Histogenesis of Alcoholic Fibrosis and Cirrhosis in the Baboon

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Sequential liver specimens of 18 baboons exposed for up to 6 years to alcohol on a nutritionally adequate diet, as well as those of pair-fed controls, were examined by light microscopy. Whereas control animals failed to develop pathologic changes, in all baboons exposed, ethanol produced initial steatosis and subsequently fibrosis. Emphasis was on the pattern of the fiber accumulations as related to lesions of the hepatocytes. Segmented neutrophilic leukocytes were rarely observed, and the picture of frank alcoholic hepatitis was absent, but diffusely increased mononuclear sinusoidal cells and interstitial clusters of such cells with PAS-positive macrophages were abundant. Fibrosis proceeding to septum formation was associated mainly with large-droplet steatosis. Septum formation was initiated by excess layers of reticulin around steatotic hepatocytes or, more frequently, by linking of fiber accumulations around the clusters of mononuclear cells, in both instances with subsequent deposition of collagen fibers. Both processes were prominent in the centrolobular zone, creating a perivenous net-like fibrosis, but septum formation also started within the lobular parenchyma and eventually linked with the barely altered portal tracts. Fifteen alcohol-fed baboons developed septums, with diffuse septal fibrosis in 5; 4 proceeded to septal cirrhosis and 1 each to micronodular and to mixed micro-macronodular cirrhosis. Cirrhosis in the baboons thus develops without the conspicuous polymorphonuclear inflammation characteristic of human alcoholic hepatitis. These observations indicate a pathway to cirrhosis over creeping fibrosis that might play a role also in man, instead of (or supplementing) the one proceeding over alcoholic hepatitis. (Am J Pathol 1980, 98:695-716)

THE COMMON KEY FEATURES of human alcoholic liver injury —steatosis, hepatitis, cirrhosis—are established, including their clinical, functional, and morphologic aspects. By contrast, the mechanisms of the formation of cirrhosis in alcoholics are less clear. Several decades ago, on the basis of primarily animal experiments,[1] it was assumed that steatosis alone may lead to fibrosis and eventually to cirrhosis. Subsequently, however, alcoholic hepatitis was recognized as a clinical and pathologic entity, and this term [2] replaced the theretofore used description of progressive alcoholic cirrhosis.[3] Since then, the opinion has prevailed that alcoholic hepatitis is an essential step in the transition to cirrhosis. The de-

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velopment of a model of chronic liver injury from alcohol intake in baboons [4] with features greatly resembling chronic alcoholic liver disease in man provided the opportunity to 1) study sequentially the morphologic pathways leading first to fibrosis and then to cirrhosis and 2) evaluate the role of alcoholic hepatitis in this transition. The purpose of this paper is to trace the light-microscopic features of fiber formation and also to relate fiber formation to other hepatic lesions in baboons exposed for several years to large amounts of alcohol with a nutritionally adequate diet. It represents, in part, a detailed reexamination of the material previously reported.[5] This study did not confirm all previous interpretations, particularly regarding the presence of alcoholic hepatitis.

Materials and Methods

Thirty-six baboons were pair-fed up to 6 years a liquid control diet or a similar diet in which carbohydrate (50% of calories) had been replaced isocalorically with ethanol as described previously.[4] The protein content (18% of total calories) corresponds to that of commonly used commercial diets that are satisfactory for the baboon and is almost twice the amount recommended for human diets. The mineral, vitamin, and choline content of the diet exceeded the amounts recommended for nonhuman primates.[6,7,8] The caloric value was 1 calorie/ml. The diet was prepared by Bio Serv Inc., Frenchtown, New Jersey, and was given to the baboons twice a day in standard drinking bottles equipped with an outlet valve. Each alcohol-fed animal was matched with a control, the dietary intake of which was identical except for the isocaloric substitution of carbohydrate by ethanol. This technique of daily pair feeding was adopted to assure a strictly equal caloric intake in ethanol-treated animals and in their individually pair-fed control animals.

The 36 adolescent or young animals used for this study were either *Papio hamadryas* or olive and yellow baboons. Twelve animals were raised in this country, and the remainder were imported from Africa and were studied after prolonged quarantine periods. They were housed in individual cages at the Laboratory for Experimental Medicine and Surgery in Primates, Tuxedo, New York. Until the actual study period, they were given a routine regimen of Purina monkey chow *ad libitum*, supplemented with a daily vitamin preparation. The animals entered the study after prolonged observation and after repeated hematologic and stool examinations had indicated the absence of disease.

Surgical or needle biopsies of the liver were performed at regular intervals under ketamine anesthesia.

This study is based on the histologic analysis of biopsy (mostly surgical) and autopsy specimens (Table 1). It represents in small part a reexamination of specimens previously described in lesser detail.[5,9–11] From paraffin blocks, sections were cut and stained by hematoxylin and eosin (H&E), periodic acid–Schiff (PAS) after glycogen digestion with diastase, Shikata's orcein stain for elastic fibers and macrophagic pigment, and iron reaction. For demonstration of connective-tissue fibers as well as of megamitochondria, chromotrope aniline blue (CAB) was used, and for connective-tissue fibers the van Gieson stain and a silver stain without gold toning in which the reticulin fibers appeared black and collagen bundles yellow. The collagen bundles seen with the latter stain were assumed to represent mostly collagen Type I, and the reticulin fibers, collagen Type III.[12] However, no immunohistochemical analysis was performed. Therefore, the collagen bundles that were stained by the van Gieson stain and appeared yellow in the silver stain used were designated as collagen in the descriptions given, while the black fibers in the silver stains were designated as reticulin. With CAB, part of the reticulin fibers were stained blue but could be differentiated from the thicker, "harder" collagen bundles. With H&E some of the collagen bundles showed birefringence.

There is some discordance in the literature concerning the definition of alcoholic hepatitis. With others,[13,14] we define alcoholic hepatitis as a lesion characterized by necrosis and prominence of segmented leukocytes within the lobular parenchyma. Criteria for cirrhosis were septums linking central with portal canals and regenerative nodules.[15]

Results

Control specimens showed no significant histopathologic findings. In the alcohol-fed baboons the appearance in the examined specimens was usually uniform.

Steatosis

The most common lesion was accumulation of either small or large fat droplets in hepatocytes. The small droplets filled the cell without displacing the centrally located nucleus, producing a foamy appearance of the cytoplasm. The large drops replaced almost the entire cytoplasm and displaced the flattened nucleus to one side. Such hepatocytes were sometimes conspicuously enlarged, but merging of fat droplets (fatty cysts) was rarely noted. Steatosis showed in general centrolobular predominance, but when it involved almost the entire lobule, only a few layers of fat-free hepatocytes surrounded the portal tracts (Table 1). Usually, small-droplet fat accumulation was followed in subsequent specimens by large-droplet steatosis. Steatosis was significantly reduced in subsequent specimens in four baboons, in association with the development of fibrosis and/or cirrhosis.

Regressive Alterations of Hepatocytes

Degenerative changes were always associated with steatosis. The cytoplasm revealed rarification with progression to hydropic transformation (clear cells). Such cells were sometimes single but often arranged in small foci with centrolobular predominance. Hydropic transformation often developed in hepatocytes with small fat droplets, resulting in conspicuous enlargement of the cells. Other, often fat-containing hepatocytes showed irregular clumping of the rest of the cytoplasm (Figure 1). The cytoplasm of hydropic hepatocytes often contained round, brightly eosinophilic bodies up to 3 μ in diameter, red in CAB, which were considered megamitochondria (Figure 2). Moreover, single hepatocytes in the vicinity of degenerative changes appeared crowded by fine red granules (mitochondrial ground-glass cells) (Figure 2). Few hepatocytes with diffusely eosinophilic cytoplasm and pyknotic nuclei lay free in the tissue spaces (2 specimens). Lipofuscin pigment was occasionally seen, mainly in centrolobular hepatocytes. Cholestasis, iron deposition, and extensive necrosis were not noted.

Table 1—Sequential Appearance of the Spectrum of Alcohol-Induced Liver Injury in 18 Baboons*

Lesions	Time in months												
	0	4	6	9	12	18	24	30	36	48	54	60	73
Normal	F I N												
Regressive alterations of hepatocytes				K L	P	R	P	Q	L		A I		
Foci of segmented leukocytes in biopsy (1–3/high power field)				K									
Megamitochondria				K L		R	K	Q	B	I	A I	Q+	
Increased sinusoidal mononuclear cells		G H	F	L	F+	C E J R	H K L	B D O+ Q	B H	E N+	A I		
Steatosis													
Scattered		J				A C							
Centrolobular		G H		K L O	N P Q	E I N	B H K L P P+	A D E Q R	B H L	D E I K N+	A B D+ H B+	Q+	K K+
Diffuse			F	M	F+	J+ M R	P+	O+					
Macrophagic nodules				M O	F+ P Q	C J+ M R	K P	A B R	B H L R+	D E K	B H B+	Q+	K+

Fibrosis	G H	F	K M O	P Q	C N	B H P P+	A D E Q R	B	D I L+ N+	A B B+ I	Q+	K K+
Septums												
Linking macrophagic nodules			M O	F+ P Q	C J M R	K P	A B R	H L R+	D E K	B B+ H	Q+	K+
Linking central canals				F+	C	P P+		H L	E	B B+ D+ H	Q+	
Linking portal tracts				F+			D+	R+	E	D+ B B+		
Diffuse septal fibrosis					J+ C		R		K		Q+	K+
Regenerative nodules or areas				F+	J+		R	R+		B B+ D+		
Cirrhosis												
Septal				F+					E	B B+ D+		
Micronodular							O+					
Macromicronodular								R+				

* Each baboon is indicated by a letter, with autopsy specimens identified (+).

Hepatocytes trapped in encircling fibrous tissue were atrophic (8 specimens) (Figure 3). Such hepatocytes were small, had dense nuclei, and showed fat droplets.

Only autopsy specimens showed hepatocytic degeneration related to alterations of microcirculation.

Hyperplastic Changes of Hepatocytes and Nodule Formation

Irregular areas composed of enlarged hepatocytes with large vesicular nuclei, sometimes arranged in two-cell-thick plates, and free of fat droplets, were found around portal tracts, but sometimes they extended in a tongue-like fashion to the center of the lobules.

Some nodules were formed by separation of part of the lobular parenchyma by septums but did not differ from the surrounding parenchyma in arrangement of the plates or in hepatocellular fat. Such nodules were found near both central canals and portal tracts (Figure 4). Three baboons had nodules consisting of fat-free large hepatocytes arranged in two-cell-thick plates.

Inflammatory and Other Reactions of Nonparenchymal Cells

The number of mononuclear sinusoidal cells was diffusely increased in areas of mild or moderate fibrosis or sometimes around hydropic hepatocytes. Few mononuclear cells were lymphocytes, and most had flat or elongated nuclei. Their cytoplasm often gave a diffuse PAS-positive diastase-resistant reaction, and some of them had distinct PAS-positive granules characteristic of macrophages (Figure 5). Some of the PAS-negative sinusoidal cells with spindle-shaped nuclei contained small fat droplets.

The most frequent reaction was interstitial accumulation of mononuclear cells (Figure 6). Many had PAS-positive diastase-resistant granules, and sometimes they contained brown pigment, which in two instances gave a brown orcein reaction. The accumulations were found near hepatocytes with large fat drops. Large clusters were considered histiocytic nodules akin to fat granulomas. They were most frequent and largest in the centrolobular zone, often in the proximity of hepatic vein tributaries. Such accumulations surrounded single, or small groups of, highly steatotic hepatocytes, which were often atrophic.

Segmented neutrophilic leukocytes were seen in small numbers intermixed with the mononuclear cells in four biopsy specimens; only in one did they exceed two per high-power field near degenerating hepatocytes (Figure 1). In another biopsy specimen they were found near atrophic hepatocytes trapped in connective tissue. Hepatocytes surrounded solely by segmented neutrophilic leukocytes were not seen in either biopsy or autopsy specimens. Scattered eosinophils were found in four baboons.

The portal tracts showed occasional accumulations of lymphoid cells in-

termixed with few macrophages, in part PAS-positive. This condition was most conspicuous in fully developed cirrhosis (Figure 7).

Changes in Fiber Distribution

Localization of Connective Tissue Changes

Pericellular excess of connective tissue was observed around fatty hepatocytes with or without degenerative changes. Hydropic changes, particularly associated with small-fat-droplet steatosis, were not necessarily accompanied by alterations of the reticulin framework.

The reticulin framework appeared rarified in areas of centrolobular large-droplet steatosis, whereas the peripheral zone showed a much denser framework, normal in amount.

Along circumscribed parts of hepatocytic plates with large fat droplets the reticulin framework was sometimes increased; diffusely thickened longitudinal layers were enforced by cross fibers (Figure 8). These straight or slightly bent layers of reticulin extended through the lobule, often mixed with bundles of collagen (Figure 9). These reticulin layers were most conspicuous in the centrolobular zone and often connected central canals, but sometimes also seemed to end blindly. When extensive, they produced a framework of straight reticulin and collagen layers around the hepatocytic plates. With the progression of these lesions, hepatocytes became scarce, and the reticulin framework, enforced by longitudinal (sometimes birefringent) (Figure 10) collagen bundles, became approximated, with the bundles covering persisting endothelial-lined vascular spaces (Figure 11). The latter sometimes had walls thick enough to qualify as arterioles.

In and around the clusters of mononuclear cells intermixed with macrophages with PAS-positive granules, reticulin fibers aggregated with thick collagen bundles, resulting in nodular accumulations of connective tissue. They were connected with each other by layers of reticulin enforced by collagen bundles, to produce eventually an intralobular knotty network of step-like or zigzag septums (Figure 12). The fibrous nodules contained atrophied hepatocytes, singly or in small groups, as well as vessels that had a distinct collagenous wall (Figure 13).

The walls of the hepatic vein tributaries were sometimes diffusely thickened, but more frequently this thickening was plaque-like, consisting mainly of collagen. Some PAS-positive macrophages were found within the plaques, suggesting that some of the perivenous fibrosis had developed from formation of fibers around perivenous histiocytic clusters. The endothelial lining of the hepatic vein tributaries showed no changes.

Centrolobular merging of the perihepatocellular fibrosis with the fibrosis around histiocytic nodules produced a net-like fibrosis of varying outline, composed mainly of collagen fibrils (Figure 14). Cellular infiltration

was limited to scattered PAS-positive macrophages and other mononuclear cells. Vessels, some of them thick-walled, also traversed this fibrotic net. Bile ductules were not observed.

Types of Connective Tissue

In all observed localizations, the connective-tissue increase consisted, if in small amounts, of longitudinal reticulin fibers connected by short cross-fibers. With increasing fibrosis, thick, van Gieson-stainable connective tissue bundles appeared that were particularly conspicuous in relation to histiocytic cell accumulations. Elastic fibers were increased only in thick septums in cirrhotic stages, and never conspicuously. Only extensive accumulations of collagen bundles both in net-like fibrosis (Figure 10) and septums, especially in cirrhosis, were birefringent.

Pattern of Connective Tissue Septums

The increased connective tissue in all localizations resulted in septum formation and, together with formation of regenerative hepatocytic nodules, cirrhosis. Several types of septums varied in shape (Figure 15). Most septums contained vessels with longitudinal collagen bundles in their walls, but bile ductules were noted only near the portal tracts.

The most frequent type represented linking of nodular connective tissue accumulations around the interstitial histiocytic clusters. These septums seemed to grow by incorporation of hepatocytes into the increased connective tissue. The septums formed within the lobular parenchyma, but commonly connected with the central canals and less often with portal tracts. Frequently they seemed to end blindly in the parenchyma.

Increased pericellular reticulin fibers condensed to septums, after disappearance of intervening hepatocytes, with persistence of some endothelial cells and macrophage-lined sinusoids. These septums appeared straight or slightly bent and were enforced also by collagenous bundles around sinusoids.

Radiation of septums from the portal tracts into the parenchyma was not frequent and only found with more advanced fibrosis, when they produced a stellate shape of the tracts. By contrast, extensions from the central zones were frequent, and septums contributed to pericellular net-like fibrosis (Figures 8 and 15). Linking of central with portal zones by both types of septum was less common. Portal–portal connections were only noted in fully developed cirrhosis.

Precirrhosis and Cirrhosis

Three types of lesions considered cirrhotic were encountered.

Four baboons had septums, usually delicate, but containing vessels, which linked central with portal canals, and the usually steatotic paren-

chyma contained nodules composed of fat-containing hepatocytes with a limited degree of hyperplasia. The lobular architecture was thus somewhat distorted, and the designation "septal precirrhosis" or "cirrhosis" was applied (Figure 16).

One baboon had at autopsy a micronodular cirrhosis. The lobular architecture was replaced by nodules of various sizes separated by connective tissue septums of variable width. They connected central with portal zones and frequently central canals as well as portal tracts with each other. In some larger hepatocytic nodules, normal portal tracts and hepatic veins were noted, with few of the portal tracts connected to the surrounding septums. A few hepatic vein tributaries were in close contact with septums (Figure 17). The connective tissue septums contained much birefringent collagen (Figure 18). The hepatocytes were usually large and in plates two or more cells thick. Some were multinucleated, and a few showed large fat droplets. In the center of the nodules, hypoxic changes were frequent. Larger septums contained single, or small groups of, hepatocytes, the latter in acinar arrangement and sometimes in syncytial arrangement without recognizable cell borders. Bile duct proliferation was subdued, while arteries and veins were frequent in the septums. The intima of the hepatic vein tributaries was thickened and fibrotic.

One partly micro-, partly macronodular cirrhosis was observed. The micronodular, mainly monolobular portion was similar to the above described specimen except that the demarcation of the nodules toward the septums was less sharp, and the inflammatory reaction within the septums was more conspicuous (Figure 19). The inflammatory infiltration was far more intensive in areas corresponding to the original central zones than in the portal tracts. Hepatocytes remained often in isolated groups in the connective tissue and sometimes appeared multinucleated because of a lack of recognizable cell borders (Figure 7). In addition, there were parenchymal nodules up to 7 mm in diameter, sharply limited from the surrounding tissue. They were composed of large hepatocytes in plates two or more cells thick that varied in thickness. Scattered hepatocytes had large fat droplets (Figure 20). Many hepatic vein tributaries, but no septums or bile ducts, were seen. The larger nodules showed in circumscribed areas small-droplet steatosis progressing to hypoxic necrosis.

Sequences in Consecutive Specimens

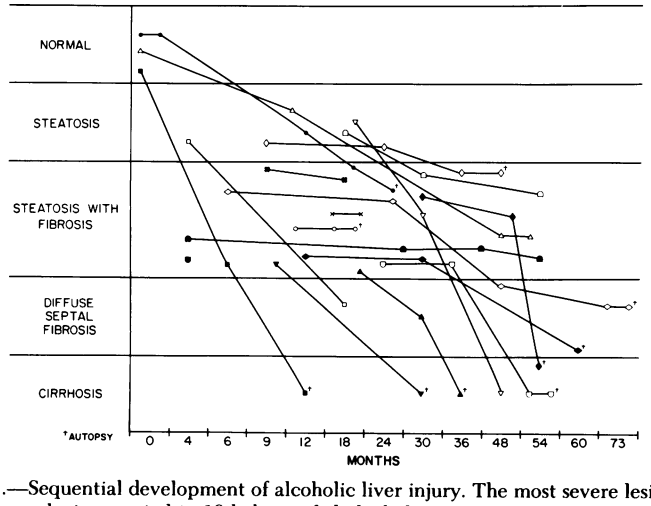
The sequences are illustrated in Text-figure 1 and detailed in Table 1.

Discussion

Examination of the histologic sequences of alcoholic liver injury in the baboon model confirmed as expected the sequential transition from normal, over increasing steatosis, to fibrosis and cirrhosis.[4,9,10] The large num-

ber of samples available permitted a sequential analysis of these tissue changes that yielded information as to the evolution of alcoholic liver injury and provided clues to the histogenesis. The emphasis is placed here on the attempt to reconstruct from serial biopsies the development of fibrosis and the pathways of transition to cirrhosis and to postulate from these observations the factors stimulating the fibroplasia.

Hepatocellular injury other than steatosis is commonly considered a strong fibroblastic stimulus[16,17] effective with or without associated inflammation. Of the two forms of hepatocellular injury encountered in the biopsy specimens of the baboons, the mainly centrolobular hydropic swelling or irregular cytoplasmic clumping was always associated with steatosis and often with megamitochondria. The lesion appeared to be a direct result of the exposure to alcohol or its metabolites and is explained at least in part by the retention of proteins normally excreted by the hepatocytes.[18] In a few instances, PAS-positive globules were noted that resembled tinctorially those seen in α_1-antitrypsin deficiency and are presumably also explained by retention of protein.[19] Degenerative lesions only exceptionally proceeded to necrosis, reflected in acidophilic bodies; extensive fallout of contiguous groups of hepatocytes resulting in obvious collapse of connective tissue framework was not observed. Since there was not always an associated alteration in the connective-tissue framework, a fibroblastic stimulus of the hepatocellular degeneration could not be proved to be an important factor in the fibrosis in the baboons. The other alteration, the atrophy of hepatocytes trapped in connective tissue, has to



TEXT-FIGURE 1.—Sequential development of alcoholic liver injury. The most severe lesion is recorded individually for each time period in 18 baboons fed alcohol up to 6 years.

be considered a consequence of the fibrosis and not a primary process, although a secondary fibroblastic stimulus cannot be excluded.

The most impressive inflammatory reaction in the baboons was mononuclear in character and may have a stimulatory effect on fibroplasia, although it was not necessarily associated with increased fibers. The diffuse mononuclear reaction was often accompanied by mild or moderate fibroplasia, while the interstitial clusters of mononuclear cells, some of them PAS-positive macrophages, were regularly associated with the most conspicuous degree of fiber formation and usually with severe steatosis.

If intralobular predominance of segmented neutrophilic leukocytes is considered the hallmark of alcoholic hepatitis, this lesion was not encountered. A few leukocytes were found, in some instances associated with degenerative hepatocytic lesions. The large number of examined specimens without accumulation of segmented leukocytes militates against the possibility that transient stages of conspicuous tissue leukocytosis have been missed, and thus a stage of alcoholic hepatitis (as commonly defined) [13,14] is an improbable cause for the cirrhosis in baboons.

Previously, lesions interpreted as alcoholic hepatitis were described.[5] In the present study, which deals with a larger baboon population followed over a long period of time, some necrosis was also found associated with an inflammatory reaction. However, the inflammation was primarily of a mononuclear nature and not restricted to the areas of necrosis. Although some polymorphonuclear leukocytes were observed, their paucity did not justify the term "alcoholic hepatitis." Moreover, the hyaline bodies reported previously [5] were considered to represent megamitochondria rather than Mallory bodies. Ultrastructural studies never revealed the typical fibrillar composition of alcoholic hyaline bodies of Mallory.

The absence of significant accumulations of lymphocytes in the earlier and septal stages of the fibrosis does not support the concept that immunologic processes play a major role in the fibrosis of the alcoholic baboons, although enhanced necrosis of cultured hepatocytes has been demonstrated when hepatocytes and lymphocytes of alcohol-fed baboons are exposed to each other *in vitro*.[20] The lymphoid cell accumulation in portal tracts and septums in the precirrhotic and the cirrhotic stages may reflect secondary alterations.

Steatosis, particularly the large-droplet type, appeared impressively associated with accumulation of excess fibers. The morphologic association suggests two mechanisms. One is increase of reticulin fibers around hepatocytic plates with steatosis. While in some, presumably earlier, stages the reticulin framework is rarefied by the distention of the hepatocytes, in others, presumably later stages, these plates are covered by layers of in-

creased connective tissue, initially reticulin and subsequently enforced by bundles of collagen. This results in long, straight or, often, slightly bent septums. The second, more frequent mechanism is associated with clusters of mononuclear cells, including macrophages and PAS-positive granules, and resembling fat granulomas.[21] They are surrounded by reticulin and collagen. The resulting nodular connective tissue accumulations are linked by connective-tissue septums, producing a knotty or crooked septal network with frequent and apparently early enforcement by thick connective tissue bundles.

The morphologic picture can only show association, not causation. However, the topographic relation of fibrosis to steatosis suggests a possible role of the latter in fiber formation. A stimulating effect on fibroplasia may be a more important factor, whether it is exerted by ethanol or acetaldehyde or by the lactate formation resulting from the reducing influence of the hepatic metabolism of alcohol.[22] This concept is supported by many studies in man and experimental animals.[23–26] However, the macrophage accumulations suggest yet another mechanism, namely, excess fat, which overwhelms the metabolic capacity of the hepatocytes and is taken up by macrophages; this process in turn may promote fibroplasia. The fact that in man alcoholic steatosis produces fibrosis and cirrhosis far more often than do other causes of steatosis, like obesity and diabetes,[27] incriminates the role of excess alcohol in either direct stimulation of fibroplasia or excess accumulation of fat-containing macrophages.

The light-microscopic examination does not permit clarification of the role of cells in the fiber formation. An excess of fat-storing cells, or Ito cells, demonstrated in other models of experimental fibrosis [28] and in human alcoholic hepatitis,[29] was also found in these baboons in an ultrastruc tural study.[30]

Both processes, formation of straight septums and formation of knotty septums, are accentuated in the centrolobular zone, and there, both types of septums combine to produce irregularly shaped areas of centrolobular net-like fibrosis not seen commonly in man. By contrast, the pericellular fibrosis seen in the baboons is common in human alcoholic liver injury. The predilection of the septums, particularly of the straight ones, for certain planes of the hepatic lobule is not fully explained. It may be related to the blood flow and the acinus of Rappaport,[31] and the septums may develop preferentially in areas farthest from oxygen-rich blood supply. The septums frequently connect central zones with each other, whereas connection with the portal tracts occurs later.

In all forms of fibrosis, reticulin appears to serve as a basis for the subsequent deposition of collagen bundles, in keeping with the general prin-

ciple [32] in both fetal development and pathologic conditions that collagen III serves as matrix for collagen I, which becomes aggregated into bundles showing birefringence. Collagen IV, a component of the basement membrane, cannot be seen by light microscopy, but its increase is suggested by a heavier layer of PAS-positive material along the sinusoids near fibrosis. Formation of elastic fibers is late and only noted in thick septums.

Wherever the septums start (in the parenchyma or near the hepatic vein tributaries), they grow, particularly in width, by incorporation of adjacent hepatocytes, which become atrophic from the encircling fibrosis and eventually disappear. Sinusoids are thus obliterated. This may, together with the pericentral fibrosis, account for the portal hypertension demonstrated in the baboons.[33] Moreover, larger vascular structures, venules or arterioles, form in the septums and are surrounded by thick collagenous bundles. They may disturb the intrahepatic circulation by permitting afferent blood to flow from the portal vein and hepatic artery directly to the hepatic vein tributaries [15] when connections between central and portal canals have been accomplished.

The eventual development of cirrhosis results, as in any form of cirrhosis, from the formation of hepatocytic nodules and connective-tissue septums. Some of the nodules in the cases designated as septal cirrhosis or precirrhosis show a steatotic parenchyma, do not alter the lobular architecture, and are formed passively by dissection of the parenchyma by the septums. Others, seen particularly in fully developed cirrhosis, are fat-free and show hyperplasia and hypertrophy. These foci of hyperplasia develop, if surrounded by septums, into active regenerative nodules. This, together with septums linking central with portal canals, completes the picture of cirrhosis.

The described hepatic lesions in alcohol-fed baboons resemble chronic human alcoholic liver injury with respect to steatosis, megamitochondria,[34,35] pattern of pericellular and central fibrosis, initial sparing of portal tracts, and progression to cirrhosis. The role of macrophages (in clusters of mononuclear cells resembling fat granulomas) in fibrosis appears to be more conspicuous in baboons than in man, while degenerative hydropic lesions of the hepatocytes, ductular proliferation, portal inflammatory reaction, and elastica formation are less conspicuous. Alcoholic hyalin of Mallory, conspicuous intralobular accumulation of leukocytes, and particularly the ringing of hepatocytes by segmented leukocytes, as well as the necrosis bridging central with portal canals, leading to their approximation,[36] are conspicuous by their absence in the baboons.

As in the case of alcoholic patients, only a minority (6 out of 18) of baboons fed alcohol progressed to cirrhosis, although all developed lesions

beyond the fatty liver stage. The difference in individual susceptibility was further illustrated by the lack of strict correlation within the groups between duration of alcohol feeding and severity of the lesions.

Differences in diet, which have been postulated to play a role in man, can be ruled out here since, except for the isocaloric substitution of ethanol for carbohydrate, the diet was identical and strictly controlled in all animals. Obviously, other factors, possibly genetic, must contribute to the variability in individual susceptibility to alcohol.

The lesion in the baboons illustrates one pathway to cirrhosis in which polymorphonuclear necroinflammation plays only a minor role, if any. This suggests the possibility that also in man, besides the currently emphasized transition of alcoholic hepatitis to cirrhosis, a creeping fibrosis with steatosis may be either the main, or at least a supplemental, pathway to cirrhosis. This assumption is supported by previous three-dimensional reconstructions of the transition of steatosis to cirrhosis in human alcoholic liver injury [37] and various clinical observations, for instance, in sake drinkers in Japan who develop cirrhosis without demonstrable hyalin of Mallory or tissue leukocytes.[38] Whether the pattern of fibrosis demonstrated in the alcoholic baboons applies to other forms of human cirrhosis remains to be investigated.

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Acknowledgments

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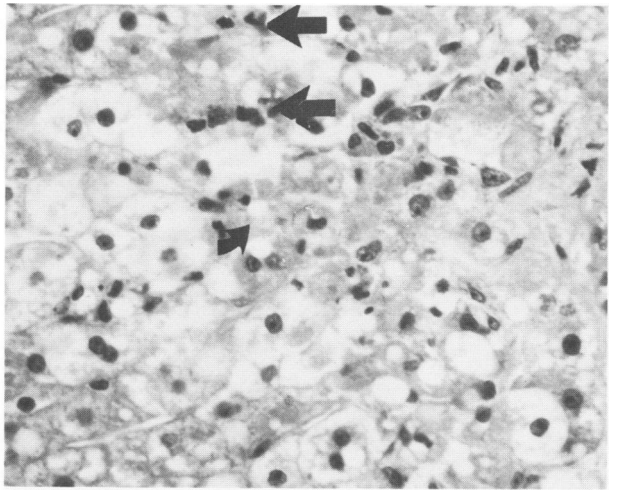
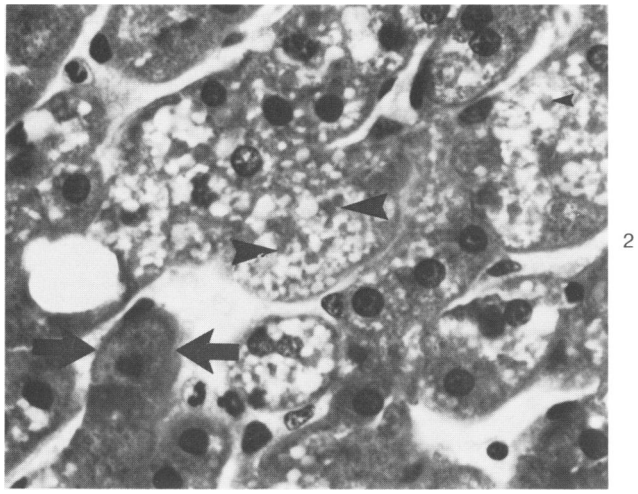
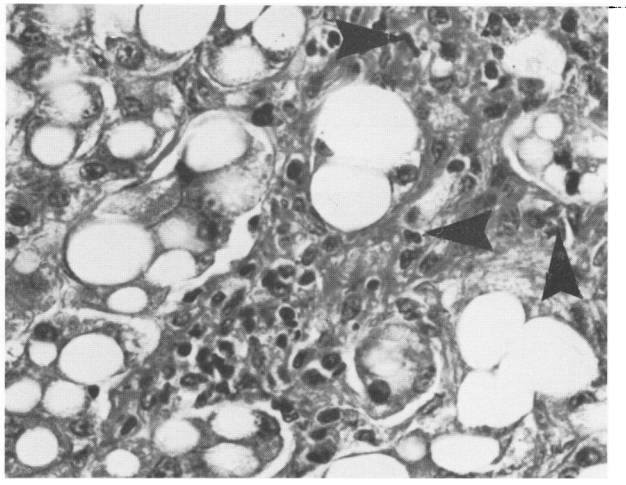






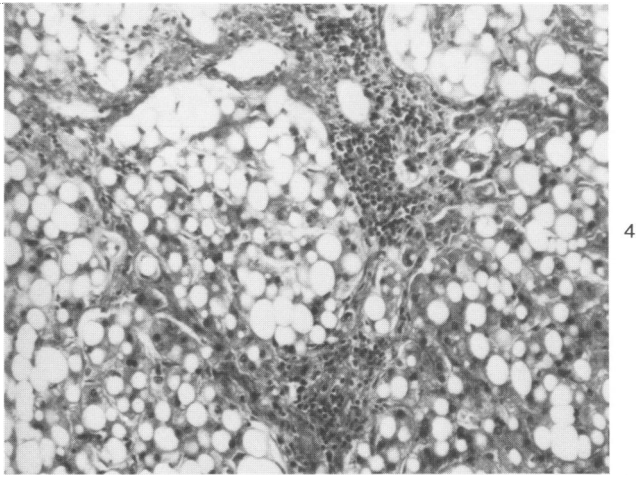
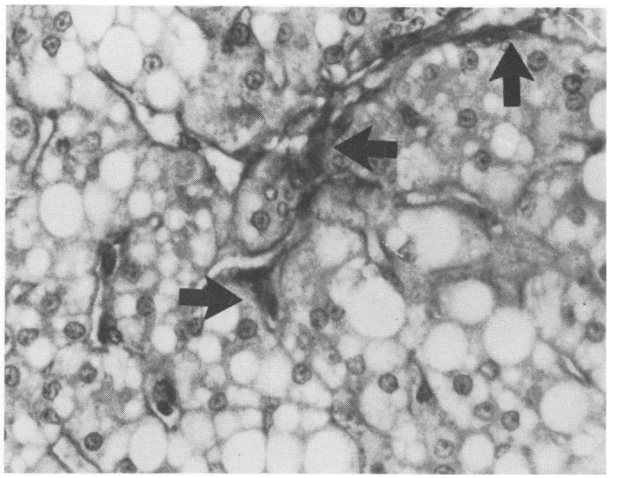


Figure 1—Hydropic swelling of hepatocytes with clumping of cytoplasm (after 10 months on alcohol). Slight increase of inflammatory cells, including an occasional segmented leukocyte (*arrows*). (H&E, ×250) **Figure 2**—Megamitochondria (*straight small arrows*) in hepatocytes showing small-fat-droplet steatosis and hepatocytes with dense eosinophilic cytoplasm (*between large arrows*) (after 9 months on alcohol). (H&E, ×400) **Figure 3**—Fat-containing hepatocytes undergoing atrophy in fibrotic tissue containing mainly histiocytic cells, but occasional segmented leukocytes (*arrows*) (after 51 months on alcohol). (H&E, ×250) **Figure 4**—Nodule consisting of hepatocytes with similar fat content as the surrounding parenchyma, encircled by connective tissue near fibrotic zone around hepatic vein tributaries (after 51 months on alcohol). (H&E, ×100)



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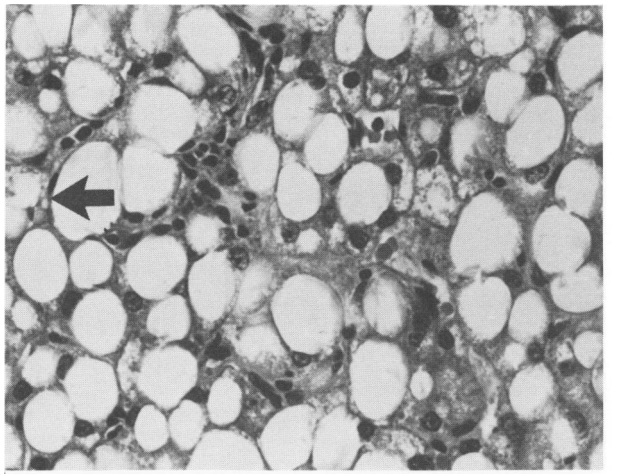

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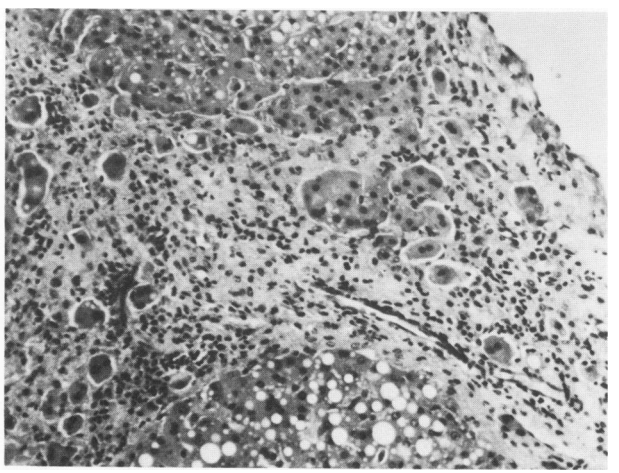

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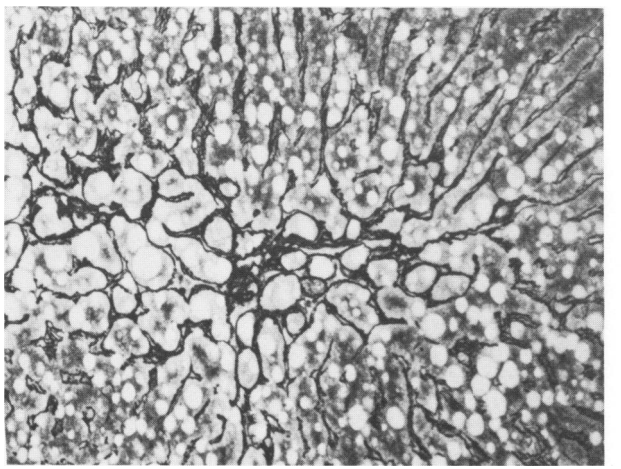

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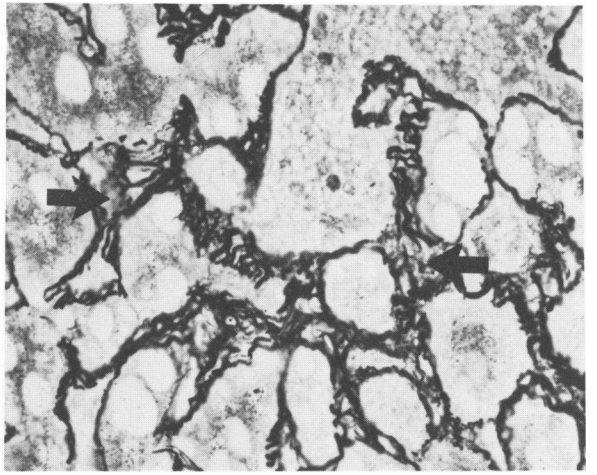
Figure 5—Small- and large-fat-droplet steatosis with increase of sinusoidal lining cells many of which contain PAS-positive granules (*arrows*) (after 3 months on alcohol) (PAS after diastase treatment, ×240) **Figure 6**—Increase of mononuclear sinusoidal cells around hepatocytes containing mainly large fat droplets (after 19 months on alcohol). A few of the sinusoidal cells show small fat droplets in their cytoplasm (fat-storing cells) (*arrow*). (H&E, × 250) **Figure 7**—Small portal tracts in micronodular portion of cirrhosis, revealing a few inflammatory cells. An arteriole extends into the connective septum, and small clusters of hepatocytes within the septums show syncytial arrangement (after 30 months on alcohol). (H&E, ×100) **Figure 8**—Centrolobular steatosis with diffuse increase of pericellular reticulin fibers (after 46 months on alcohol). (Silver impregnation, ×100)



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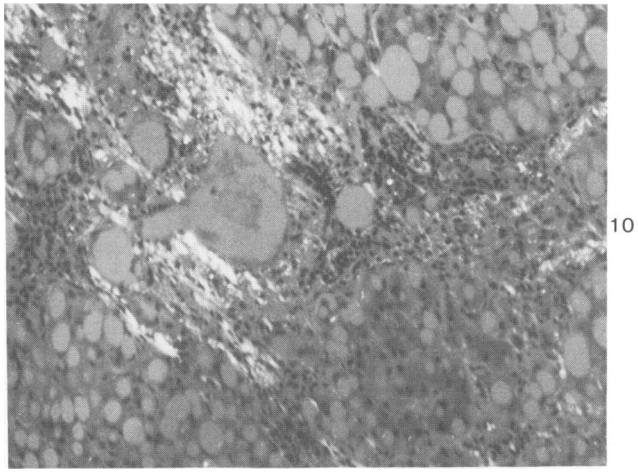

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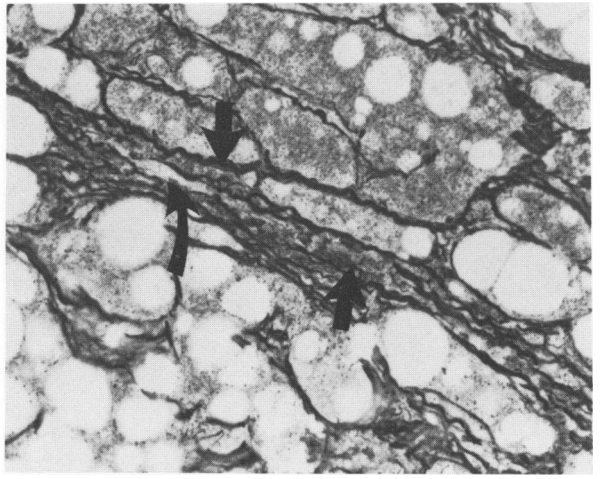

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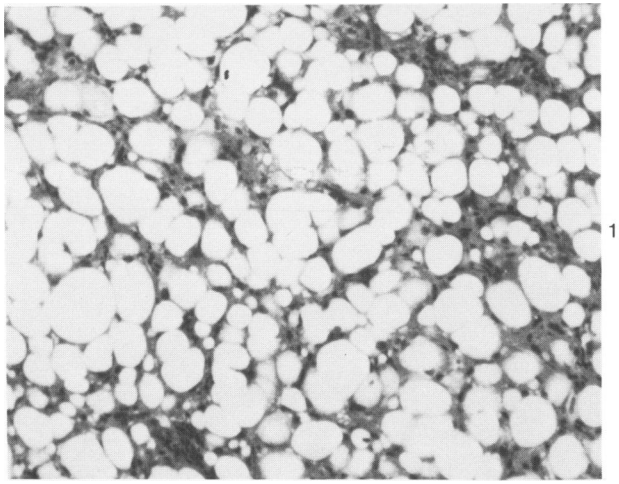

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Figure 9—Pericellular accumulation of reticulin in form of longitudinal and cross fibers. It is enforced in places by lighter stained bundles of collagen (*arrows*) (after 46 months on alcohol). (Silver impregnation, ×250) **Figure 10**—Birefringent fibers of collagen surround steatotic hepatocytes in centrolobular zone (after 51 months on alcohol). (H&E, ×100, polarized light) **Figure 11**—Increased reticulin around hepatocytes with large fat droplets. Note enforcement by lighter stained collagen (*straight arrows*) surrounding small vascular space (*curved arrow*) (after 39 months on alcohol). (Silver impregnation, ×250) **Figure 12**—Fibrotic nodules containing macrophages, connected by septums in knotty arrangement (after 9 months on alcohol). (CAB, ×100)

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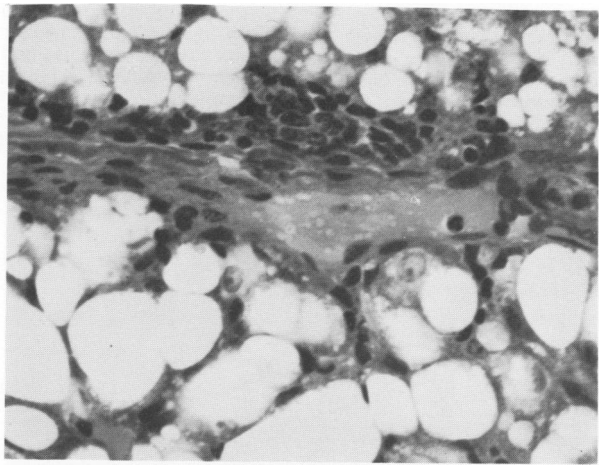

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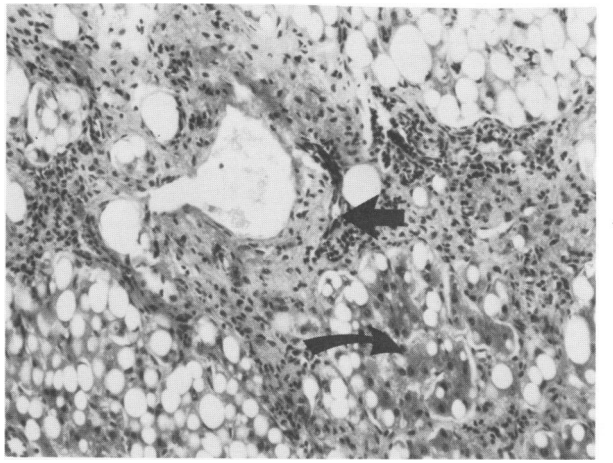

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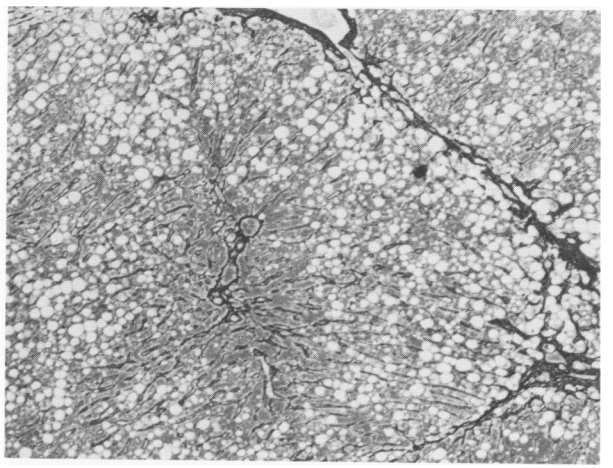

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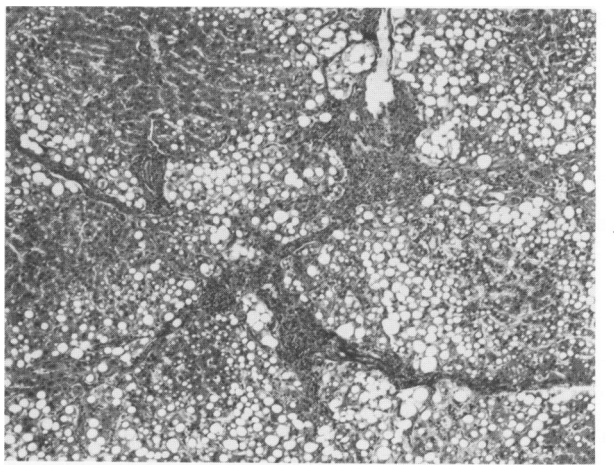

Figure 13—Septum surrounded by hepatocytes with large fat droplets containing mononuclear cells, and a vascular space lined by endothelial cells (after 9 months on alcohol). (CAB, ×250) **Figure 14**—Extensive fibrosis around hepatic vein tributaries. The fibrotic area, infiltrated by mononuclear cells, contains fat-containing hepatocytes, partly atrophic, and vessels, some with thick walls, indicating arteriolar character (*straight arrow*). Some fat-free hepatocytes show evidence of regeneration (*curved arrow*) (after 51 months on alcohol). (H&E, ×100) **Figure 15**—Various types of septums. On the top, perivenous fibrosis connected by straight septum, crooked septums on the right, and straight septums within parenchyma, partly ending blindly in the central portion (after 16 months on alcohol). (Silver impregnation, ×60) **Figure 16**—Septal type of cirrhosis with formation of nodules surrounded by septums in the central portion. Note also some fat-free hepatocytes in two-cell-thick plates (after 51 months on alcohol). (CAB, ×60)

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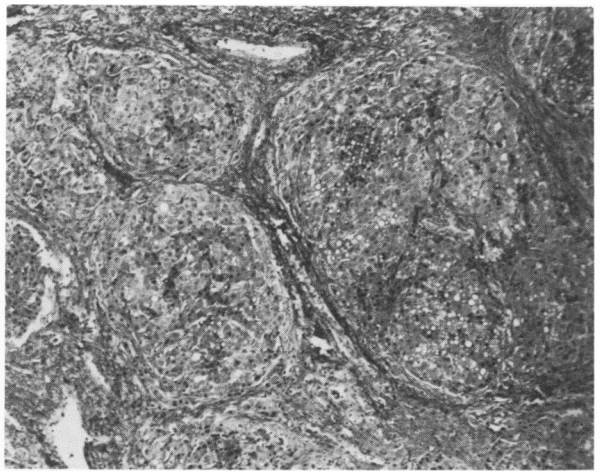

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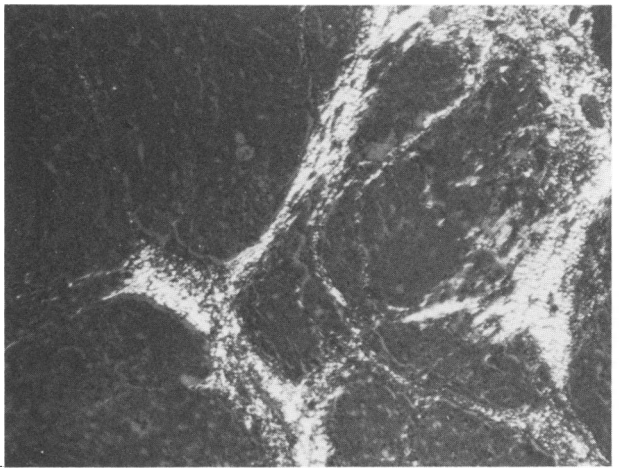

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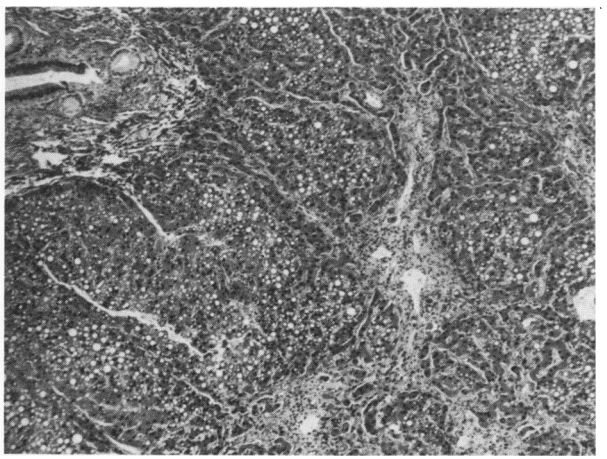

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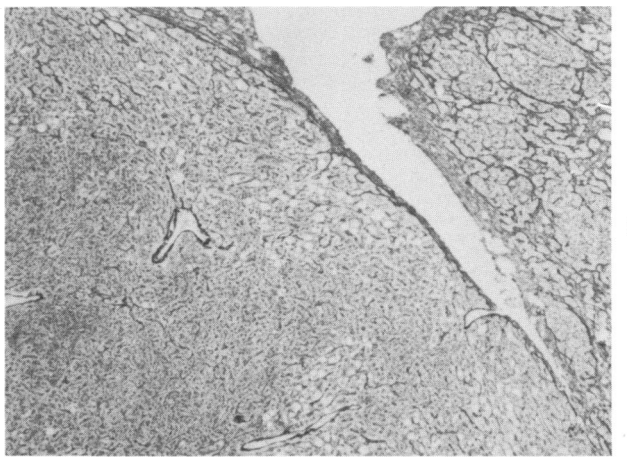

Figure 17—Micronodular cirrhosis with focal steatosis and hypoxic necrosis in center of nodules. Note hepatic vein tributaries adjacent to septum in upper portion (after 26 months on alcohol). (CAB, × 60) **Figure 18**—Birefringent collagen bundles in the septums of micronodular cirrhosis (after 26 months on alcohol). (H&E, × 60, polarized light) **Figure 19**—Micronodular portion of mixed micro-macronodular cirrhosis. Occasionally fat droplets accumulate in the nodules, which consist of hepatocytes in two-cell-thick plates. The septums, which contain isolated groups of hepatocytes, are infiltrated by mononuclear inflammatory cells (after 30 months on alcohol). (H&E, ×60) **Figure 20**—Large hyperplastic nodule revealing mainly efferent veins, occasional steatosis, and a sparse reticulum framework, in contrast to the micronodular portion in the right upper corner (after 30 months on alcohol). (Silver impregnation, ×40)

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