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Pathological Anatomy and Histopathology

Morphometry of terminal hepatic veins*

2. Follow up in chronically alcohol-fed baboons

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Summary. Alcohol induced perivenular fibrosis of terminal hepatic veins (THV) is claimed to be a precursor lesion leading to fibrosis development in man and baboon. The nature and significance of the THV lesions found in four baboons chronically fed with alcohol were studied in liver biopsies obtained during a three year period. The surface of THV wall and the number of mesenchymal cells were assessed with a semi-automatic image analyser. Histologically, THV were characterized as (a) phlebitic, in the presence of an inflammatory cell infiltrate involving the venous wall; (b) oedematous, when the connective tissue of the THV wall was disrupted or dissociated and (c) fibrotic, when perivenular scarring appeared as an increased rim. These lesions were present simultaneously and their intensity and distribution were independent of the duration of alcohol intoxication. Morphometric data obtained from these THV confirmed the thickening of the THV wall (WS/IS in: oedematous 1.05 ± 0.36; phlebitic 1.65 ± 1.04; fibrotic 1.47 ± 0.36); and revealed an increased number of mesenchymal cells in fibrotic (439 μm^2/cell; $p < 0.01$) and in phlebitic THV (510 μm^2/cell; $p < 0.05$). A constant relationship was found between phlebitis and the presence of inflammatory infiltrate within the hepatic acini. Fibrotic THV was a less frequent finding and the lesion disappeared in the sequential biopsies of one of the baboons. In conclusion, THV lesions were heterogeneous and the collagen deposition in the THV wall was potentially reversible during the three year alcohol intoxication period, regardless the inflammatory reaction and, thus, did not indicate early irreversible hapatic fibrosis.

Key words: Alcoholic liver disease – Terminal hepatic veins – Baboon – Fibrosis – Morphometry

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Introduction

Alcohol toxicity, particularly that derived from its metabolic product acetaldehyde, has been largely demonstrated at the hepatocyte level (Lieber 1985a, b; Ugarte and Iturriaga 1976). Sometimes, four progressive lesions develop simultaneously: steatosis, hepatitis with hepatocellular necrosis and mesenchymal proliferation, fibrosis and, finally, cirrhosis (Christoffersen and Nielsen 1972; Desmet 1985; MacSween and Burt 1986; Scheuer 1982). The two first changes are known to be reversible, but cirrhosis is generally accepted as irreversible, even after alcohol withdrawal (Baptista et al. 1981; Fleming and McGee 1984). The role of alcoholic hepatitis in fibrogenesis and fibrosis reversibility is still debated (Galambos 1972; Hall 1985; Rojkind 1985). The spectrum of the human lesions can be reproduced in baboons, which are therefore considered as the best animal model for alcoholic disease (Lieber and deCarli 1976; Lieber et al. 1975).

Centrolobular fibrosis is recognized as an early event in the development of cirrhosis. Characterized by Edmonson et al. (1963) this lesion was extensively studied by Lieber (1983) in man and baboons subjected to alcohol intoxication. The suggestion was made that perivenular fibrosis is a reliable index for the development of liver fibrosis

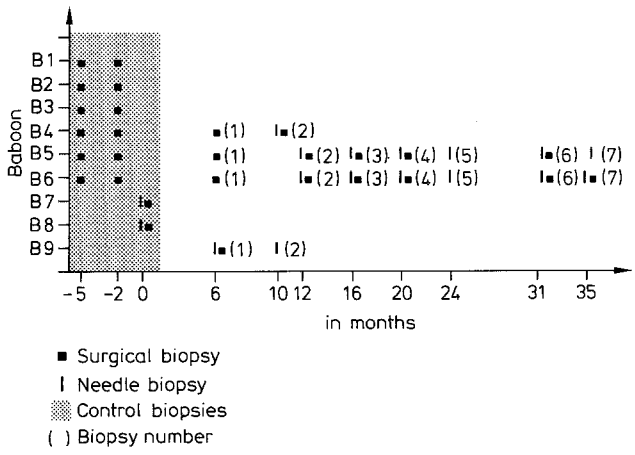

Fig. 1. Schedule of surgical and needle liver biopsies in baboons before and during alcohol intoxication

Table 1. THV number (n) measured and related to liver biopsy surface (bs) in square millimeters

Baboon n°		Biopsies						
		1	2	3	4	5	6	7
B4	n	7	12					
	bs	13.1	28.3					
B5	n	11	20	9	15	4	15	3
	bs	15.6	21.1	10.6	20.4	4.5	13.6	4.9
B6	n	11	21	19	17	6	8	13
	bs	11.1	19.7	19.2	16.0	3.6	11.2	13.7
B9	n	44	2					
	bs	35.1	2.3					

(Nakano et al. 1982; Van Waes and Lieber 1977). This was further confirmed by Worner and Lieber (1985) after the follow up of 34 alcoholics with sequential biopsies, all patients with perivenular fibrosis showing progression towards extensive fibrosis or cirrhosis.

The purpose of the present study was to follow and to assess the early THV changes in baboons by morphometric and histological methods during a three year period of chronic alcohol intoxication. The morphometric characterization was obtained by means of two variables which were selected during the first part of this work (Porto et al. 1989) concerning normal THV measurements in man and baboon.

Materials and methods

Four male baboons Papio Papio born and raised in France, weighing between 14 and 18 kg, were fed chronically with a liquid diet according to Lieber and deCarli (1974), in which ethanol represented 45 percent of total caloric intake. A commercial diet, (Alburone, Sopharga Co.) with mineral and vitamin supplementation, was used. Protein content represented 27 percent of total calories, and lipid and carbohydrate 14 percent, each. The daily consumption of the liquid diet was 63 cal/kg, representing an ethanol intake of 4.06 g/kg of body weight per day. Animals entered the experiment after repeated haematological and faecal examinations which failed to show abnormalities. They had a 6% weight-loss (1020 ± 630 g) in the first three months, but remained in constant weight up to the end of the intoxication period. Animals were submitted to sequential percutaneous needle and/or surgical liver biopsies, under ketamine anesthesia, during three years (see Fig. 1). Fourteen liver biopsies performed in 8 normal baboons (Porto et al. 1989), served as control. Each animal was indicated by B and its number (B1...B9).

All specimens were fixed in Bouin's solution and embedded in paraffin for light microscopy. Histological sections were stained with haematoxylin and eosin (HE), Masson's trichrome, Gordon and Sweet's silver method for reticulin, orcein for elastic fibers and sirius red for collagen. Occasional serial sections were performed for the tridimensional study of THV wall.

Each liver biopsy was evaluated for the presence of fat, megamitochondria, portal and acinar inflammatory infiltrate and cell necrosis. These were subjectively graded on a scale of 0 to 3+ as 0 = absent, 1+ = minimal or few, 2+ = moderate and 3+ = marked or many. The hepatic veins at the periphery of the hepatic acini (centrolobular position) having sinusoidal openings and with a lumen diameter non exceeding 150 µm were accepted as THV. THV walls were characterized as: *normal* representive of those with the same histological characteristics found in control biopsies; *oedematous* when the connective tissue of the vein wall appeared just dissociated; fibrotic consisting in the THV with increased perivenular scarring as an increased rim and *phlebitic* in the presence of an inflammatory cell infiltrate involving the venous wall. All THV encountered in each biopsy was histologically characterized, their number and the surface of the biopsy are summerized in Table 1.

THV were measured by contour tracing of their external and internal limits ($\times 25$ or $\times 40$ objective) as seen with a Leitz-ASM 68K semi-automatic image analysis system. From the basic variables obtained by the computer program, we retained: internal surface (IS), internal diameter (ID), maximal internal diameter (IDmax), minimal internal diameter (IDmin) and wall surface (WS) – the difference between the external surface and the internal surface. Slides stained with haematoxylin and eosin or by Masson's trichrome were used to quantify the cellularity in the perivenous connective tissue. Endothelial nuclei were counted in the internal limit of the veins, and a distinction was made between polymorphonuclear, mononuclear (lymphocyte and monocyte) and mesenchymal cell (Mc) nuclei in the vein's wall. THV were accepted as transversely sectioned when IDmin/IDmax was superior to 0.67. The two ratios, WS/IS and WS/Mc previously selected for morphometric assessment of THV (Porto et al. 1989), were used for evaluation of wall thickness and wall cellularity, respectively.

Variations of the mesenchymal cell number (WS/Mc) in THV from control ($WS/Mc = 558 \pm 183$ µm^2/cell, $n = 200$) and intoxicated animals were compared by ANOVA – analysis of variance. Evaluation of wall thickness (WS/IS) of the different classes, as related to controls ($WS/IS = 0.63 \pm 0.23$; $n = 62$) was tested with a non parametric test – Kruskall-Wallis and Wilcoxon (Milton and Tsokos 1983). Distribution of THV lesions as related to time of alcohol intoxication were analysed, individually, for each baboon.

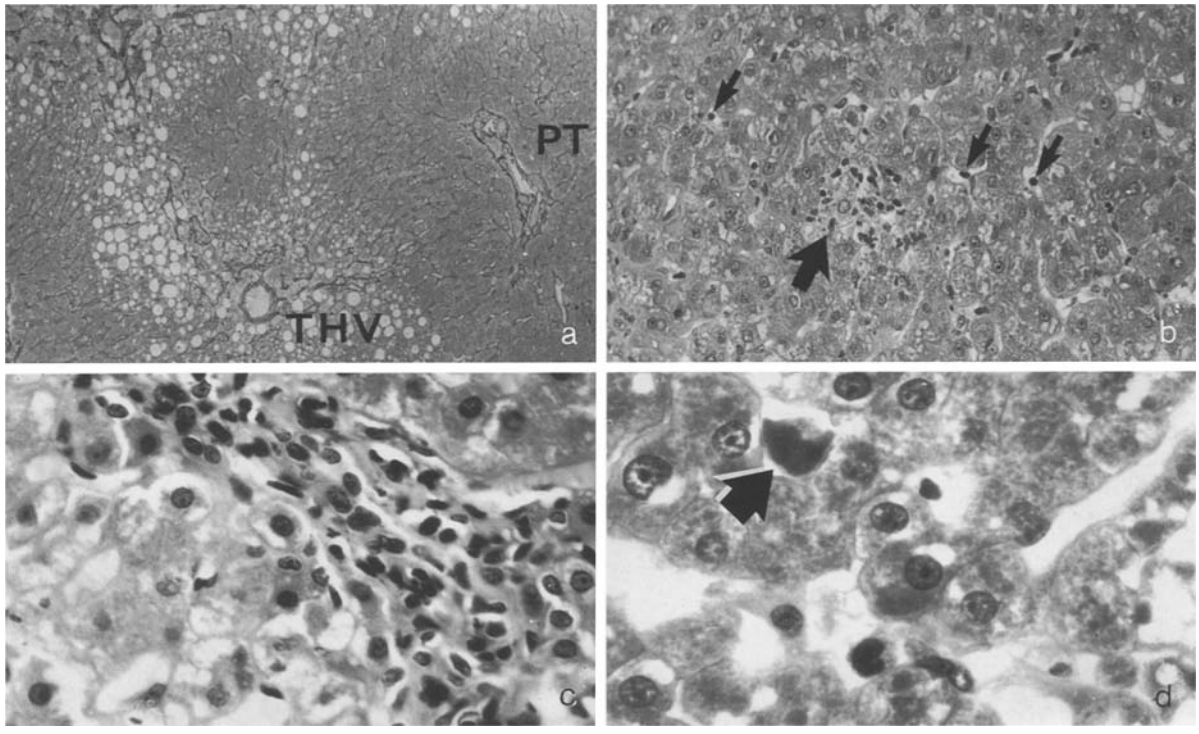

Fig. 2. **a** Liver general archictecture. Fibrosis is present mainly around terminal hepatic veins (THV) and at the periphery of hepatic acini, concomitantly with perivenular fibrosis. Portal tracts (PT) and parenchyma in zone 1 and 2 are preserved. Baboon 5 after 6 months of alcohol intoxication. Sirius red (×50); **b** Acinar inflammation, lymphocytes and monocytes are found isolated (*arrows*) or forming small granulomas (*large arrow*). Baboon 6 after 35 months of alcohol intoxication. HE (×120); **c** Portal inflammation, cell infiltrate are principally constitued by mononuclear cell. Baboon 9 after 6 months of alcohol intoxication. HE (×320); and **d** Cell necrosis (*arrow*) and presence of mononuclear cell in the sinusoids. Baboon 4 after 12 months of alcohol intoxication. Masson's trichrome (×640)

Results

Histopathological findings during alcohol intoxication in baboons showed steatosis and megamitochondria to be predominant features in all biopsies. Acinar and portal inflammation were characterized predominantly by lymphomononuclear cells and hepatocytic necrosis (Fig. 2). No classical signs of alcoholic hepatitis, for example, Mallory bodies and neutrophils, were found. Moreover, no signs of cirrhosis or extensive fibrosis, defined as fibrosis with loss of lobular architecture and parenchymal nodularity, were obtained during the three year experimental period. Fibrosis was found in the perivenular zone predominantly, affecting the THV and causing thickening of the perisinusoidal space of zone 3 of the hepatic acini. Pericellular or perisinusoidal fibrosis was observed in association with phlebitic or fibrotic THV. Figure 3 shows the evolution of portal or acinar inflammation and cell necrosis for each baboon.

The percentage of the three classes of the vein lesions has been assessed for each animal, as illustrated in Fig. 4. THV alterations were intense in the beginning of the alcohol intoxication (6 months) in 2 animals (B5 and B6) comprising wall infiltration by lymphomononuclear cells, disruption and disorganization of connective tissue fibers. Perivenular fibrosis was already present in both baboons. These findings were concomitant with moderate or intense acinar and portal inflammation, fatty infiltration, hepatocyte ballonization, necrosis and megamitochondria. Signs of acinar and portal inflammation persisted until the end of the experiment (35 months) but fibrosis in THV regressed in one baboon (B5). The two others baboons (B4 and B9) showed mainly THV wall dissociation in a small number of veins, during the one year experimental period, associated with signs of THV fibrosis in one single case. Parenchymal lesions were represented by fatty infiltration, cell necrosis and megamitochondria.

Oedema of the THV wall was the most constant lesion throughout the experiment (Fig. 5). The phlebitic lesion was mainly observed in the presence of acinar inflammatory infiltration. Monocytes and lymphocytes, isolated or forming cell clusters, were present in the THV wall. The

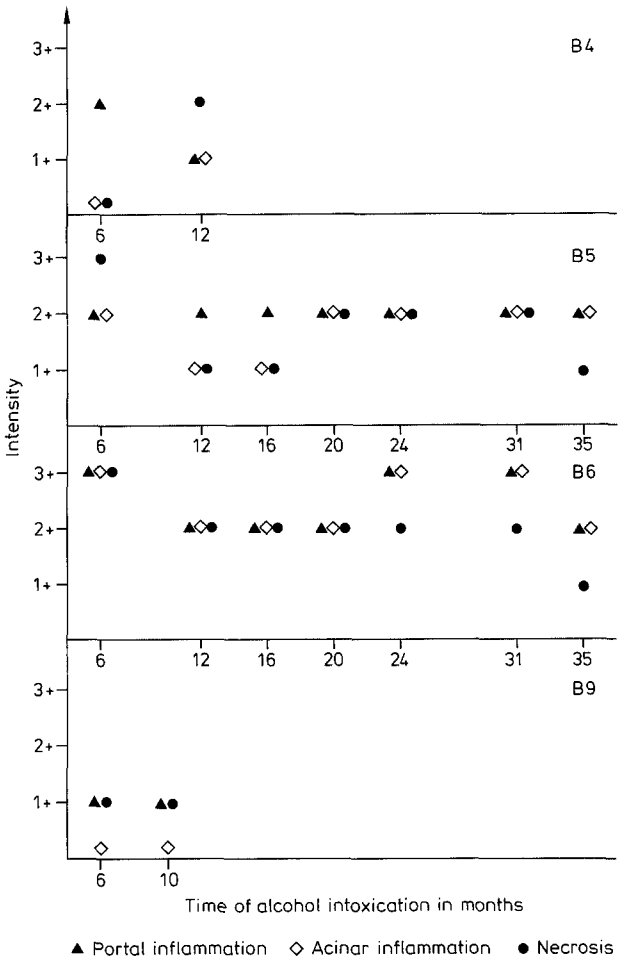

Fig. 3. Semi-quantitative evaluation of the necrosis, portal and acinar inflammation on sequential biopsies obtained from four alcohol-fed baboons. Graded on a scale of 0 to 3+ as 0=absent, 1+=minimal or few, 2+=moderate and 3+=marked or many

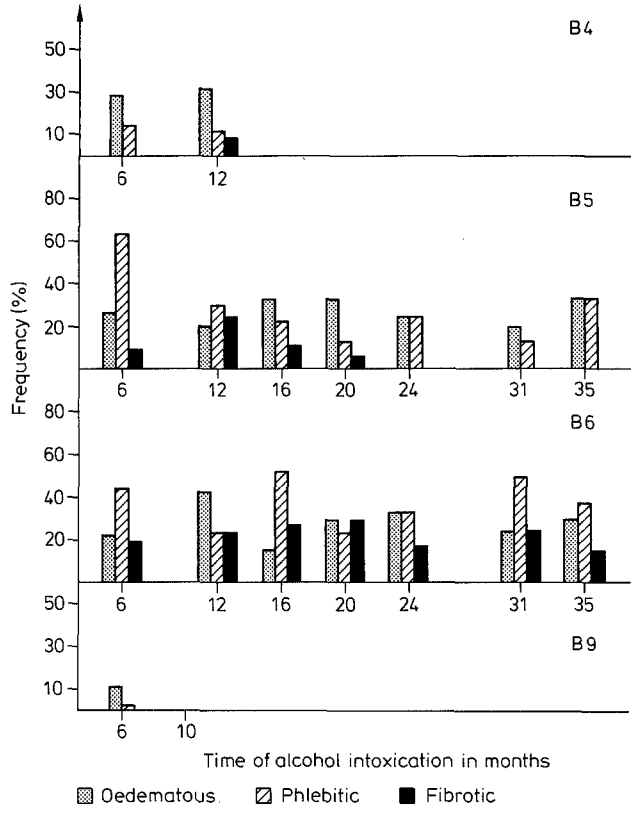

Fig. 4. THV histological lesion: distribution, frequency of oedematous, phlebitic and fibrotic THV during the three year period of alcohol intoxication in baboons

latter appeared dissociated and was usually found with an enlarged collagen rim. Histological examination of these veins in serial sections showed that wall inflammation was segmental, sometimes asymmetrical (Fig. 6) and associated with local collagen deposition. Such lesion appeared early and, like oedema, remained stable. In contrast, the starfish appearance or the regular necklace aspect of the fibrotic THV wall (Fig. 7) appeared independently of other alcohol-related lesions, such as the inflammatory infiltrate. It was not a constant feature.

Comparison of the wall thickness from the THV histological classes (normal, oedematous, phlebitic and fibrotic) during alcohol intoxication of baboons are found in Table 2. These THV measurements have significantly higher values of WS/IS that are 1.5 times greater in oedematous THV (range 0.48 to 1.67), 2 times greater in fibrotic THV (range 0.94 to 2.10) when compared to controls. For phlebitic THV, a large range of modified WS/IS values (0.48 to 3.54) was obtained, that corresponded to histological definition of this group by the presence of leucocytes in the venous wall, whatever the thickness of that wall. Fibrotic THV had an increased number of mesenchymal cells in their walls when compared with controls (WS/Mc = 439 ± 143 μm^2 WS/cell; $p < 0.01$) and those veins with an inflammatory infiltrate had also a significant decrease in the value of WS/Mc when compared to controls (WS/Mc = 510 ± 172 μm^2/cell; $p < 0.05$).

Discussion

Examination of the histological sequencies of alcoholic liver injury in the baboon model revealed steatosis and megamitochondria as the two constant findings during the entire experiment. These lesions were markers of recent alcohol intoxication (Junge et al. 1987; Lane and Lieber 1966; MacSween and Burt 1986) and confirmed the efficiency

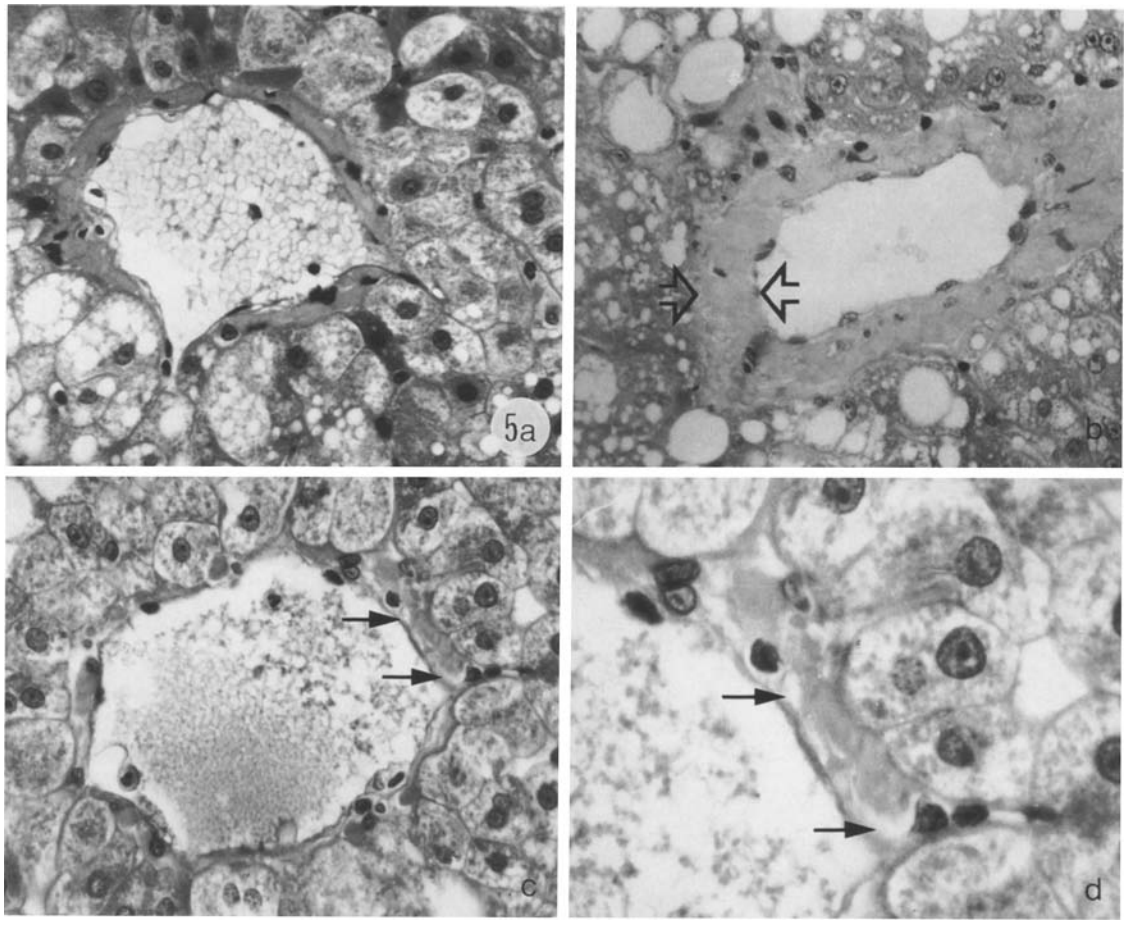

Fig. 5a–d. Histological lesions of THV in alcoholic intoxicated baboons. **a** No change in THV wall. Baboon 5 after 24 months of alcohol intoxication. Masson's trichrome (×280); (**b**) Fibrotic change in an enlarged THV wall (*arrows*). Baboon 6 after 20 months of alcohol intoxication, Masson's trichrome (×220); **c, d** Oedema of THV wall. Baboon 9 after 6 months of alcohol intoxication. Masson's trichrome; **c** Loose organization of the perivenous connective tissue (*arrows*) (×320) and **d** Higher magnification of (**c**), intramural low density of collagen bundles (*arrows*) (×640)

of the diet. The cell necrosis and inflammatory reaction in the portal tract and in acinar location corroborate previous results (Davidson 1986; Lieber 1982; Lieber and Leo 1986) demonstrating a direct toxic effect of alcohol upon the liver, with this equilibrated diet.

The different intensity of parenchymal lesions was probably due to individual variation. This variation in the spectrum of alcoholic disease was reported by Lieber (1982, 1983). Liver fibrosis or cirrhosis developed only in one fourth of the animals submitted to the same conditions of alcohol intoxication. Classic alcoholic hepatitis, as defined by the presence of Mallory bodies surrounded by polymorphonuclear leucocytes, was never found in the baboons during three years of alcohol intoxication. The same findings were pointed out by Popper and Lieber (1980) in the follow up of 18 alcohol-fed baboons, over 6 year period. However acinar inflammation, consisting predominantly of lymphomonuclear cells isolated in sinusoids or regrouped as small granulomas, could be considered to be a special type of alcoholic hepatitis (Baptista et al. 1981) and probably play a role in the development of liver fibrosis (French et al. 1979; MacSween 1985). THV phlebitis has not been previously reported in alcohol-fed baboons. Goodman and Ishak (1982) described a lymphocytic phlebitis characterized by a chronic inflammatory infiltrate of the THV or sublobular hepatic vein walls in human alcoholic liver disease. The infiltrate reported by these authors involved the entire venous wall. In our series, in contrast, it was found to be a patchy periphlebitis associated with degeneration and necrosis of hepatocytes in zone 3 of the hepatic acini. Such phlebitis was associated with collagen deposition in a scar formation that thickened the THV wall. Oedematous THV character-

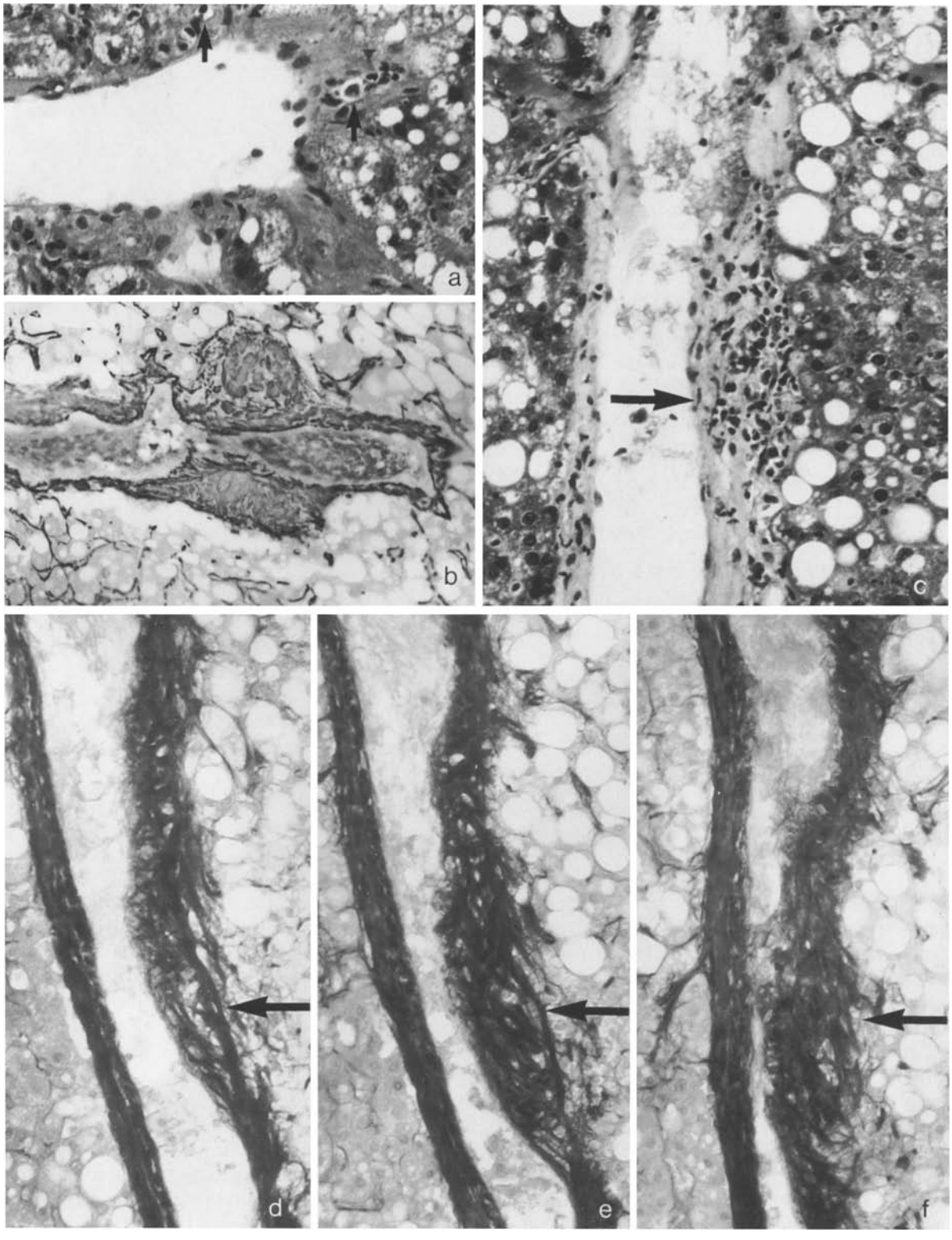

Fig. 6. a THV phlebitis. Polymorphonuclear and lymphomonocytes (*arrows*) in the periphery of the THV wall. Baboon 6 after 12 months of alcohol intoxication. Masson's trichrome (×250); **b** THV focal and asymmetrical fibrosis in THV from baboon 6 after 16 months of alcohol intoxication. Reticulin (×165); **c–f** Patch inflammation (*arrow*) and collagen deposit in THV wall. Serial slides (5 μm interval). Baboon 6 after 24 months of alcohol intoxication (×200). **c** Masson's trichrome; **d–f** Collagen deposit in inflammatory focus. Sirius red

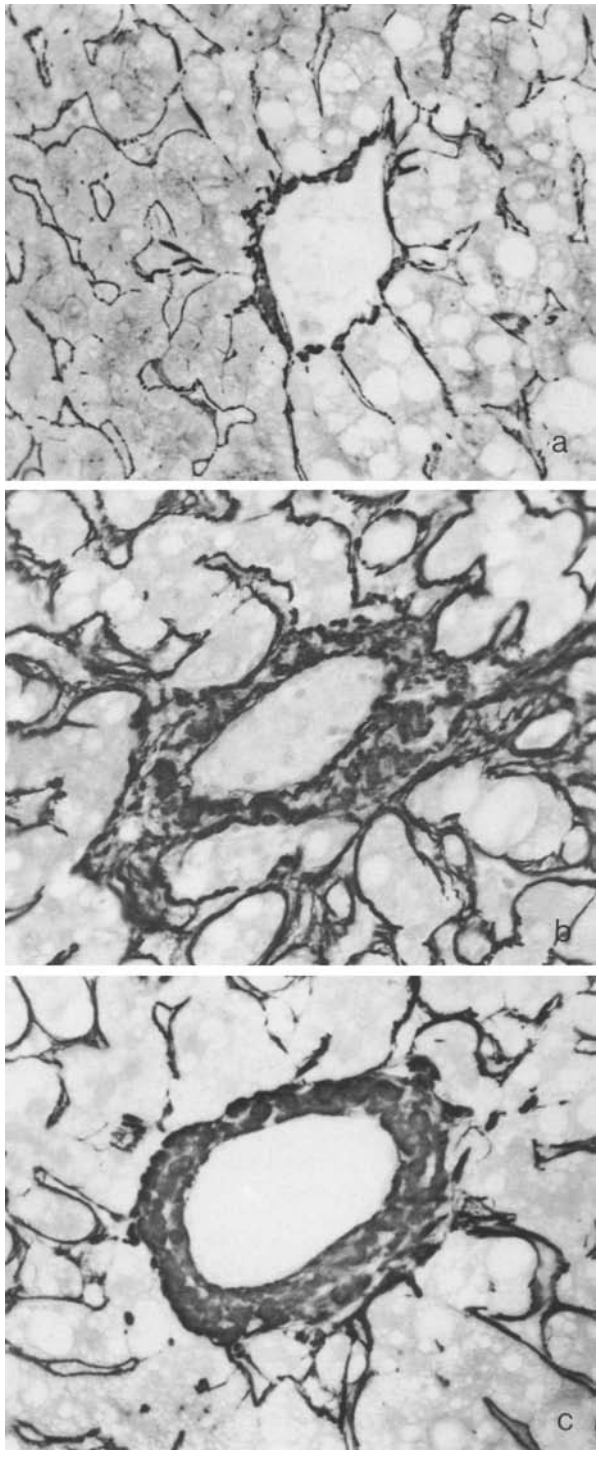

Fig. 7. **a** Normal THV. Baboon 5 after 31 months of alcohol intoxication. Reticulin (×220); **b** THV perivenular fibrosis. Difuse perivenular fibrosis associated with perisinusoidal and pericellular fibrosis. Baboon 6 after 12 months of alcohol intoxication. Reticulin (×200). **c** THV perivenular fibrosis. Limited regular necklace aspect of THV fibrosis. Baboon 5 after 20 months of alcohol intoxication. Reticulin (×350)

Table 2. Morphometrical evaluation of wall thickeness (WS/IS) of THV histological classes from alcoholic intoxicated baboons

		Alcoholic intoxication			
	Control	Normal	Oedematous	Phlebitic	Fibrotic
WS/IS*	0.63 ±0.23	0.62 ±0.14	1.05 ±0.36[a]	1.65 ±1.04[a]	1.47 ±0.36[a, b]
n	62	33	15	12	13

* values as mean ± SD; non homogenous histological classes, Kruskall and Wallis test ($H = 56.09$, $p < 0.001$)

[a] Significantly different, $p < 0.001$, Wilcoxon test between each histological class and control THV

[b] Significantly different, $p < 0.001$, Wilcoxon test between normal, oedematous and fibrotic classes

Table 3. Morphometric evaluation of the cellularity on the THV wall (WS/Mc) of THV histological classes from alcoholic intoxicated baboons

		Alcoholic intoxication			
	Control	Normal	Oedematous	Phlebitic	Fibrotic
WS/Mc* (μm/cell)	558 ±183	552 ±189	540 ±168	510 ±172[a]	439 ±143[b]
n	200	94	63	60	33

* values as mean ± SD; non homogenous histological classes, Anova test ($F = 7.55$, $p < 0.01$)

[a] Significantly different, $p < 0.05$

[b] Significantly different, $p < 0.01$, Kramer extension of Duncan's multiple range test between each histological class and control THV

ized by dissociated walls were also responsible for enlarged THV rim, with value of WS/IS statistically different from control and fibrotic THV. This feature that could be seen either independently or in association with other THV lesions may represent the first modification of THV in alcoholic intoxication. Fibrotic THV, i.e., perivenular fibrosis, as described by Van Waes and Lieber (1977) and by Nakano et al. (1982) was also found in the baboon biopsies during alcohol intoxication. The heterogeneity of the THV lesions found in alcohol-fed baboons could reflect two different processes in liver fibrogenesis, firstly, perivenular scarring following phlebitic and hepatocytic lesions which was the most impressive and frequent feature found in this experimental material. Fibrosis, in this first case, retained the characteristics of a late residual process (Fallon 1982). Secondly, the increasing collagen rim on the THV wall free of inflammatory reaction might represent part of fibrosis directly

enhanced by alcohol (Lieber 1985b; Orrego et al. 1979). These two mechanisms of development of fibrosis lead to a common lesion which is referred to phlebosclerosis by several authors (Burt and MacSween 1986; Goldman and Ishak 1980).

Morphometrical assessment of these lesions, from transversely sectioned THV, showed statistically different WS/IS values that in some cases exceeded 5 times those found in the control THV. So, thickening of the venous wall when assessed by morphometric methods only is not sufficient to foretell extensive liver fibrosis since oedematous and phlebitic lesions could be also responsible for such thickening. In the experiment, fibrotic THV were seen as early as 6 months after the beginning of alcoholic intoxication and since extensive fibrosis did not appear with time, our results did not confirm perivenular fibrosis as a predictive sign for the development of cirrhosis, as emphasized by Lieber reports (1982, 1983) and by Worner et al. (1985). Moreover, fibrotic THV were no longer found on later biopsies from one baboon. This reversibility of THV fibrosis was also remarked on by Nasrallah et al. (1980) in human biopsies from alcoholic patients. Furthermore, it has been reported that young collagen is susceptible to degradation in various pathological conditions (Rojkind 1985). Remodeling of liver connective matrix, including reversibility of early collagen deposition were studied by Grimaud and coworkers (Grimaud 1985) using immunolabelling of collagen isotypes and associated proteins.

The mesenchymal cell population in the wall of fibrotic or phlebitic THV was increased when compared with controls. Nakano and Lieber (1982) showed that myofibroblasts were the most common mesenchymal cell in fibrotic THV with a thickened rim. This ratio did not show an increased number of these cells in normal or oedematous THV under alcohol intoxication. Modification of the mesenchymal cell ultrastructural characteristics such as the transformation of lipocytes into myofibroblasts (Mak et al. 1984; McGee and Patrick 1972; Minato et al. 1983; Okanoue et al. 1983) within the perisinusoidal space, could be postulated as a primary event before proliferation and/or migration of collagen secretory cells to the THV wall.

In the alcohol-fed baboon "pre-fibrotic" liver, THV alterations included wall disruption, disorganization of connective tissue and collagen deposition. Associated perivenular inflammatory reaction due to zone 3 acini degeneration and necrosis of hepatocytes, a toxic effect of alcohol, also contributed to the fibrogenesis which was evident morphologically and by morphometry. Moreover, such fibrotic process failed to develop in all animals and appeared transitory on sequential biopsies. Finally, the development of fibrosis in the wall of THV of alcohol-fed baboons disclosed a potential for reversibility and so its morphology lacks prognostic significance as far as the development of liver fibrosis is concerned.

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References

Baptista A (1981) Alcoholic liver disease: morphological manifestations. Lancet i: 707–711

Burt AD, MacSween RNM (1986) Hepatic vein lesion in alcoholic liver disease: retrospective biopsy study and necropsy study. J Clin Pathol 39(1):63–67

Christoffersen P, Nielsen K (1972) Histologic changes in liver biopsies from chronic alcoholics. Acta Path Microbiol Scand (Section A) 80:557–565

Davidson CS (1986) Changing concepts in the pathogenesis of alcoholic liver disease. Alcohol Clin Exp Res 10 (6):3S–4S

Desmet VJ (1985) Alcoholic liver disease. Histological features and evolution. Acta Med Scand S703:111–126

Edmondson HA, Peters RL, Reynolds TB, Kuzma OT (1963) Sclerosing hyalin necrosis of the liver in the chronic alcoholic. A recognizable clinical syndrome. Ann Inter Med 59:646–672

Fallon HJ (1982) Alcohol and terminal hepatic venule. Early lesion or late residual? Gastroenterology 83:930–932

Fleming KA, McGee JO'D (1984) Alcohol induced liver disease. J Clin Pathol 37:721–733

French SW, Burbige EJ, Tarder G, Bourke E, Harkin CG, Denton T (1979) Lymphocyte sequestration by liver in alcoholic hepatitis. Arch Pathol Lab Med 103:146–152

Galambos JT (1972) Natural history of alcoholic hepatitis. III. Histologic changes. Gastroenterology 63:1026–1035

Goodmann ZD, Ishak KG (1982) Occlusive venous lesions in alcoholic liver disease. A study of 200 cases. Gastroenterology 83:786–796

Grimaud J-A (1985) Immunoelectron microscopy for collagen types differentiation in portal hepatic fibrosis. In: Gerlach U, Pott G, Rauterberg J, Voss B (eds) Connective tissue of the normal and fibrotic human liver. George Thieme Verlag, Stuttgart, pp 30–33

Hall PM (1985) Pathology and pathogenesis of alcoholic liver diseases. In: Hall P (ed) Alcoholic liver diseases. Edward Arnold, London, pp 41–68

Junge J, Horn T, Christoffersen P (1987) Megamitochondria as a diagnostic marker for alcohol induced centrilobular and periportal fibrosis in the liver. Virchows Arch [A] 410(6):553–558

Lane BP, Lieber CS (1966) Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. Am J Pathol 49(4):593–603

Lieber CS (1982) Ethanol, steatosis and fibrosis. In: Gerlach U, Pott G, Rauterberg J, Voss B (eds) Connective Tissue of the Normal and Fibrotic Human Liver. George Thieme Verlag, Stuttgart, pp 107–111

Lieber CS (1983) Precursor lesions of cirrhosis. Alcohol Alcoholism 18(1):5–20
Lieber CS (1985a) Alcohol and the liver: metabolism of ethanol. Metabolic effects and pathogenesis of injury. Acta Med Scand S703:11–55
Lieber CS (1985b) Alcohol metabolism. In: Hall P (ed) Alcoholic Liver Diseases. Edward Arnold, London, pp 3–40
Lieber CS, deCarli LM (1974) An experimental model of alcohol feeding and liver injury in the baboon. J Med Prim 3:153–163
Lieber CS, deCarli LM (1976) Animal models of ethanol dependence and liver injury in rats and baboons. Fed Proc 35:1232–1236
Lieber CS, deCarli LM, Rubin E (1975) Sequential production of fatty liver, hepatitis and cirrhosis in sub-human primates fed ethanol with adequate diets. Proc Nat Acad Sci USA 72:437–441
Lieber CS, Leo MA (1986) Interaction of alcohol and nutritional factors with hepatic fibrosis. In: Popper H, Schaffner F (eds) Progress in Liver Diseases, vol. VIII, chapter 14. Grune and Stratton, pp 253–272
MacSween RNM (1985) Alcohol and liver injury: genetic and immunological factors. Acta Med Scand S703:57–65
MacSween RNM, Burt AD (1986) Histologic spectrum of alcoholic liver disease. Sem Liver Dis 6 (3):221–232
Mak KM, Leo MA, Lieber CS (1984) Alcoholic liver injury in baboons. Transformation of lipocytes to transitional cells. Gastroenterology 87:188–200
McGee JO'D, Patrick RS (1972) The role of perisinusoidal cells in hepatic fibrogenesis. Lab Invest 26 (4):429–440
Milton JS, Tsokos JO (1983) Statistical methods in the biological and health sciences. McGraw-Hill, New York
Minato Y, Hasumura Y, Takeuchi J (1983) The role of fat-storing cells in Disse space fibrogenesis in alcoholic liver disease. Hepatology 3:559–566
Nakano M, Lieber CS (1982) Ultrastructure of initial stages of perivenular fibrosis in alcohol-fed baboons. Am J Pathol 106:145–155
Nakano M, Worner TM, Lieber CS (1982) Perivenular fibrosis in alcoholic liver injury: ultrastructure and histologic progression. Gastroenterology 83:777–785
Nasrallah SM, Nassar VH, Galambos JT (1980) Importance of terminal hepatic venule thickning. Arch Pathol Lab Med 104:84–86
Okanoue T, Burbige EJ, French SW (1983) The role of the Ito cells in the perivenular and intralobular fibrosis of alcoholic hepatitis. Arch Pathol Lab Med 107:459–463
Orrego H, Medline A, Blendis LM, Rankin JG, Kreaden DA (1979) Collagenisation of the Disse space in alcoholic liver disease. Gut 20:673–679
Popper H, Lieber CS (1980) Pathogenesis of alcoholic fibrosis and cirrhosis in the baboon. Am J Pathol 98 (3):695–715
Porto LC, Chevallier M, Grimaud J-A (1989) Morphometry of terminal hepatic veins in man and baboons. Virchows Arch [A] 414:129–134
Rojkind M (1985) Collagen metabolism in the liver. In: Hall P (ed) Alcoholic Liver Disease. Edward Arnold, London, pp 91–112
Scheuer PJ (1982) The morphology of alcoholic disease. Br Med Bull 38 (1):63–65
Ugarte G, Iturriaga H (1976) Metabolic pathways of alcohol in the liver. Front Gastroenterol Res 2:150–193
VanWaes L, Lieber CS (1977) Early perivenular fibrosis in alcoholic fatty liver: an index of progressive liver injury. Gastroenterology 73:646–650
Worner T, Lieber CS (1985) Perivenular fibrosis as precursor lesion of cirrhosis. JAMA 254:627–630

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