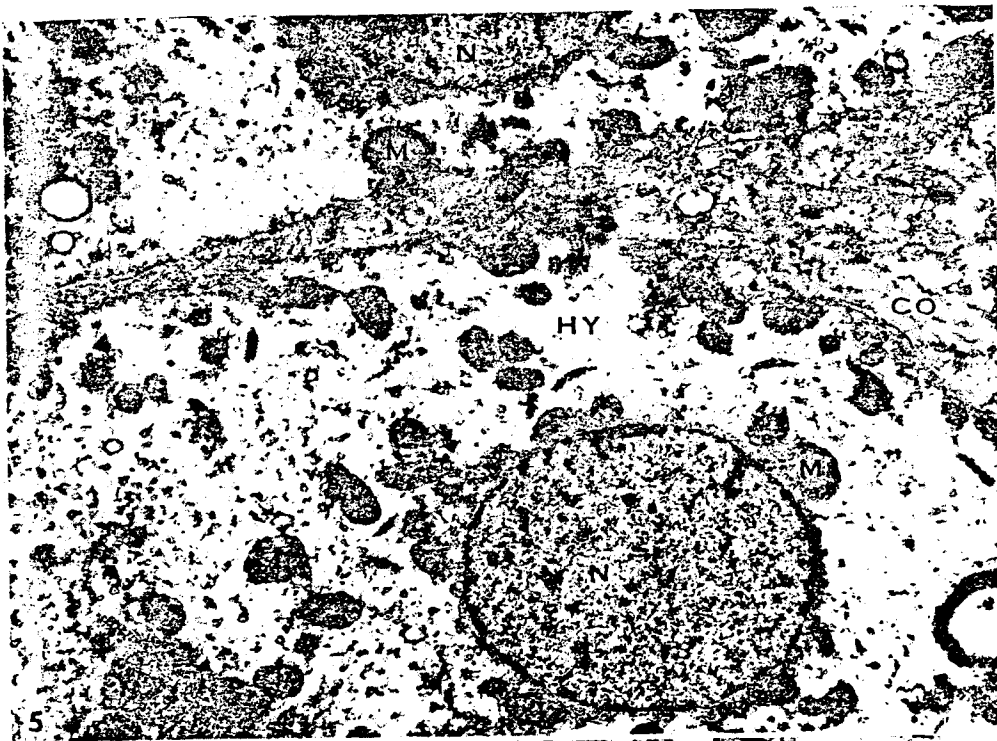


FIG. 5. Low-power electron photomicrograph shows the displacement of cell organelles to the cell membranes and to the nuclear membrane. The endoplasmic reticulum is almost completely destroyed. A few strands of collagen between 2 liver cells can be recognized. Nucleus (N); mitochondria (M); hydropic change (HY); collagen (CO). (Uranyl acetate stain; $\times 11,700$)

FIG. 6. Hyaline necrosis and neutrophilic exudate is seen at the lower left. Intense sclerosis of sinusoids, diffuse hydropic swelling and moderate fatty change are also present. The patient, a 55-year-old woman, had been an alcoholic for 10 years. (Hematoxylin and eosin stain; $\times 350$)

sels was present in only one patient (Fig. 6). A type of hyaline change involving only occasional cells and characterized by small round hyaline bodies within the cytoplasm was seen in 3 biopsy specimens (Fig. 7). These bodies are similar to those reported by Porta and associates (8) in rats that had received 36% of their total calories as ethanol for 2 to 12 weeks. The hyaline bodies were believed to be megamitochondria when examined with the electron microscope. To date we have been unable to identify the small round hyaline bodies in our material with the electron microscope.

Most interesting were the changes in the mesenchymal tissue. Although 20 patients, or 44.4%, had no centrolobular mesenchymal changes (Fig. 8), the other 25 patients, or 55.6%, did show some degree of connective tissue proliferation. The milder degrees of proliferative changes were unexpected because biopsies on alcoholics without symptomatic liver disease had not been done on more than an occasional patient. An increase of connective tissue that stained positively for collagen often thickened the walls of the central veins and sinusoids (Figs. 9 and 10). The lumen of the vessels did not seem to be compromised in any way and the sclerotic process was not in every case associated with fatty, hyaline, or hydropic change in the parenchymal cells. In more advanced lesions, the collagen surrounded individual liver cells or groups of liver cells and at the same time obliterated the sinusoidal lumens (Fig. 11). The presence of collagen in the space of Disse was observed on examination with the electron microscope (Fig. 12). The amounts of collagen varied; in some specimens it was an outstanding feature and penetrated the spaces between individual liver cells (Fig. 5). Although the centrolobular sclerosis might occur alone, in most instances there was an increase of periportal connective tissue of a lesser or an equivalent amount; rarely was it greater.

A summary of the microscopic findings is given in Table 1.

DISCUSSION

In this study, biopsies were performed on 45 of 100 patients, none of whom had cirrhosis, so there was an opportunity to study the early

necrosis was rarely observed; it occurred in only 3 of 45 biopsy specimens and in one patient at autopsy. In one of the specimens, the



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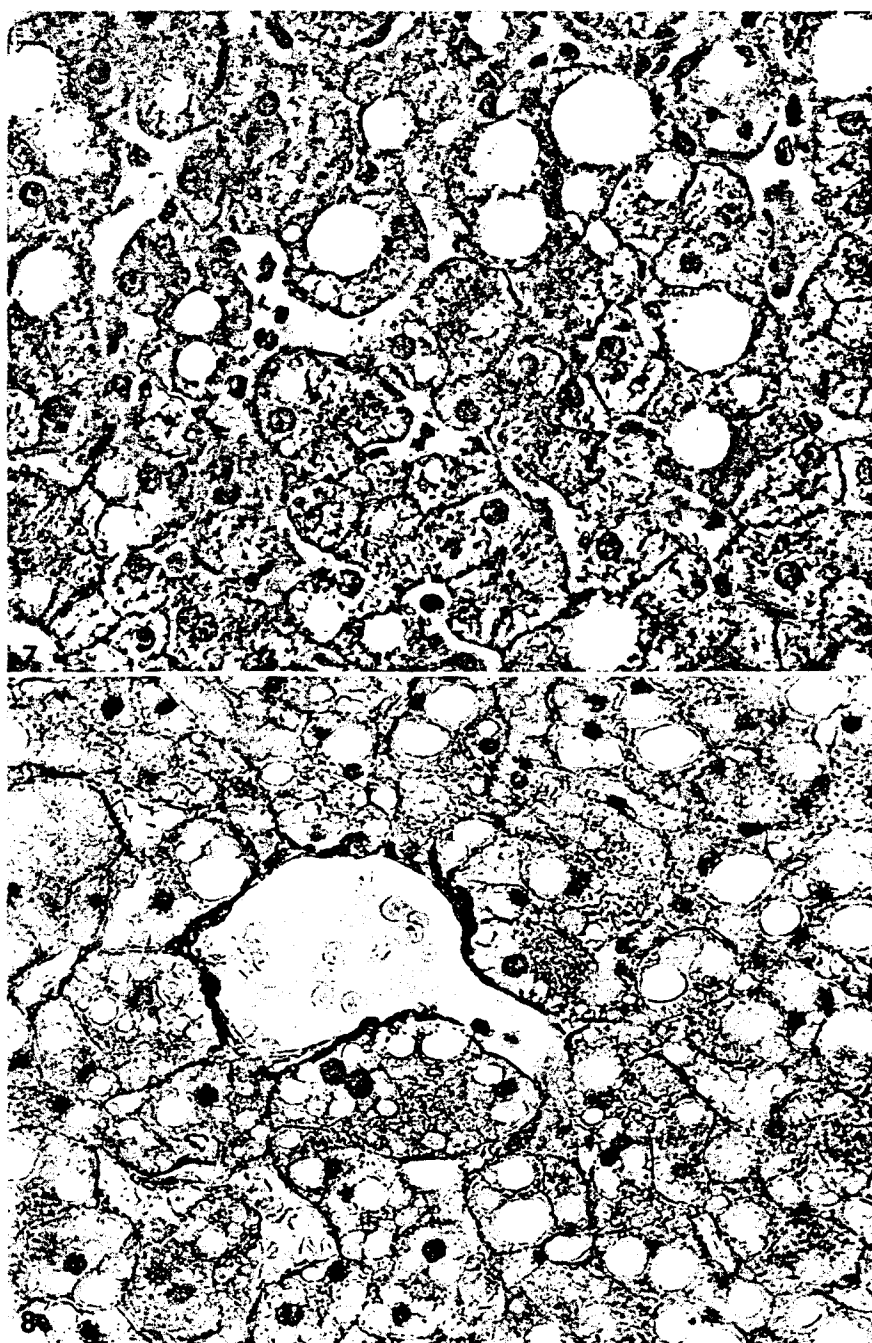


FIG. 7. Small round hyaline bodies are present in two liver cells at the upper right. There is mild fatty change. The patient had been on a 4-day spree, and without food. He claimed to have been drinking one gallon of wine per day. (Hematoxylin and eosin stain; $\times 500$)

FIG. 8. This central vein appears normal, even though marked fatty change involves the liver cells. The patient, an alcoholic for 20 years, had been on a spree for 2 weeks without any food intake. (Masson trichrome stain; $\times 500$)

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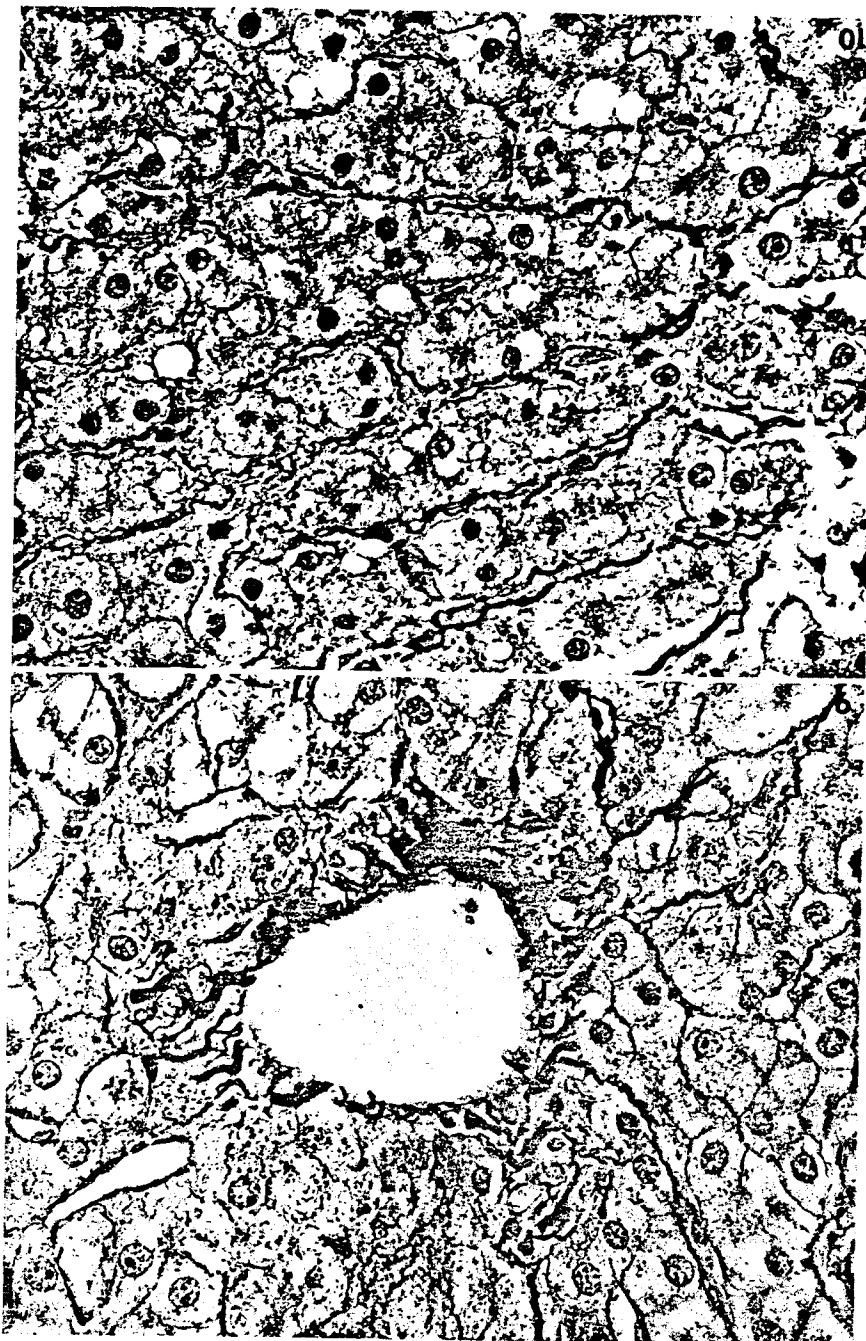


Fig. 9. The wall of this central vein is thickened and spurs of connective tissue extend outward along the surrounding sinusoids. Same patient as in Fig. 1. (Masson trichrome stain; $\times 500$)

Fig. 10. Early thickening of sinusoidal walls in a liver that contains only a few fat vacuoles. The patient had been an alcoholic for 15 years but he had been eating fairly well. (Masson trichrome stain; $\times 500$)

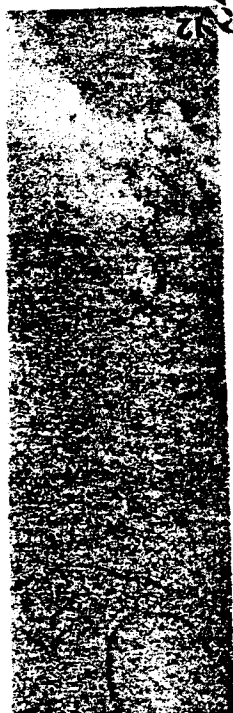


Fig. 11. The central vein is a 2+ fat vacuolated. There is a 2+ fat vacuolation. (Lead citrate stain; $\times 300$)



Fig. 12. Prominent picture. Parenchyma (M). (Lead citrate stain; $\times 300$)

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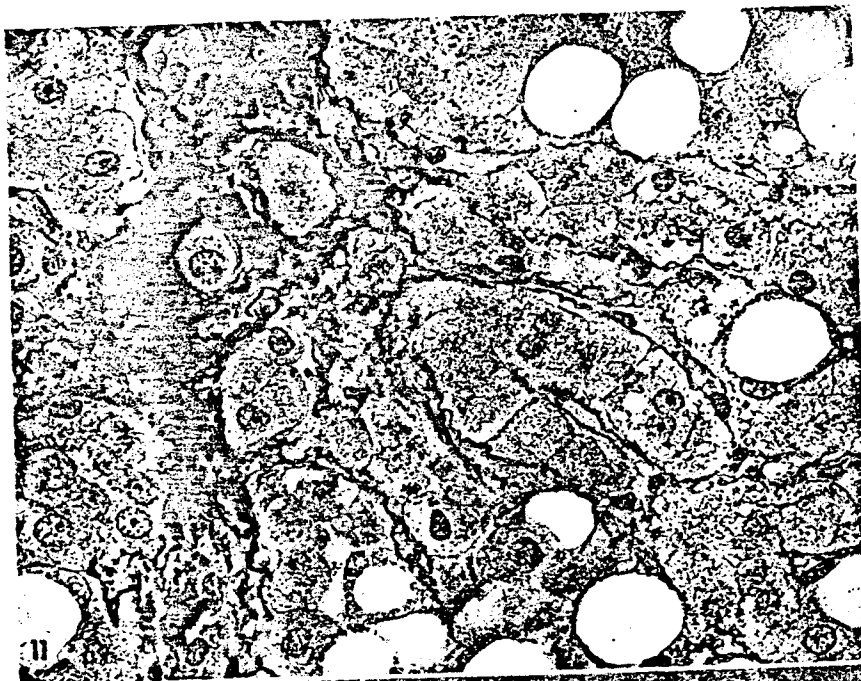


FIG. 11. The connective tissue has encircled individual liver cells and obliterated sinusoids. There is a 2+ fatty change. The patient had been drinking heavily for 1 month and had not eaten for 3 days previous to hospitalization for delirium tremens. (Masson trichrome stain; $\times 300$)

FIG. 12. Prominent collagen deposition in space of Disse (SD). Sinusoid (S) is at top of picture. Parenchymal cell microvilli extend around collagen (CO) fibrils. Mitochondrion (M). (Lead citrate stain, $\times 46,000$)

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TABLE 1
Microscopic Findings in 45 Patients

Fat	Hydropic change	Hyaline necrosis	Centrolobular sclerosis	Periportal fibrosis
0-1+ in 9	4			
1+ in 22	8	1	1+ in 5	1+ in 2
2+ in 11	6	2	1+ in 1	1+ in 2
			2+ in 3	2+ in 2
3+ in 3			1+ in 1	

potassium levels did not have any hydropic change. The possibility of widespread damage to the cell membranes cannot be ruled out, even though the only direct evidence of this was seen in the disruption of the mitochondrial membranes. At a recent symposium on the "Forms of Water in Biological Systems," Hechter (3) discussed the intracellular water structure and mechanism of cellular transport. He presented a schematic representation of the "resting membrane," showing the protein layers interlocked by water layers in an icelike arrangement. Taking into consideration both the light and the electron microscopic findings in our biopsy sections, it seems inevitable that the normal relationship of water to the membrane systems is disturbed. Further investigation of the plasma membrane as well as the membranes of the organelles is needed.

Another possible cause for the hydropic change in the liver cells is suggested by the experiments of Kalant and co-workers (4) who showed that ethanol affects the transport of cations across cell membranes, leading to an increase in intracellular water and sodium and a possible fall in intracellular potassium.

Severe mitochondrial changes in the livers of chronic alcoholic patients have been reported (2, 7, 9, 10). Enlargement of mitochondria, with abnormality of shape, changes in the cristae, myelin figures and degeneration were reported by Porta and associates (7). Svoboda and Manning (10) noted crystal-like inclusions, often with mitochondrial enlargement. Abnormally large, dense bodies in the mitochondrial matrix, cavitation of mitochondria and rearrangement of cristae were also observed. Lane and Lieber (5) used human subjects who, after a period of normal diet, were given an analogous diet in which

the carbohydrate was replaced isocalorically by ethanol to the extent that 46% of the total caloric intake was supplied by ethanol. The diets were continued for 16 to 18 days. The authors noted on biopsy that a minority of the mitochondria exhibited some degree of damage.

The small number of biopsy specimens showing hyaline necrosis indicates that this type of injury is infrequent in patients who do not have symptoms of liver disease. The small, round hyaline bodies which are morphologically dissimilar to "alcoholic hyalin" seen in otherwise normal appearing cells may or may not have any significance. It is probable that liver cell injury, short of necrosis, in the alcoholic is reversible, even after repeated "binges" over a period of many years.

The question of whether or not hydropic change and small hyaline bodies affecting the liver cells may, if the patient continues to drink and abstains from food, progress to sclerosing hyaline necrosis is left unanswered. It seems to us that patients who enter the hospital with this disorder most likely go through a stage in which their liver shows only the milder changes. Perhaps the onset of either delirium tremens or nausea and vomiting, followed by hospitalization, prevents the more serious liver disorder. However, there may be unknown factors that lead to the onset of acute sclerosing hyaline necrosis, as we have occasionally seen the disease become clinically manifest several days or weeks after the patient has been hospitalized.

The presence of increased connective tissue around the central veins and along the sinusoidal walls in the absence of any other significant lesion was unexpected. Although it cannot be definitely stated that this excess connective tissue forms without any concomitant liver cell injury, we were unable, in some biopsy specimens, to observe any such injury with the light microscope. We know that more advanced fibrosis is often associated with hyaline necrosis of parenchymal cells. The exact relationship of the two types of injury is not clear. Some of the material in the walls of the sinusoids that stains with Masson's trichrome stain may be material other than collagen; but, on electron microscopic studies, collagen fibers were easily demon-

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strated in our material. Although the proliferative change may undergo slow resolution when the patient abstains from ethanol, it is likely that the sclerosing process is more or less progressive in many alcoholic patients, leading eventually to symptoms of chronic liver disease. The stimuli responsible for the proliferation of cells and collagen along the sinusoids are unknown. The remarkable variation in the intensity of the process from one patient to another is most difficult to explain.

Although a more detailed report will be made of the clinical and laboratory aspects of this study, a few findings are worthy of comment. The mortality rate of 6% among alcoholic patients who had been on a "spree" and who did not appear to be seriously ill on entry to the hospital was rather surprising. We hope to study further this group of patients if a significant number of them are hospitalized again for acute alcoholism. The fact that nearly one-fourth of those who complained of abdominal pain had laboratory findings suggesting pancreatitis emphasizes the frequency of this complication and shows the importance of determining the serum amylase level on all such patients.

The marked disturbances in serum electrolytes and death due to cardiac arrest in one such patient implies that electrolyte deficiencies should be promptly treated and not allowed to remain until the patient can eat enough to regain electrolyte balance.

Lastly, the absence of a palpable liver does not mean the organ may not contain excess fat. Five patients had 1+ to 2+ in their liver biopsy study, but the liver was not enlarged on physical examination. However, if the patient is jaundiced, the liver is nearly always enlarged.

SUMMARY

One hundred chronic alcoholic patients without evidence of cirrhosis were studied at the time of an episode of acute alcoholism.

Six patients died, each of a different complication that was not related to alcoholic liver disease. The frequency of serum electrolyte deficiency was high and should be considered as a possible factor in sudden death. Biopsy specimens of the liver were taken from 45 patients. Microscopically, although fatty change was present in over 90%, there was a lack of severe hepatic alterations. More than one-third of the patients had swollen hydropic liver cells that were characterized by organelle disorganization when viewed with the electron microscope. About one-half of the patients had some centrilobular intrasinusoidal collagen which seemed to have its origin in the spaces of Disse but often could be found in the intercellular spaces by electron microscopy.

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