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Morphometry of terminal hepatic veins*

1. Comparative study in man and baboon

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Summary. In the present study, hepatic venous distribution per unit of liver surface area on normal wedge biopsies from man ($n=11$) and baboon ($n=8$) were analysed and compared. Terminal hepatic veins (THV – man: $n=100$; baboon: $n=200$) morphometric size variables were obtained with a Leitz ASM 68K morphometric equipment. THV, defined as hepatic veins up to 150 μm in internal diameter (ID), in the centrolobular position and with sinusoidal openings, represented 84% and 74% of hepatic veins of man and baboon, respectively. Four or more THV were generally found on 8 mm^2 of liver surface. Transversely sectioned THV selected by the ratio IDminimum/IDmaximum >0.67, was found to be only 25% of the total THV. In baboon, THV merge with other terminal veins and the interlobular veins present sinusoidal inlets. The baboon THV wall surface (WS) and wall thickness (WT) values were higher than in man. Positive correlations between the number of mesenchymal cells (Mc) in the vein wall and wall surface of terminal hepatic veins (man: $r=0.79$; baboon: $r=0.83$) and between wall surface and internal surface (IS) (man: $r=0.80$; baboon: $r=0.72$) were found. Two ratios were selected as the most reliable parameters: (1) for the THV wall rim, wall surface/internal surface (WS/IS – man: 0.43 ± 0.16; baboon: 0.63 ± 0.23), regarding transversely sectioned THV; and (2) for the evaluation of wall cell density (WS/Mc-man: 550 ± 231; baboon: 558 ± 183 μm^2/cell) as they did not depend on THV caliber.

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Key words: Liver – Hepatic veins – Man – Baboon – Morphometry

Introduction

Perivenular fibrosis of terminal hepatic venule (THV) was claimed to be one precursor lesion leading to liver fibrosis in alcoholics (Worner and Lieber 1985). Using the baboon model, Lieber (1978, 1983) described the various steps of THV lesions under chronic alcohol intoxication and their good correlation with human lesions in alcoholism. No work has previously been carried out on the THV sampling quality of liver biopsies, i.e., the THV number per liver biopsy surface and the selection of transversely sectioned THV for morphometric comparisons. Also, little is known about the normal values for THV variables, either in man or in baboon under normal conditions. Moreover, liver venous distribution in man and in various species of experimental animals, other than baboon, has been demonstrated to exhibit different patterns (Elias and Popper 1955). It therefore seemed important to search for major dissimilarities between man and baboons, since this should be taken into consideration in future experiments with baboons.

The purpose of the present study was to compare normal THV from man and baboon and to determine which morphometric venous variables were the most reliable, for the definition of the normal morphological pattern of the vein in order to make subsequent comparisons with pathological material.

Materials and methods

Eleven surgical wedge biopsies of the liver were obtained from 11 patients (4 men and 7 women, ages range between 28 and

76 – $\bar{x}=54$) submitted to choledococystectomy, but showing a normal liver histologically. None were alcoholics (<20 g/day in women and <60 g/day in men) and laboratory data on liver functions were within normal limits.

Fourteen wedge biopsies of the liver were obtained from 8 baboons (*Papio papio*). They were born and raised in France and weighed between 14 and 18 kg. All hepatic function tests showed normal values and the animals were free of parasitic diseases.

All specimens were fixed in Bouin's solution for light microscopy and processed through conventional histological methods. Slides were stained with haematoxylin and eosin, Masson's trichrome, Gordon and Sweet's silver method for reticulin, orcein for elastic fibers and sirius red for collagen.

Morphological measures were performed using a semiautomatic image analysis system (LEITZ-DIALUX microscope, VARIOSCAN V16 camera, LEITZ ASM 68K computer with black and white monitor, digitizing tabled and HEWLETT-PACKARD THINKJET printer). LEITZ ASM 68K MEASURES, GRAPHICS AND STATISTICS program was used.

Hepatic veins were measured by contour tracing of their external and internal image limits taken from reticulin or sirius red stained sections. Large hepatic veins were measured on the monitor from the microscope image with a ×10 objective and the smaller ones with ×25 or ×40 objectives. The measured hepatic veins were identified and numbered on a ×17.5 photograph from each liver section. The following THV variables were obtained by using the computer program: external surface (ES), external diameter (ED), internal surface (IS), internal perimeter (IP), internal diameter (ID), maximal (IDmax) and minimal (IDmin) internal diameters. We considered the wall surface (WS) to be the difference between the external and internal surface, and the wall thickness (WT) half of the difference between the external and the internal diameters. Correlations between diameter, perimeter and surface, were performed by the internal formulae in the program.

Adjacent serial slides stained by haematoxylin and eosin or Masson's trichrome were used to count cell nuclei in the perivenous connective tissue. A distinction was made between endothelial cell (Ec) nuclei and mesenchymal cell (Mc) nuclei counted in the internal limits of the veins and in the wall of the vein, respectively.

Fourteen representative frames of 8 mm^2 (4×2) were randomly drawn on the photographs of the liver sections, in general one per biopsy and as far from the liver capsule as possible. When identified and measured, the hepatic veins inside the frames were selected to evaluate the number of THV found on a 8 mm^2 liver surface. We defined the group of THV with internal diameter up to 150 μm according to classical anatomical data. Transverse sections of THV were selected by the ratio IDmin/IDmax >0.67. The number of transversely sectioned THV was also obtained from each frame.

The hepatic veins found in the frames were separated by their ID into 50 μm classes. The THV, selected by ID<150 μm, were distributed into 10 μm classes.

Reproducibility of measurements was estimated by measuring 40 veins twice by the same observer, testing the results with linear regression analysis and the Student's *t*-test. Intra-observer variance was calculated from the standard deviation of three measurements on 20 veins (Rodbard 1974).

Two hundred THV from baboons and one hundred THV from men were measured. The proportionality of THV rim to THV caliber was tested by means of linear regression between: 1) WT×ID and 2) WS×IS. The correlation between THV rim and wall cellularity was calculated from Ec×IP and Mc×WS. Differences between THV parameters in man and baboon, expressed as mean±SD, were estimated by the Student's *t*-test in a IBM PC computer and Lotus 123 (Sundqvist and Enkvist 1987) with appropriated formulas (Milton and Tsokos 1983).

Results

Reproducibility tests showed a good correlation with r>0.99 for IS, IP, ID, WS and WT measurements, retained for the characterization of the THV with the semi-automatic equipment, and no significant differences were found between first and second measurements. The intra-observer coefficient of variation ranged between 0.78 to 4.32% as shown in Table 1.

Ninety-three hepatic veins were found in man and ninety-nine in baboons, on 112 mm^2 of liver surface (fourteen frames of 8 mm^2). The ID-class distribution of these hepatic veins (Fig. 1a) showed no frequence difference between man and baboon when large ID classes were defined. THV represented 74% and 84% of all the hepatic veins for man and baboon, respectively. The number of terminal hepatic veins between 30 to 70 μm ID was basically constant (Fig. 1b). Such number decreased between 70 and 100 μm ID and the reduction was significant after 100 μm ID ($t=4.05$, $p<0.001$).

Variation of THV number found in the frames (man=3 to 7; baboon=3 to 9) was not statistically significant ($t=1.78$). In 90% of the biopsies, 4 or more terminal hepatic veins were found for a surface of 8 mm^2, but only 25% of them were found to have an IDmin/IDmax superior to 0.67. The frequence distribution of the THV number and of the transversely sectioned THV in frames of 8 mm^2 appear in Fig. 2.

Table 1. Reproducibility and intraobserver variance of THV morphometric parameters

Parameters	Reproducibility 1a/1b		Intraobserver variance mean
	r	*t*	% CV_w
Internal surface	0.997	NS*	2.16
Internal perimeter	0.995	NS	1.41
Internal diameter	0.997	NS	0.78
Wall surface	0.993	NS	3.85
Wall thickness	0.990	NS	4.32

r: correlation coefficient; *t:* *t*-value from paired Student's *t*-test; NS*: non significant at 0.05 confidence value

$$\% CV_w = \frac{Sw}{x} 100 \qquad S_w = \sqrt{\frac{\Sigma \sigma_i^2 (r_i - 1)}{r_i \Sigma (r_i - 1)}}$$

σ_i^2: variance of each observation; r_i: number of observations (triplicate); x: mean

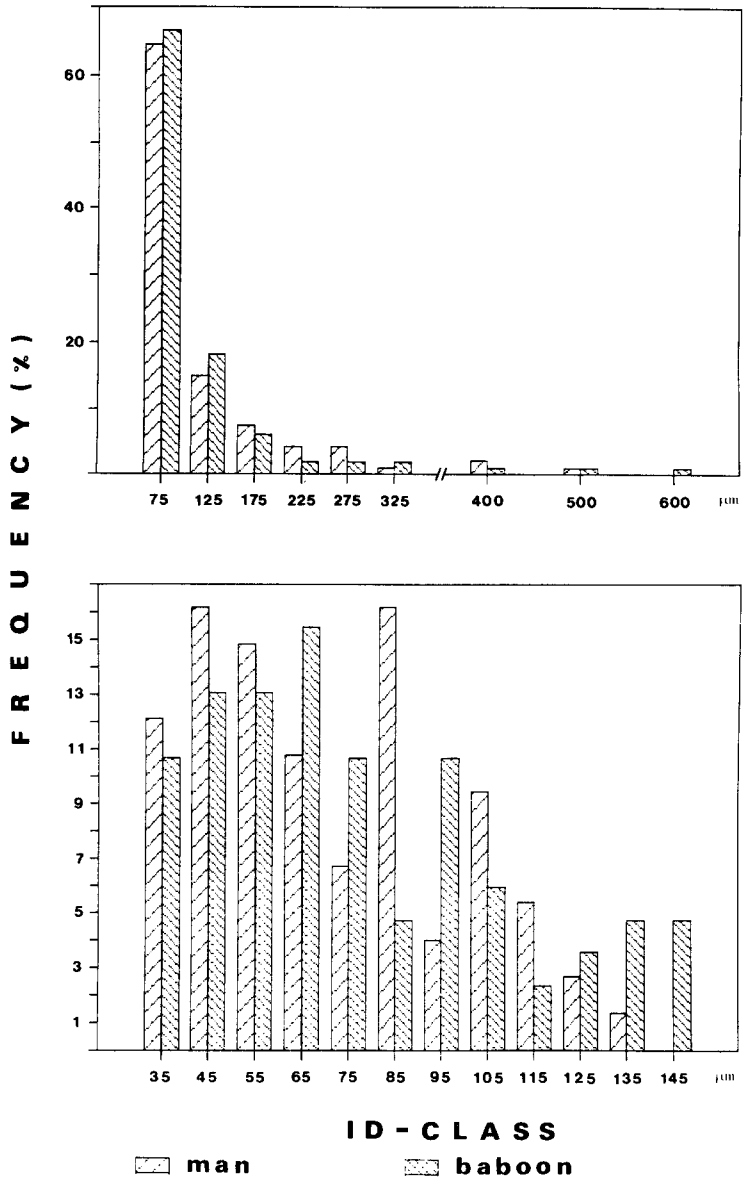


Fig. 1. Histogram of distribution of the hepatic veins found on 112 mm^2 (14×8 mm^2) per class of internal diameter.
a All hepatic veins (man, $n=93$; baboon, $n=99$).
b terminal hepatic veins (ID < 150 µm)

Correlations between internal diameter versus wall thickness and between internal surface versus wall surface showed positive correlation coefficients when two dimension parameters were used (man: r = 0.80 and baboon: r = 0.72) if THV transverse sections were selected (Fig. 3).

Correlations between THV endothelial cells × IP (man: Ec = 0.26 IP − 0.87, r = 0.73; baboon: Ec = 0.27 − 0.28, r = 0.62) and between mesenchymal cell × WS (man: Mc = 0.0016 WS + 0.054, r = 0.79; baboon: Mc = 0.0018 WS + 0.247, r = 0.83) were not affected by the section angle of THV (man, $n=100$; baboon, $n=200$).

As THV caliber increased from 25 to 150 µm its wall increased in size. This is schematically illustrated in Fig. 4, where four segments with ID varying from: 1) up to 43 µm; 2) 44 to 66 µm; 3) 67 to 98 µm; and 4) 99 to 150 µm are represented with the nuclear figures of endothelial and mesenchymal cells and their respective WT and WS. The comparison of THV parameters showed that the wall surface, expressed by the ratio WS/IS, was significantly greater ($t=4.31$; $p<0.001$) in baboon (0.63 ± 0.23) than in man (0.43 ± 0.16). No difference was found between the ratio WS/Mc in man (550 ± 231 μm^2/cell) and baboon (558 ± 183 μm^2/cell) THV (Table 2).

Discussion

Reproducibility and intra-observer error measurements in the present study were in accordance with

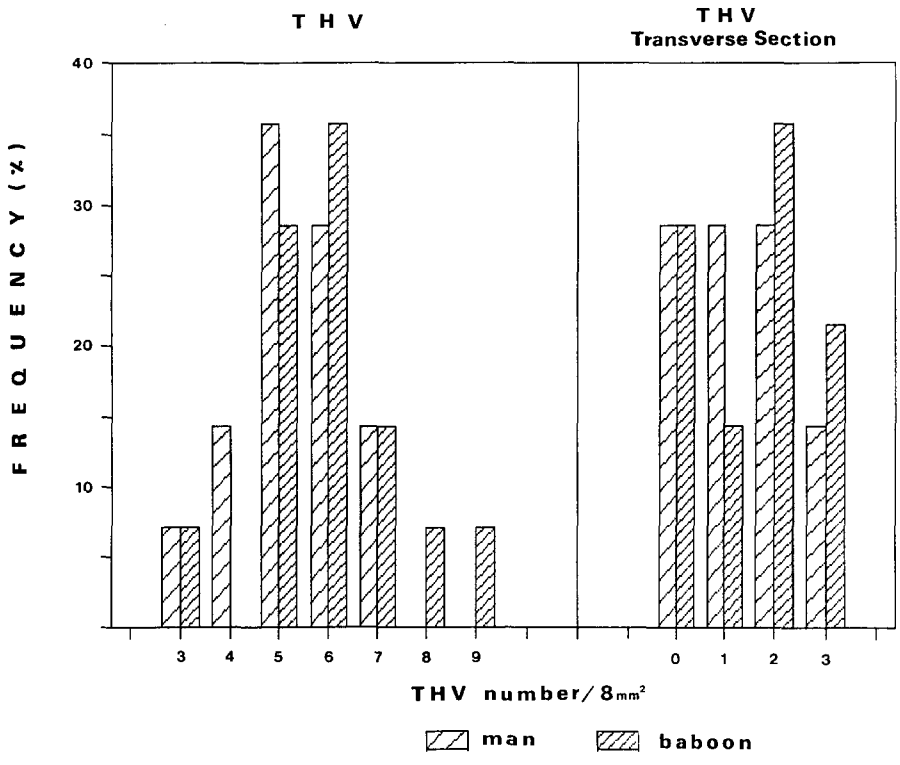
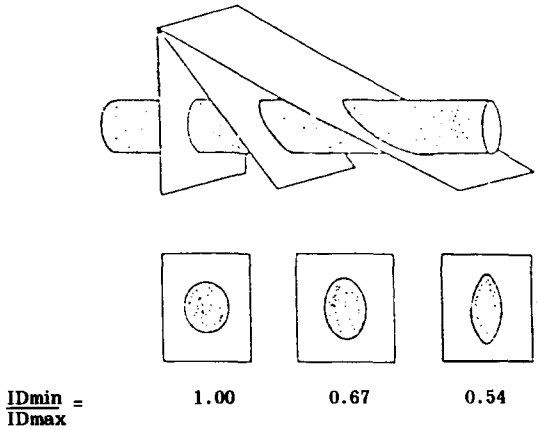


Fig. 2. Distribution of terminal hepatic vein number found in 8 mm^2 of liver surface of man and baboon. Selection of transversely sectioned THV by IDmin/IDmax >0.67 restricted the available THV number found in 8 mm^2 of liver surface



INTERNAL DIAMETER VERSUS WALL THICKNESS

IDmin / IDmax	man
> 0.54	WT = 0.08 x ID - 0.18 (r = 0.53, n = 63)
> 0.67	WT = 0.09 x ID - 0.28 (r = 0.54, n = 29)
	baboon
> 0.54	WT = 0.11 x ID + 0.83 (r = 0.29, n = 123)
> 0.67	WT = 0.14 x ID - 1.44 (r = 0.43, n = 62)

INTERNAL SURFACE VERSUS WALL SURFACE

	man
> 0.54	WS = 0.38 x IS + 143 (r = 0.79, n = 63)
> 0.67	WS = 0.38 x IS + 129 (r = 0.80, n = 29)
	baboon
> 0.54	WS = 0.58 x IS + 375 (r = 0.54, n = 123)
> 0.67	WS = 0.63 x IS - 56 (r = 0.72, n = 62)

Fig. 3. Correlation between THV morphometric parameters. No satisfactory correlations were obtained between ID and WT, even when only tranversally sectioned THV were included. Correlation between surface parameters (IS x WS) showed a better correlation coefficient, respected IDmin/IDmax >0.67

those found by others with comparable equipment (Dardick and Caldwell, personal communication).

Counts and measurements of the hepatic veins should be related to a surface-unit to be used as reference. The choice of 8 mm^2 represents half of a needle biopsy mean surface usually required and obtained for a reliable diagnosis (Bateson et al. 1980; Sherlock et al. 1984). Variation of THV

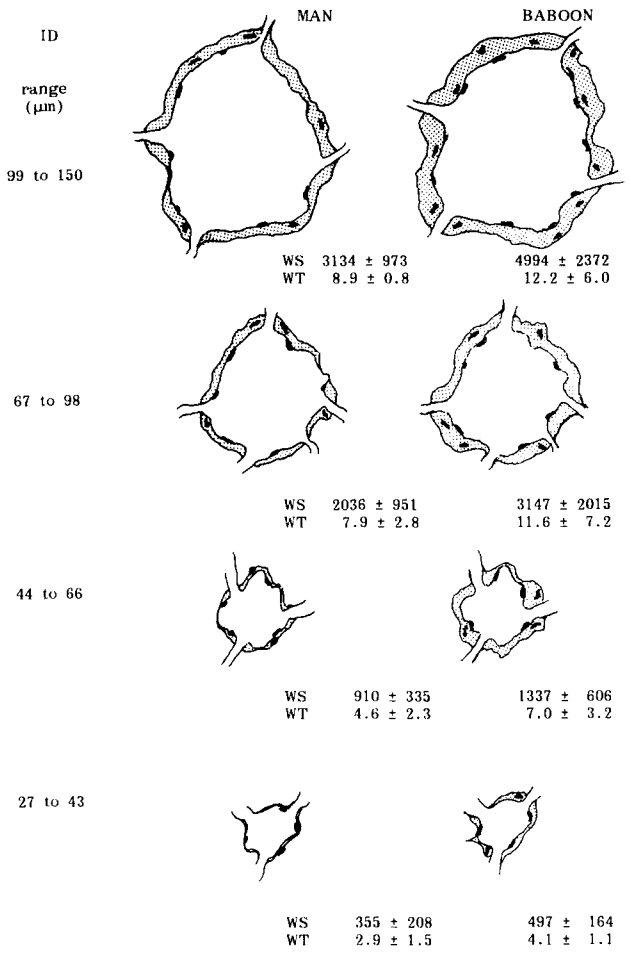


Fig. 4. Comparative representation of THV segments of the same diameter in man and baboon. WT in μm and WS in μm^2. Baboon THV had WS and WT higher than comparable man THV segments

Table 2. Comparison of the wall surface/internal surface (WS/IS) and wall surface/mesenchymal cell (WS/Mc) ratios of man and baboon

	man	baboon	t	p
WS/IS	0.43 ± 0.16 $n=29$	0.63 ± 0.23 $n=62$	4.31	<0.001
WS/Mc μm^2/cell	550 ± 231 $n=100$	558 ± 183 $n=200$	0.03	NS*

Values expressed as mean ± SD; t: t-value from Student's t-test
NS*: non significant at 0.05 confidence value

number found in the frames probably reflected the section angle of the liver tissue, since it was not statistically significant. Although four or more THV were generally found in the frames, only 25% of them were accepted as transversely sectioned. Liver fragments with 60 to 80 mm^2 surface sections must be obtained if a large number of transversely sectioned THV are planned for comparison.

Injection-corrosion vascular casts of hepatic veins from human specimens showed that only the centrilobular veins had sinusoidal inlets and that these veins might join intercalated veins or a major trunk vein (Elias 1949; Elias and Popper 1955; Hales et al. 1959). In histological sections, characterization of a terminal hepatic venule should take into account the peri-acinar (centrolobular) position, sinusoidal inlets (Rappaport et al. 1983) and an internal diameter up to 150 μm. The ID class distribution of THV suggested that, in man, THV of less than 70 μm ID represented the final portion of these centrilobular veins and 70–150 μm ID veins were a continuation of terminal hepatic venules that still occupied a centrolobular position. In the baboon, we found THV merging one another and also with the junction of terminal hepatic veins with intercalated veins. The baboon "terminal veins" of 70 to 150 μm ID were particularly variable in their wall thickness and wall surface, although with the presence of sinusoidal openings. It could therefore be assumed that venous distribution in baboons is similar to that of other experimental animals with regard to the sinusoidal openings on the intercalated hepatic veins (Elias and Popper 1955).

The correlation coefficients of the internal diameter versus wall thickness, and wall surface versus internal surface showed a greater variability of the former and confirmed that measurements of non-regular circles were better expressed by their surface than by their diameters (Bradbury 1978). Wall thickness and wall surface values were greater in baboon than in man, as already shown by Van Waes and Lieber (1977) and Nakano and Lieber studies (1982). However, WT values from baboon THV were quite different from those reported by these authors. That difference could be explained by the choice of basic data acquisition method in the evaluation of ID and WT, since our ID data resulted from an average of 24 diameters traced with an interval angle of 7.5% as opposed to Nakano manual measurements which might directly approximate the diameter along some arbitrary direction; by inclusion of terminal veins in the proximity of the capsular region, which were often richer in fibrous tissue (Petrelli and Scheuer 1967); and by interspecies variations. We worked exclusively with *Papio papio,* whereas Lieber's group studied either *Papio hamadryas* or olive and yellow baboons (Lieber et al. 1985).

These discrepancies with respect to basic values for THV could greatly affect the interpretation of experimental results. Nakano and Lieber (1982) accepted 8 μm as a threshold for normal WT and considered as indicative of perivenular fibrosis all the values exceeding 8 μm in THV up to 125 μm in diameter. In our study, the thickness of the THV rim was found to be more than 11 μm for THV segments between 67 to 150 μm in ID. These normal values could be misinterpreted as already being abnormal.

The evaluation of cellular density in the perivenular connective tissue gave us a constant relationship between the amount of connective tissue and the number of cells. Such regularity seemed to reflect the stability of connective tissue cells and that of the extracellular matrix within these veins. Identification of the venous cell population (endothelial and mesenchymal cells) and of their number correlated well with other venous wall parameters and were of interest, since striking myofibroblast proliferation has been reported to occur in early stages of THV thickening (Nakano et al. 1982).

The scarcity of THV of comparable size in the tissue sections obtained from needle biopsies make it difficult to apply ID and WT for the diagnosis of terminal hepatic vein fibrosis. For further THV morphometric assessment the relation WS/IS, obtained from THV with IDmin/IDmax >0.67, seems to be the most valuable parameter for subsequent quantification of perivenular fibrosis during chronic alcoholic intoxication in baboons. Moreover, cellularity of THV wall, expressed as WS/Mc, can be used as a morphometric parameter for diagnosis of THV wall alteration.

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