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The sinusoidal barrier in rats with portacaval anastomosis: a morphometric study*

L. Dubuisson, F. Sztark, C. Bedin, P. Bioulac-Sage, and C. Balabaud
Laboratoire des Interactions Cellulaires – Université de Bordeaux II, 146, rue Léo Saiquat, F-33076 Bordeaux Cedex, France

Summary. Shunting of portal blood in the rat leads to liver atrophy and to an increase in arterial blood flow with microcirculatory disturbances. The aim of this study was to investigate the effects of these disturbances on the liver sinusoidal barrier (endothelial and perisinusoidal cells) using morphometric techniques. Rats with portacaval anastomosis (PCA) and sham operated pair-fed controls were studied 3 months after the shunt. Sinusoidal volume density in PCA increased but not significantly and the volume density (V_v) of total endothelial (EC) and perisinusoidal cells (PSC) increased by 104.54% compared to sham operated pair-fed rats. The increase of EC V_v was not associated with an increase in surface density (S_v) suggesting a fall in the number of small fenestrations and an increase in cell thickness. This interpretation supports the morphological observations. The increase of PSC V_v was mainly related to the increase in their subendothelial processes V_v and not to that of the cell body V_v. Lipids V_v and RER S_v expressed per sinusoidal cells remained unchanged suggesting that the balance between the 2 hypothetical functions of the PSC, namely fibrogenesis and storage of vitamin A, was maintained.

In conclusion, changes of EC and PSC after PCA result mainly in thickening of the sinusoidal barrier. This increase may impair exchanges between the sinusoidal lumen and Disse space and contribute to functional abnormalities.

Key words: Perisinusoidal cell – Endothelial cell – Portacaval anastomosis – Rat – Morphometry

Introduction

Sinusoidal cells have been the subject of extensive studies in the past 10 years (Wisse and Knook 1977, 1982). The sinusoidal barrier plays a major

Offprint requests to: L. Dubuisson at the above address

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role in regulating the exchanges between plasma and the sinusoidal membrane of the hepatocyte. This barrier is defined as consisting of the endothelial (EC) and perisinusoidal cells (PSC). The EC form the blood barrier and are characterized by a thin endothelium with sieve plates and some gaps (Fraser 1980; Wisse et al. 1983). PSC participate in the storage of vitamin A and probably in fibrogenesis (Ito and Shibasaki 1968; Wake 1971) and are also part of the sinusoidal barrier. They support the endothelial lining with their "subendothelial processes" (Vonnahme 1982).

Portacaval anastomosis (PCA) is an interesting model for the study of these 2 sinusoidal cell types as the liver is by-passed by portal blood and supplied only by the hepatic artery. In PCA, apart from an absolute increased hepatic arterial blood flow (More et al. 1984) sinusoidal haemodynamic disturbances have also been reported (McCuskey et al. 1983). In the centrolobular zones, the velocity of blood flow in the dilated sinusoidal network tends to be slow whereas in the so-called nodular foci scattered in the periportal areas it is near normal. In addition, blood flows towards the central venules as well as to the portal venules in the short sinusoidal segments supplied by arterio-sinus twigs (McCuskey et al. 1983). The haemodynamic disturbances are associated with structural changes: atrophy of hepatic cords in the mid-centrolobular zones and hypertrophy of hepatocytes, which sometimes form plates two cells thick along narrow sinusoids in the foci (Dubuisson et al. 1984). In previous studies qualitative alterations of sinusoidal cells after PCA were shown (Dubuisson et al. 1982a; Dubuisson et al. 1982b).

The aim of this work was to measure changes occurring in the sinusoidal barrier (which represents the zone of exchange between blood and hepatocytes) using morphometric techniques.

Materials and methods

Male Wistar rats (250–300 g) bred in our laboratory and submitted to an artificial day light cycle, with free access to water and food (AO_4, Villemoisson-sur-Orge, France) were used.

End-to-side portacaval anastomosis (Lee and Fisher 1961) was performed on groups of 5 rats. They were housed in metabolic cages for one month and then in individual plastic cages for another period of 2 months.

Sham operated rats (sham PCA) were pair-fed during the 3 month study period.

Perfusion of the liver. 5 PCA and 5 sham-PCA animals were anesthetized with pentobarbital between 9 and 11 am. in order to minimize circadian variations in morphology and functions. Livers were perfused via the aorta with a constant infusion pump at a flow rate of 3 ml/min/100 g b.wt. for the PCA group allowing for liver atrophy and at 5 ml/min/100 g b.wt. for the controls. Perfusion pressure was measured in the 2 groups. The perfusion was performed in 2 stages: first a washing with 0.1 M phosphate buffer for 1 minute followed by the perfusion of 1.5% glutaraldehyde in the same buffer (pH 7.4, mosm 345) for 3 minutes. At the end of the first step the left lobe was removed and processed for routine light microscopy (H&E). At the end of the second step the liver was quickly removed, weighed and systematically cut into small blocks, then processed for transmission electron microscopy (TEM).

Morphometric analysis. 3 blocks were used for each animal. One semi-thick section (1 μm) per block was stained with toluidine blue. Ultrathin sections were chosen independently of

the acinar localization in zones devoid of foci and vascular spaces. 10 micrographs showing more than 50% of reference tissue were taken for each section and studied under a Philips EM 300 at 3 different levels ($\times 1{,}900$; $\times 12{,}400$; $\times 34{,}000$) according to Weibel et al. (1969).

At a magnification of $\times 1{,}900$, volume densities (V_v) of sinusoids and cells (EC, PSC) were estimated with a coherent double lattice system (CP = 30) and expressed per liver parenchyma and per sinusoid respectively.

At a magnification of $\times 12{,}400$, volume densities of EC were estimated with a coherent double lattice system. Surface densities (S_v) and surface to volume ratio (S/V) were estimated with a multipurpose test system (50 points, 100 points). All the measurements were expressed per sinusoid.

In perisinusoidal cells, total V_v as well as V_v of cellular body only, and S_v of perisinusoidal processes were estimated with the same lattices and expressed per sinusoid.

At the highest magnification ($\times 34{,}000$) V_v and S_v of lipid droplets, RER S_v of PSC were measured on micrographs showing more than 55% of perisinusoidal tissue with a multipurpose lattice system.

No attempt was made to correct for possible errors introduced by section thickness, angle and specific membrane configuration which may result in either over-or under estimation of membranes.

V_v were expressed in cm^3/cm^3 or % of sinusoids or intralobular tissue or perisinusoidal tissue; S_v in m^3/cm^3 of sinusoids.

Results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using Student's t test for unpaired data. P values < 0.05 were considered significant.

Results

Rats lost weight shortly after PCA but regained their preoperative weight 1 month later. 3 months after the shunt, body weight increased by $31.3 \pm 7.5\%$ in PCA and $33 \pm 8.0\%$ in sham pair-fed animals. Liver weight/body weight ratio decreased by $29 \pm 12.5\%$ in PCA compared to sham pair-fed rats.

In PCA animals perfusion pressure was significantly decreased changing from 106.5 ± 17 cm H_2O in controls to 55.6 ± 14.5.

By light microscopy, after PCA sinusoids seemed enlarged throughout the parenchyma except in hyperplastic focal areas. They occupied a larger volume than in the controls (Table 1). However the difference was not significant.

Table 1. Volumetric composition of liver parenchyma in PCA rats compared with sham operated controls

	PCA $n=5$	Sham pair-fed PCA $n=5$
Sinusoids V_v[a]	0.29 $\pm$0.05[b]	0.24 $\pm$0.08
Endothelial cells V_v	0.038 $\pm$ 0.015*	0.022 $\pm$ 0.017
Perisinusoidal cells V_v	0.052 $\pm$ 0.006*	0.022 $\pm$ 0.005

[a] Volume density (V_v) is expressed as cm^3/cm^3 of liver parenchyma ($\times 1{,}900$) (double lattice test system)

[b] Mean $\pm$ SD

* Significantly different from the control $P < 0.05$

Table 2. Morphometric data concerning endothelial cells in PCA rats compared with sham operated controls

	PCA $n=5$	Sham pair-fed PCA $n=5$
(1) V_v	0.13 ± 0.02[a]*	0.09 ± 0.02
(2) S_v	57.0 ± 8.9	65.6 ± 17.3
(3) S/V	0.12 ± 0.09	0.13 ± 0.03

(1) Volume density (V_v) of endothelial cells expressed as cm^3/cm^3 of sinusoidal space ($\times 12{,}400$) (Coherent double lattice test system)
(2) Surface density (S_v) of endothelial cells expressed as m^2/cm^3 of sinusoidal space ($\times 12{,}400$) (Multipurpose test system)
(3) Surface to volume ratio (S/V) of endothelial cells expressed as cm^2/cm^3 of sinusoidal space ($\times 12{,}400$) (Multipurpose test system)
[a] Mean $\pm$ SD
* Significantly different from the controls $P<0.05$

Table 3. Morphometric data concerning perisinusoidal cells (PSC) in PCA rats compared with sham operated controls

	PCA $n=5$	Sham pair-fed PCA $n=5$
A		
V_v (T)	0.18 ± 0.02[a]*	0.09 ± 0.02
V_v (CB)	0.02 ± 0.01	0.03 ± 0.092
S_v (PR)	39.1 ± 0.7*	22.7 ± 0.4
S/V (PR)	0.19 ± 0.07	0.18 ± 0.08
B		
V_v (L)	0.08 ± 0.04	0.08 ± 0.04
S_v (L)	0.14 ± 0.02*	0.09 ± 0.04
S_v (RER)	0.27 ± 0.09	0.22 ± 0.04

Volume density (V_v) surface density (S_v) and surface to volume ratio (S/V) are expressed in cm^3/cm^3, m^2/cm^3 and cm^2/cm^3 respectively.
A = Values expressed per sinusoid ($\times 12{,}400$) (Coherent double lattice test system, Multipurpose test system)
V_v (T) of total PSC; V_v (CB) of PSC cellular body;
S_v (PR) of PSC processes; S/V of PSC processes.
B = Values expressed per mean PSC ($\times 34{,}000$) (Multipurpose test system)
V_v (L) of lipid vacuoles; S_v (L) of lipid vacuoles
S_v (RER) of RER
[a] Mean $\pm$ SD
* Significantly different from the control, $P<0.05$

Endothelial cells (Table 2). On TEM, endothelial cells looked quite normal. However they seemed to form a more continuous and thicker barrier than in sham PCA with less fenestrations (Fig. 1 and 3).

Their volume density whether expressed as cm^3/cm^3 of liver parenchyma or of sinusoidal space was significantly increased (Table 1, 2) but not their surface density or their surface to volume ratio (Table 2).

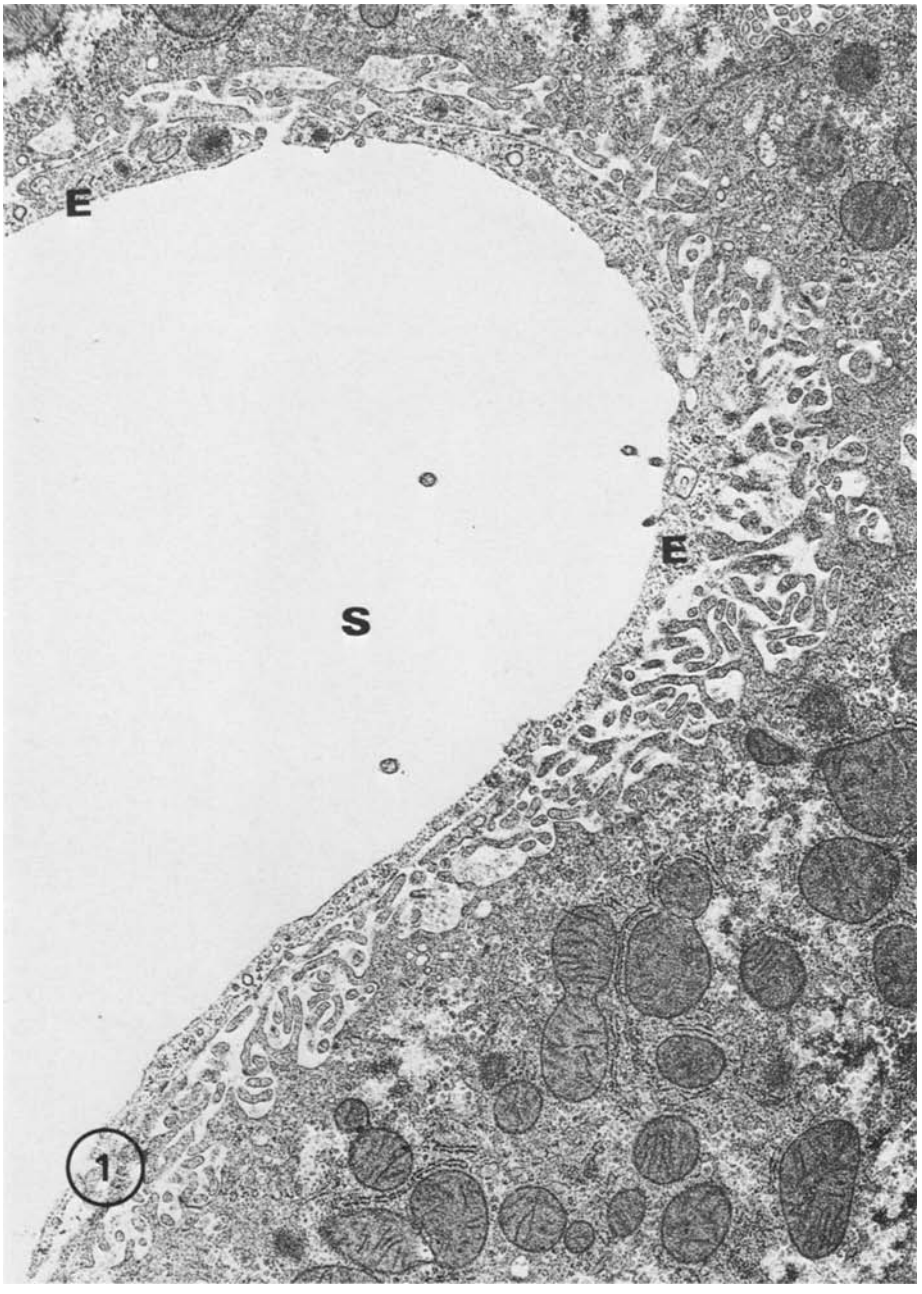

Fig. 1. 3 months PCA rat liver. A thick endothelial barrier (*E*) almost completely surrounds a sinusoid (*S*). ×14,800

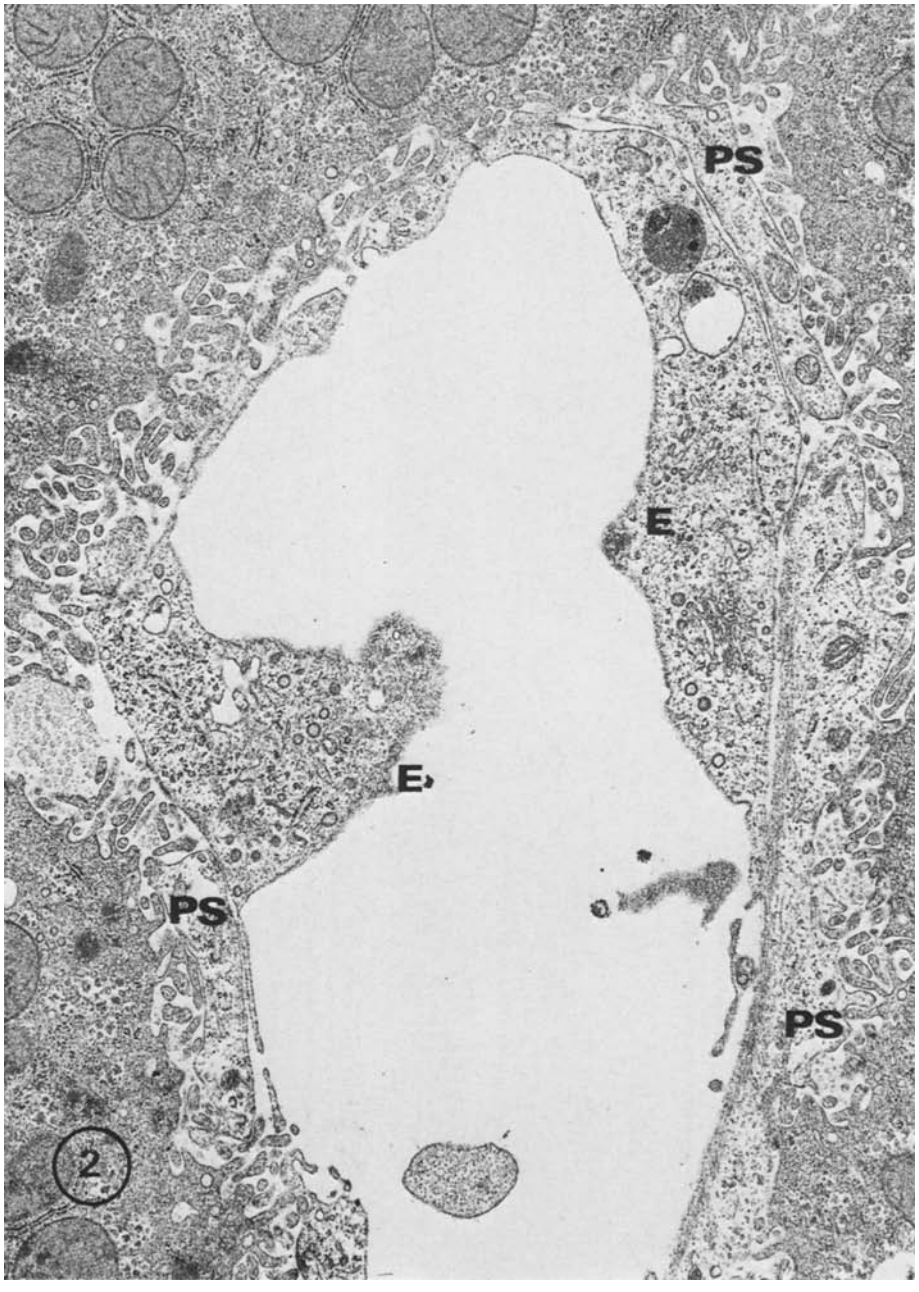

Fig. 2. 3 months PCA rat liver. A thick endothelial barrier (*E*) supported by the numerous subendothelial processes of a perisinusoidal cell (*PS*) can be observed. × 14,800

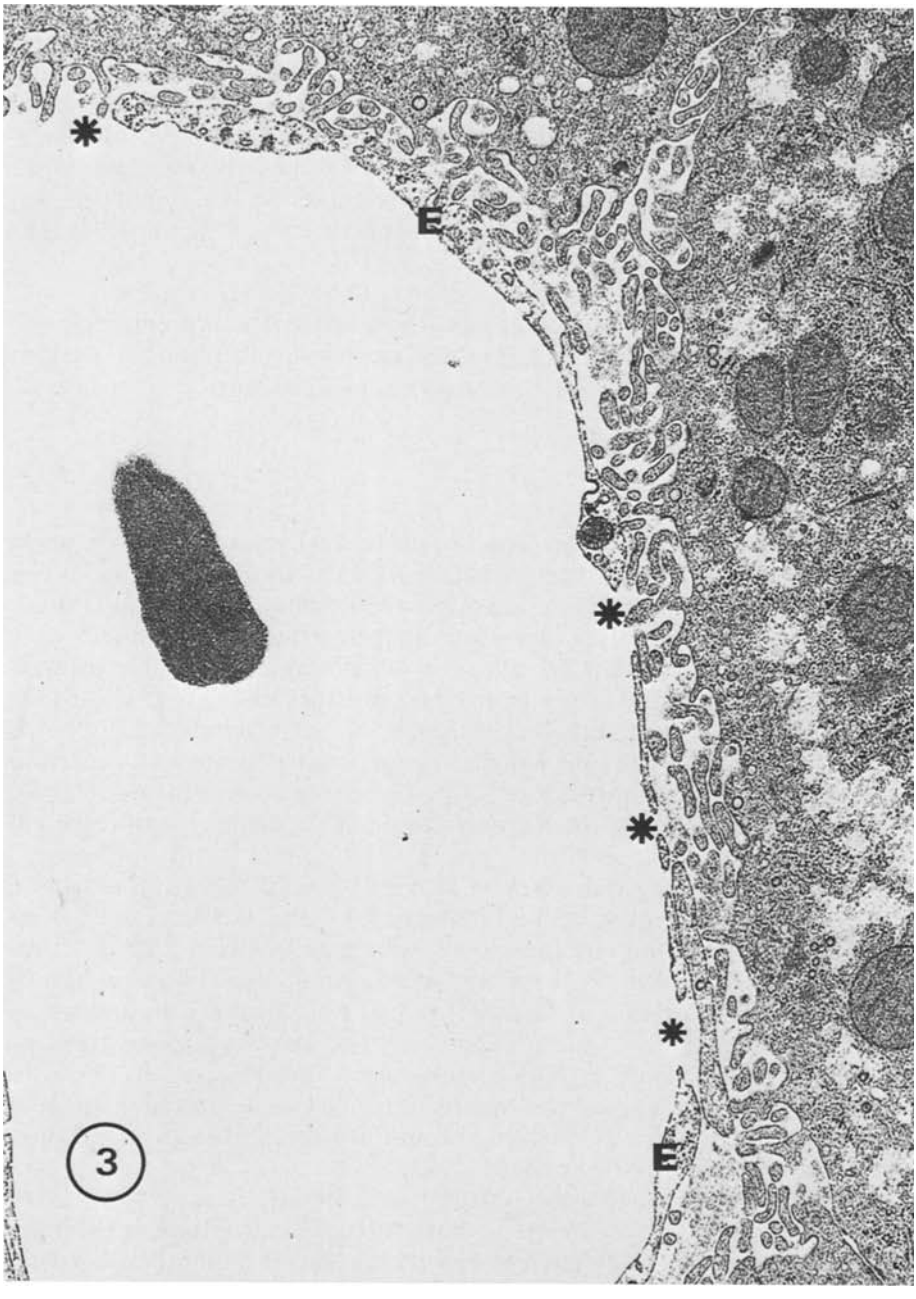

Fig. 3. 3 months sham PCA rat liver. The endothelial barrier (*E*) is thin and discontinuous. Numerous fenestrations can be observed (*). × 14,800

Perisinusoidal cells. After PCA TEM showed an increase in PSC tissue (Fig. 2) when compared with the controls (Fig. 3) particularly in the subendothelial processes. Indeed, their volume density whether expressed as cm^3/cm^3 of liver parenchyma or sinusoidal space, was significantly increased (Table 1, 2). The perisinusoidal cell body V_v and the subendothelial processes S_v were increased while the S_v and S/V of these cells were unchanged (Table 3). The S_v but not the V_v of lipid vacuoles in the cytoplasm was significantly increased compared to sham operated rats. S_v of RER was not significantly increased.

Disse space. On semi-thick sections the Disse space was not enlarged and the number of collagen bundles was not increased. On ultrathin sections no basement membrane was seen beneath endothelial cells.

Discussion

Liver atrophy is the main consequence of portacaval anastomosis. It reached 25 to 30% compared to sham pair-fed animals. In PCA hepatic blood flow expressed per g liver tissue is normal (More et al. 1984). This is made possible through an absolute increase in hepatic arterial blood flow (More et al. 1984). At the moment no data is available on how the liver protects itself against the hypothetically high blood pressure in PCA. It is possible that (Grisham and Nopanitaya 1981) part of the arterial blood flow is diverted, as in the control rats, towards the peribiliary plexus and the terminal portal vein which represents a large blood reservoir. However, direct openings of arterioles into the periportal zone of the acinus might represent a real threat.

As shown recently (McCuskey et al. 1983) the dilatation of sinusoids, the increased number of intersinusoidal sinusoids and the possibility of reversed flow towards the terminal portal vein may be defense mechanisms against high pressure in PCA. There is no proof, however, that this is the case. In fact dilatation of sinusoids remains moderate (Kyu and Cavanagh 1970) whether it is due to hypothetically increased blood pressure or to other causes, such as hyperoestrogenia (Van Thiel et al. 1983). In our study sinusoids V_v was not signficantly increased, probably because liver samples were taken at random and not in zone 3 where the dilatation is the most obvious (McCuskey et al. 1983).

In this study which focused on the barrier it was important to know whether there were any artifacts. In both models sinusoids were correctly washed, contained none or few red blood cells and the endothelial barrier was not disrupted on TEM, as perfusion pressure was low enough to avoid this kind of problem (Wisse et al. 1983). The fact that the perfusion pressure in PCA was half the pressure obtained in controls was due to the dilatation of the hepatic artery and possibly also to the dilatation of sinusoids.

As suspected the sinusoidal barrier did show changes. Morphometry

is a convenient tool to assess these changes quantitatively. Total volume density of EC and PSC was double that of sham operated rats whose value was in the control range (Blouin et al. 1977).

The increased V_v of EC not followed by changes in S_v and S/V could perhaps be explained by a) a decrease in the number of sieve plates or of their pores, b) an increased number of gaps and c) an increase in cell thickness. Our observations by TEM as well as others by SEM (McCuskey et al. 1983) support this interpretation.

The increased V_v of PSC was not due to an increase in cell body V_v but to the extension of subendothelial processes as demonstrated by the increase in their S_v (Dubuisson and Vonnahme 1983). The slight increase in the number of PSC observed after PCA should also be taken into account (Dubuisson et al. 1982b; Ronchetti et al. 1983). Extension of subendothelial processes have been reported in fibrosis (McGee and Patrick 1972), liver cell necrosis (Vonnahme 1982) and human cirrhosis (Vonnahme and Dubuisson 1983; Lamouliatte et al. 1985). Processes can proliferate and encircle the endothelial lining like pericytes in capillaries (Courtoy and Boyles 1983). Indeed PSC are considered to belong to the fibroblast class. Extension of perisinusoidal processes could be related to necrosis occurring immediately after the shunt or represents, associated with the thickening of EC, a response to the hypothetical increase in sinusoidal pressure.

PSC are involved in fibrogenesis and participate with other hepatic cells (Clement et al. 1984) in the formation of various types of collagen (mainly type III and IV). After PCA there was no significant increase of the RER S_v suggesting that collagen synthesis was not enhanced. However an increase of the reticulin network after PCA has previously been shown (Dubuisson et al. 1982b). Although it is not possible to rule out an enhancement of collagen synthesis by other hepatic cells it is likely that the reticulin staining partly corresponds to the perisinusoidal processes as shown recently (Vonnahme 1982). In addition to their similarities with fibroblasts, PSC are involved in the storage of vitamin A inside lipid droplets (Wake 1971). The increase of their S_v without change in the V_v suggests a difference in the distribution of lipid vacuoles inside the PSC. Although the exact amount of vitamin A inside PSC is unknown, the present results seem to indicate that PSC normally store vitamin A. According to the Wake hypothesis (Senoo et al. 1982) the balance between the 2 hypothetical functions of the PSC namely fibrogenesis and storage of vitamin A would not be impaired in PSC.

In conclusion, after PCA in spite of major changes in hepatic arterial blood flow and in the microcirculation, changes in EC and PSC are moderate. They mainly consist in the thickening of the sinusoidal barrier, leading to a difference in local resistance against deformation (Wisse et al. 1983) and probably to the impairment of exchanges between the sinusoidal lumen and the Disse space. More data are needed to evaluate the contribution of this thickening to the functional liver abnormalities observed after the shunt (Lauterburg et al. 1976).

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