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CHANGES IN HEPATIC STRUCTURE IN WILSON'S DISEASE

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The morphologic characteristics of the cirrhosis in Wilson's disease have not been clearly established from the scattered reports in the literature frequently based on single cases. From the time of the earliest reports, the majority of observers have described a coarse, nodular cirrhosis that is now conventionally designated as postnecrotic cirrhosis. Others have described a fine granular liver, now usually called portal, Laennec's, or diffuse septal cirrhosis. Most of the histologic descriptions are indeed sketchy by present standards. The present investigation of a series of cases of Wilson's disease was carried out in an attempt to gain an understanding of the pathogenesis of the cirrhosis in this disorder. Since it seems to be one of the few types of cirrhosis in which at least one etiologic factor, namely, a disturbance of copper metabolism, is established,[1] an investigation of the histologic pattern in fully developed cases or in presumed precursor stages assumes added importance.

MATERIAL AND METHOD

Tissue fixed in formalin or Zenker's solution, paraffin blocks, or prepared sections were obtained with the co-operation of pathologists and clinicians from various areas in the United States. Where possible, in addition to hematoxylin and eosin stained preparations, the following special methods were utilized: the periodic acid-Schiff reaction before and after removal of glycogen with diastase, the Gomori silver impregnation for reticulin, chromotrope-aniline blue, Prussian blue reaction for iron, and Sudan black and oil red O for neutral lipids in frozen sections.

Most of the patients were in the younger age group and exhibited, except where specifically stated, the characteristic neurologic and biochemical manifestations of Wilson's disease.

OBSERVATIONS

The specimens examined consisted of 19 cases with cirrhosis and 2 without. The sections with cirrhosis exhibited variations in the size of the hepatic nodules, ranging from small aggregates of few cells about 300 μ in diameter to nodules with a maximum diameter of 3 cm. In the smaller nodules, as a rule, the cell plates converged toward the center of the nodule, whereas in the larger nodules a lobular architecture was apparent, with persistent portal tracts and central veins. Occasionally, as many as 4 of each were visible on cross section in one nodule. The central veins appeared more abundant than in the normal liver, and some were found at the periphery of the nodule near the connective tissue septum. In most of the fatal cases, the liver cells near the central veins were necrotic. The degree of degeneration and regeneration of liver cells varied, depending upon the circumstance. The portal tracts occasionally appeared to be connected by perilobular fibrosis. The connective tissue septums between the nodules exhibited considerable variation in width and extent. Veins were abundant in all of them. In the wider septums, forming broad connective tissue bands, "ghost lobules" were apparent following loss of the liver cell plates; in these areas central and portal canals were closely approximated. The former central and intermediate zones consisted of a collapsed framework with only a few cells of mesenchymal character remaining. The peripheral zone was marked by extensively proliferated ductules. The original border of the portal tract, when stained with connective tissue stains, was almost always distinguishable from the collapsed framework since the heavy collagen fibers of the former contrasted well with the wavy membranes in the collapsed parenchyma. The bile ducts and the blood vessels failed to reveal significant alterations. These basic features were modified from case to case, depending upon the activity of the lesion (Table I). In the material examined, several stages could be recognized.

Arrested Stage (4 Necropsies, 1 Biopsy Specimen)

The liver cells revealed little abnormality except for focal necrosis. The cell plates were more than one cell thick in only a few of the smaller nodules. The parenchymal nuclei had the normal vesicular appearance. In the biopsy specimen the cytoplasmic glycogen content appeared average, judging from its vacuolation and granulation. The border between the nodules and the septums was sharp, and the limiting plate was

TABLE I

HISTOLOGIC FEATURES IN VARIOUS STAGES OF CIRRHOSIS IN WILSON'S DISEASE

	Arrested	Partially arrested	Active	Acute parenchymal breakdown	Pre-cirrhotic	Sibling of patient with Wilson's disease
Number of cases	5	6	7	1	1	1
Multilobulated lobules	+	+	+	+	0	0
Broad bands of collapse	0	+	+	+	0	0
Collapse with ghost lobules	0	+	+	+	0	0
Thin connective tissue septums	+	0	0	0	0	0
Conspicuous hepatocellular regeneration	0	+	+	+	0	0
Conspicuous ductular cell proliferation	0	0	+	+	0	0
Defect in limiting plate	0	+	+	+	0	0
Fatty metamorphosis						
Large droplets	0	+	+	+	+	0
Small droplets	0	+	+	+	0	0
Large Kupffer cells	0	+	+	+	0	0
Giant nuclei with glycogen	0	0	0	0	0	0
Moderate nuclei with glycogen	0	+	+	+	0	+
Few nuclei with glycogen	+	0	0	0	+	0
Necrotic liver cells	0	+	+	+	+	0
Nonspecific necrosis	0	0	0	0	0	0
Bile pigment in liver cells	0	0	+	+	0	0
Iron pigment in liver cells	0	0	0	0	0	0
Heavy inflammatory reaction in portal tract	0	0	0	0	0	0

well defined (Fig. 1). The portal tracts showed little inflammatory reaction.

Partially Arrested Stage (6 Necropsies)

Most of the nodules were similar to those described in the previous stage except that many of the peripherally located liver cell nuclei were markedly distended and enlarged up to 20 μ. The nuclear border appeared sharp and refractile. The central portion was unstained, and the periphery of the nuclear droplet gave a positive reaction for glycogen. The nucleolus was displaced to the periphery of the nucleus. In addition, particularly in the smaller nodules, large regenerating liver cells with giant nuclei were common. The hepatic cells were usually arranged in several rows. This was associated with blurred nodular margins and an absence of a limiting plate (Fig. 2). Small numbers of hepatic cells with pigmented and vacuolated cytoplasm lay isolated in the surrounding connective tissue; others appeared in continuity with proliferating ductules. The latter were surrounded by a mononuclear inflammatory exudate containing some neutrophils. In some instances, usually in those with lesser degrees of nuclear degeneration, the disintegrating liver cells exhibited conspicuous fatty metamorphosis. The fat appeared in the form of both large and small droplets.

Active Stage (7 Necropsies)

In all instances except one, large nuclei containing glycogen were prominent features, and in 5 cases these were abundant. The largest of the nuclei measured more than 35 μ in diameter (Figs. 3 and 4). In all but one case, fatty metamorphosis was frequently in the form of small droplets more or less crowding the cytoplasm but not displacing the nucleus (Fig. 7). In some instances, large, single fat droplets filled the entire cell and pushed the nucleus to the side (Fig. 8). In 2 cases almost all liver cells in all nodules exhibited severe fatty metamorphosis. Disintegration of the fatty cells was characterized by acidophilic coagulation and bile pigmentation as well as by the accumulation of nonglycogenic PAS-positive intracytoplasmic granules. Neighboring Kupffer cells were conspicuously enlarged and contained cellular breakdown products, some of which were PAS-positive. Fat droplets up to 6 μ in diameter and amorphous acidophilic material also appeared within Kupffer cells (Fig. 9). The fatty metamorphosis and disintegration of liver cells, as well as the Kupffer cell reaction, were particularly marked in smaller nodules. The parenchyma bordering upon the connective tissue was not sharply outlined. Regeneration was prominent and characterized by giant liver cells with large nuclei. In sections impregnated

with silver, recent collapse of the pre-existing reticulin framework was noted. Ductular proliferation and inflammatory reaction were generally prominent, and plasma cells were, in some cases, frequently gathered about the proliferated ductules. Bile stasis with plugs in bile ductules was found in 4 cases.

Acute Parenchymal Breakdown (One Necropsy)

While glycogen degeneration of the nuclei and fatty metamorphosis were prominent but less widespread than in other cases, necrosis of liver cells was reflected by conspicuous brown cytoplasmic pigmentation and PAS-positive granulation. Neighboring Kupffer cells were heavily loaded with bile pigment and PAS-positive material. Approximation of the reticulin framework, indicating recent collapse, was noted in many areas (Fig. 6). The collapsing zone appeared to merge with the neighboring connective tissue septums (Fig. 5). They contained irregularly proliferating ductules, many scavenger cells and isolated bile pigment-laden liver cells.

Precirrhotic Fatty Metamorphosis (One Biopsy Specimen)

The liver of a patient with the clinical and biochemical features of Wilson's disease showed patchy areas of fatty metamorphosis mainly in the form of large droplets without specific lobular distribution (Fig. 10). There was practically no liver cell necrosis, and the Kupffer cells contained only scant amounts of PAS-positive material in diastase-treated sections. Very few of the liver cell nuclei exhibited glycogen change. Small amounts of copper were demonstrated in the parenchymal cells, using the technique of Uzman.[2]

Sibling of a Patient with Wilson's Disease (Two Biopsy Specimens)

The surgical biopsy specimens were obtained from one child at the ages of 3½ and 4½ years. The sister of this patient had evidence of fully developed hepatolenticular degeneration. Ceruloplasmin had been absent from the serum of the patient since birth, but no other clinical or laboratory evidence of hepatic or neurologic dysfunction was detectable.[3] Both specimens exhibited normal hepatic structure except for the presence of enlarged glycogen nuclei in the periphery of the lobules (Fig. 11).

Control Group

For comparison, 15 examples of active postnecrotic cirrhosis not associated with Wilson's disease were gathered from the necropsy files of the Department of Pathology, Mount Sinai Hospital. Glycogen-contain-

ing nuclei were observed in 3 instances. In one this feature was fairly prominent though not as extensive as in most of the cases of active Wilson's disease. The nuclei in these cases did not attain the unusual size observed in the active stages of Wilson's disease. Fatty metamorphosis of some degree was noted in 8 of the control cases. Necrosis and breakdown of fatty cells was noted in only one instance, and this occurred without enlarged Kupffer cells.

DISCUSSION

In all necropsy specimens from cases of hepatolenticular degeneration, the typical lesion of postnecrotic cirrhosis was found. This was characterized by 3 cardinal manifestations [4,5]: (a) irregular distribution of the postnecrotic alterations as reflected by preserved lobular architecture in many nodules; (b) stromal collapse with the formation of septums of varying width; (c) conspicuous parenchymal regeneration. The existence of postnecrotic cirrhosis as a regular feature of Wilson's disease is confirmed by those cases reported in the literature, in which either the illustrations or the histologic descriptions provide adequate information as to the character of the cirrhosis. [6-10]

Postnecrotic cirrhosis may result, at least in some instances, from viral hepatitis. Several factors are incompatible with the assumption that the cirrhosis of Wilson's disease might be the sequel of intercurrent viral hepatitis. Some of the manifestations of acute viral hepatitis seen in postnecrotic cirrhosis, i.e., acidophilic bodies and single cell necrosis, with accumulation of mononuclear cells in place of the liver cells that have disappeared, [11,12] were not observed in Wilson's disease. There were two features which were conspicuous, however, in Wilson's disease. These were not evident as prominently or as frequently in examples of postnecrotic cirrhosis, particularly those following viral hepatitis. One was the presence of both large and small fat droplets in hepatic cells. This is occasionally found in postnecrotic cirrhosis, presumably as the result of complicating nutritional disturbance. In Wilson's disease, however, fatty metamorphosis is a regular feature and apparently is associated with the activity of the process, particularly in relation to foci of degenerating liver cells. The appearance of large Kupffer cells in such areas is also noteworthy and would seem to indicate an irritation of these mesenchymal cells by fat. This is not common in other types of cirrhosis. The fatty metamorphosis seemed to precede the cirrhotic alterations. This was suggested by one of our biopsy specimens and has been indicated by other recorded observations. [9,13]

A second important feature is glycogen degeneration of nuclei, which appears to be outstanding in the active stages of the cirrhosis in Wilson's

disease. Accumulation of glycogen within hepatic nuclei has been observed with significant frequency in diabetes.[14] It has been recorded as an incidental occurrence in other conditions and has been produced experimentally by the *in vivo* perfusion of rat liver with hypotonic saline.[15] The physiologic implications of this phenomenon are not wholly clear. It, too, occurs in other types of postnecrotic cirrhosis but not with the frequency and regularity that is manifested in Wilson's disease. In the majority of our cases the nuclear change appeared in many more cells than was usually the case in the livers of patients with diabetes. Even more remarkable was the large size of the glycogen-containing nuclei, particularly in the active stages of the cirrhosis. Possibly the glycogen degeneration is, in some way, related to the underlying metabolic abnormality in this condition. This is suggested by the occurrence of this alteration in the liver of a clinically normal sibling of a patient with Wilson's disease. This child exhibited no abnormality except for the absence of serum ceruloplasmin. To our knowledge, glycogen degeneration of hepatic nuclei has not been specifically emphasized in previously reported cases of Wilson's disease. However, nuclear vacuolation has been mentioned by several authors,[8,16,17] and in at least one report[7] it was ascribed to the presence of intranuclear glycogen. The vacuolation can be recognized in the illustrations in several reports.[17-19] The possibility that the nuclear vacuolation is an incidental feature attributable to the parenteral administration of supportive fluids (intravenous glucose or saline infusions) must also be entertained. This would apply particularly in the case of patients receiving supportive care in the terminal stages of their illnesses.

Although the relation of fatty metamorphosis and nuclear glycogen degeneration to the metabolic disturbance in Wilson's disease is not clear, these alterations appear to be associated with the breakdown of hepatic parenchyma that leads to the postnecrotic cirrhosis in Wilson's disease.

The specimens investigated reveal various features in the cirrhosis of Wilson's disease, indicating a range from arrested to active stages. In addition to the other recognized criteria of activity in postnecrotic cirrhosis, fatty metamorphosis and glycogen degeneration of the liver nuclei occur in Wilson's disease. In one case in which massive necrosis and beginning collapse reflected the active development of characteristic features of postnecrotic cirrhosis, the most actively degenerating cells appeared bile-laden. It is likely, therefore, that in the massive necrosis underlying postnecrotic cirrhosis, other processes are involved as well. The observations cited indicate that the cirrhosis seen in Wilson's disease may develop through stages that differ from those observed following viral hepatitis.

SUMMARY

Histologic observations of the liver in 20 examples of hepatolenticular degeneration (Wilson's disease) indicate that the cirrhosis associated with this condition is postnecrotic in type, with stages varying from those which are completely arrested to others which are very active. Features not found, at least with significant regularity or extent, in other types of postnecrotic cirrhosis are degeneration of fat-containing liver cells, the appearance of excessively large Kupffer cells, and glycogen degeneration of the liver cell nuclei. The fatty metamorphosis and nuclear alterations apparently precede the development of cirrhosis and appear to contribute at least in part, to the necrosis and stromal collapse characteristic of postnecrotic cirrhosis.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Except where indicated, the photographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1.   Arrested stage of postnecrotic cirrhosis in Wilson's disease. The limiting plate of the nodule is well defined, and the inflammatory reaction in the broad septum is subdued. $\times$ 63.

FIG. 2.   Partially arrested stage. The margins of the nodules are not sharp. Hepatic cell nuclei distended with glycogen are present at the periphery of the nodules (arrow) or near portal tracts. $\times$ 63.

FIG. 3.   An active stage with destruction of the limiting plate producing ragged nodule borders. Many liver cell nuclei are vacuolated and contain glycogen. The septum exhibits a diffuse inflammatory reaction and ductular proliferation. $\times$ 63.
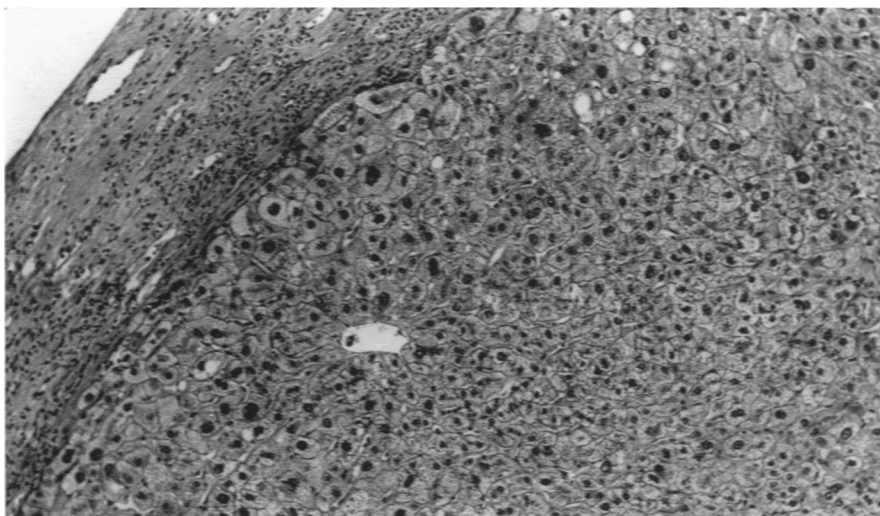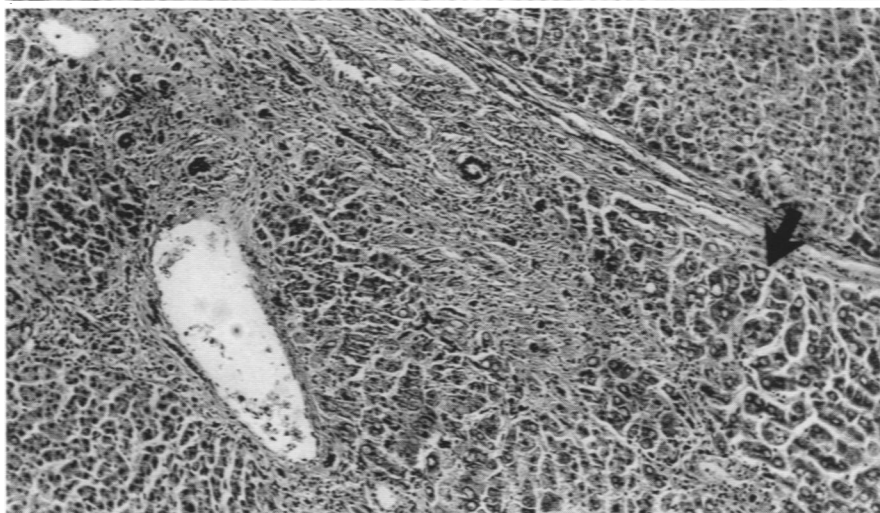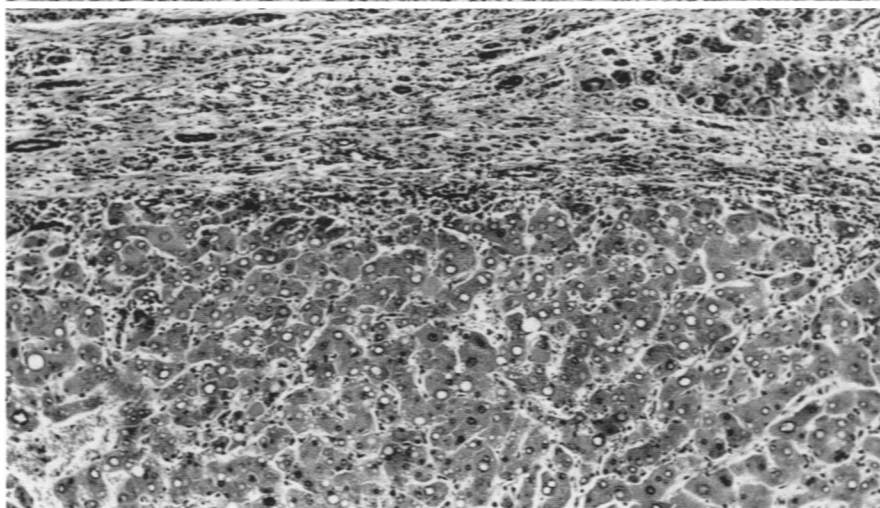
Fig. 4.   Nuclear glycogen degeneration in an active stage of cirrhosis in Wilson's disease. The nuclei are extremely enlarged and ballooned; nucleoli are displaced to the periphery. × 400.

Fig. 5.   Stage of acute parenchymal breakdown. Necrosis is widespread, and the collapsing hepatic parenchyma appears to merge with adjacent septums. Fatty metamorphosis (straight arrow) and glycogen degeneration of nuclei (curved arrow) are noted. × 120.

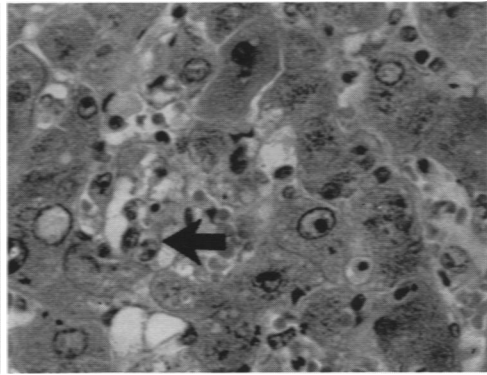Fig. 6.   Acute parenchymal breakdown. The reticulin framework is closely approximated (arrow). Gomori reticulin stain. × 120.
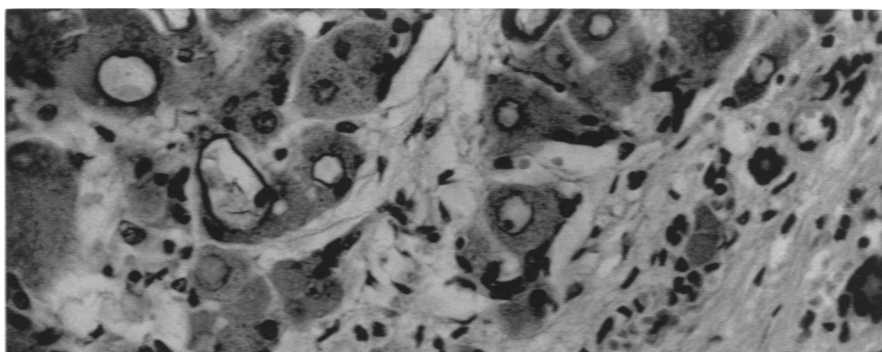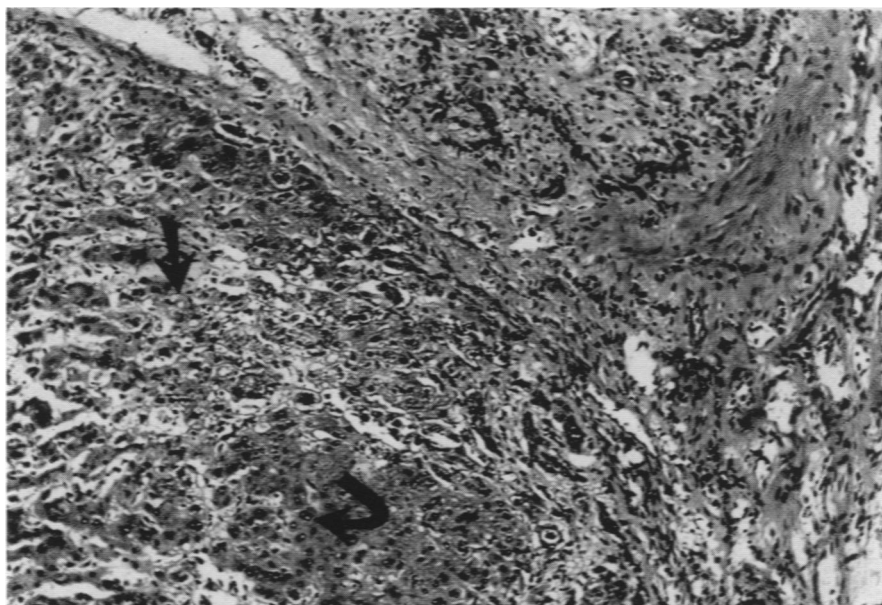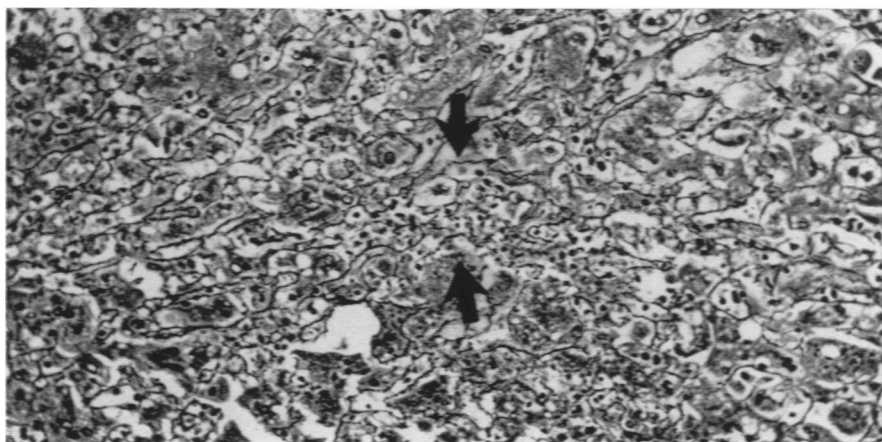
Fig. 7.   Active stage. Fatty metamorphosis and nuclear glycogen degeneration is typically seen at this stage. × 120.

Fig. 8.   Active stage. Large fat droplets appear within the cytoplasm of parenchymal cells, and a large Kupffer cell has a granular cytoplasm (arrow). × 240.

Fig. 9.   Active stage. Enlarged Kupffer cells with vacuolated cytoplasm are associated with fatty metamorphosis and pigment deposition in liver cells. × 240.

Fig. 10.   Precirrhotic stage. Liver biopsy from a patient with the clinical and biochemical features of Wilson's disease. Only extensive fatty metamorphosis is evident. × 63.

Fig. 11.   Liver biopsy from an asymptomatic sibling of a patient with Wilson's disease. The sibling showed only absence of serum ceruloplasmin. Large nuclei containing glycogen are present in some of the hepatic c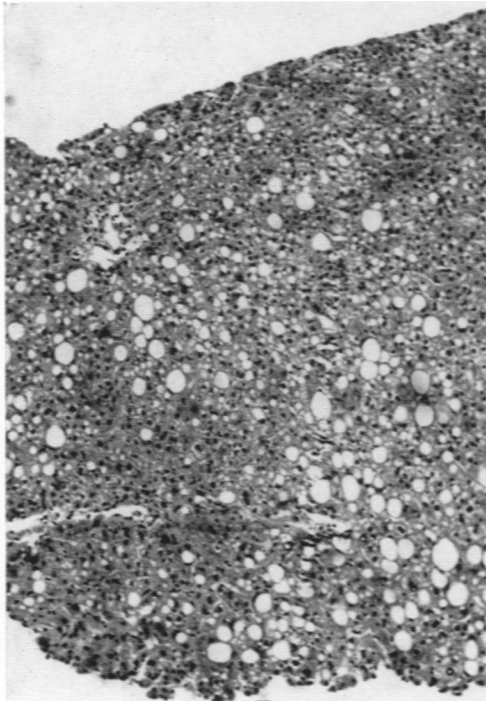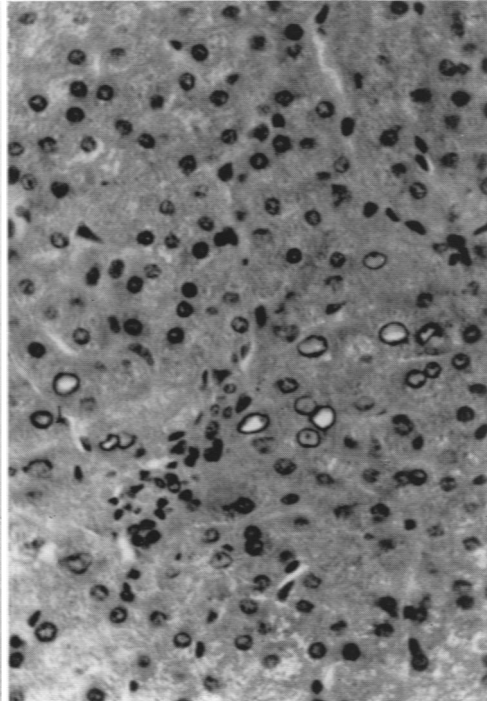ells at the periphery of lobules. × 120.