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Perisinusoidal fibrosis of the liver in patients with thrombocytopenic purpura

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Summary. 10 patients with thrombocytopenic purpura (TP) underwent splenectomy. Eight of these patients had idiopathic TP (certain or probable). All had normal liver function tests. Liver histology of the surgical biopsy was normal with the exception of a non specific mild portal infiltration in 6 cases. On Sirius red staining the perisinusoidal network was normal in 3 cases, mildly or moderately increased in 5 cases and often associated with perivenular fibrosis. Collagen types I, III, IV, laminin and fibronectin were increased in the 8 biopsies tested. On semi-thin sections, numerous Kupffer cells were observed. Under the electron microscope, sinusoidal abnormalities were very similar in all 7 patients studied: numerous Kupffer cells containing abundant lysosomes, numerous collagen bundles in the Disse space, active endothelial cells, transformation of some perisinusoidal cells into cells with some of the characteristics of fibroblasts (increased RER) and myofibroblasts (peripheral condensations of the filamentous network), increased fragments of basement membrane-like material. In two cases there was an increase in the number of perisinusoidal cells loaded with lipids. The similarity of the lesions and the absence of other fibrogenic causes (except in 2 cases) suggest that TP may represent another group of diseases with perisinusoidal fibrosis. The aetiology of fibrosis remains unknown but platelet derived growth factor and activated macrophages may play a major role.

Key words: Thrombocytopenic purpura – Idiopathic thrombocytopenic purpura – Liver sinusoidal fibrosis – Electron microscopy – Immunocytochemistry

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Increasing numbers of cases of perisinusoidal fibrosis (PSF) in patients showing no clinical, biochemical or pathological evidence of liver disease are reported. Recently, other researchers, as well as ourselves, have reported the occurrence of PSF in patients with agnogenic myeloid metaplasia (Degott et al. 1985; Bioulac-Sage et al. 1986b). We suggested that the platelet derived growth factor (PDGF) liberated by ineffective liver megakaryocytosis and the activation of Kupffer cells might play a role in the fibrogenic process (Bioulac-Sage et al. 1986b). In the present study, we have investigated whether thrombocytopenic purpura (TP), which is also characterized by massive platelet destruction and activation of Kupffer cells, could be associated with PSF. We observed that patients with severe TP requiring splenectomy had mild to moderate PSF.

Patients and methods

Ten patients with TP requiring splenectomy were studied. Eight showed idiopathic thrombocytopenic purpura (ITP) and two a purpura of different immunological origin (systemic lupus). None were excessive alcohol consumers (alcohol intake <20 g/day in women and <40 g/day in men). All relevant clinical data are presented in Table 1. At the time of surgery liver function tests which included total bilirubin (normal <17 µmol/L), aspartate and alanine aminotransferases (normal <40IU/l) and alkaline phosphatase (normal <120 µmol/L) were normal.

A wedge liver biopsy was performed during splenectomy. Patients had given written informed consent and the study was approved by the Ethics Commitee of the Regional University Hospital. The biopsy was fixed in Bouin's reagent, processed for light microscopy studies, and stained with H & E and Sirius red. In eight cases, part of the biopsy was frozen in nitrogen cooled isopentane for immunocytolocalization of collagens type I, III, IV, laminin and fibronectin (Clément et al. 1986). In addition, for seven of these patients, part of the biopsy was perfusion-fixed with 1.5% glutaraldehyde and processed for electron microscopy (Bioulac-Sage et al. 1986a). Semi-thin sec-

Table 1

Case	Age	Sex	Discovery and initial platelet count/ml	Missing criteria of diagnosis (1)	Cortico-therapy initial dose and duration	Other treatment related to TP	Major antecedents, drugs or diseases	Other abnormal test or associated disease at time of surgery	Date of splenectomy	Outcome (2)
ITP										
1	16	M	Cutaneous and mucosal purpura since June 1985 (4000)	–	120 mg/day 7 months	Immunoglobulins Plasma exchanges	–	–	March 1986	Favorable
2	22	F	Cutaneous purpura since October 1985 (10000)	–	60 mg/day 3 months	Plasma exchanges	Oestroprogestative contraception for 1 year (ADEPAL)	–	March 1986	Mediocre
3	48	F	Auditive discomfort in February 1984 (10000)	–	65 mg/day 4 months	–	Oestroprogestative contraception for 15 years (STEDIRIL)	AN-AB (+1/100)	October 1984	Mediocre
Probable ITP (some missing criteria)										
4	31	F	Cutaneous purpura and heavy periods after salicylic treatment in February 1986 (4000)	Antiplatelet antibodies Isotopic double population	60 mg/day 3 months	Immunoglobulins	Oestroprogestative contraception for 8 years (ADEPAL)	Associated focal nodular hyperplasia	August 1986	Favorable
5	53	F	Cutaneous purpura since July 1981 (15000)	Antiplatelet antibodies	None (B hepatitis)	Immunoglobulins	B hepatitis 2 months before splenectomy numerous stays in tropical areas	Gamma globulins 30 g/l	July 1986	Favorable
6	46	M	Discrete purpura since October 1985 (16000)	Isotopic exploration was not performed	60 mg/day 3 months	Immunoglobulins	Chronic use of anti-inflammatory drugs for coxarthrosis	–	October 1986	Mediocre
Other pathologies associated with purpura										
7	22	F	Mucosal bleeding and heavy periods in July 1985 (8000)	–	None (pregnancy)	Immunoglobulins	Minor B thalassemia Overweight (72 kg for 1.60 m)	–	September 1985	Favorable
8	51	F	Purpura in 1982 and 1986 (with heavy periods)	Isotopic exploration was not performed	several in 1982 in 1986 90 mg/day 5 months	Immunoglobulins Plasma exchanges	Overweight (130 kg) Salicylic and glafenine abuse	ESR (40/82)	September 1986	Mediocre
Immunologic purpura										
9	54	F	Cutaneous purpura since April 1984 (34000)	Isotopic exploration was not performed	in 1984 in 1985	Plasma exchange	Probable systemic lupus	AN-AB (+1/300)	June 1986	Favorable
10	54	F	Cutaneous purpura in 1980, 1982 May 1986 (43000)	–	in 1980, 1982 in 1986: 70 mg/day 5 months	None	Systemic lupus	ESR = 50/83 AN-AB (+1/500)	October 1986	Favorable

(1) Criteria of diagnosis: absence of splenomegaly; bone marrow cytology in favor of ITP; presence of antiplatelet antibodies; absence of other auto immune antibodies (for ex. antinuclear antibodies (AN-AB)); isotopic study of platelet kinetics with spleen sequestration and existence of a double population
(2) Outcome: favorable: normalization of platelet blood count; mediocre: transitory normalization followed by a platelet decrease (long term corticotherapy was then started)

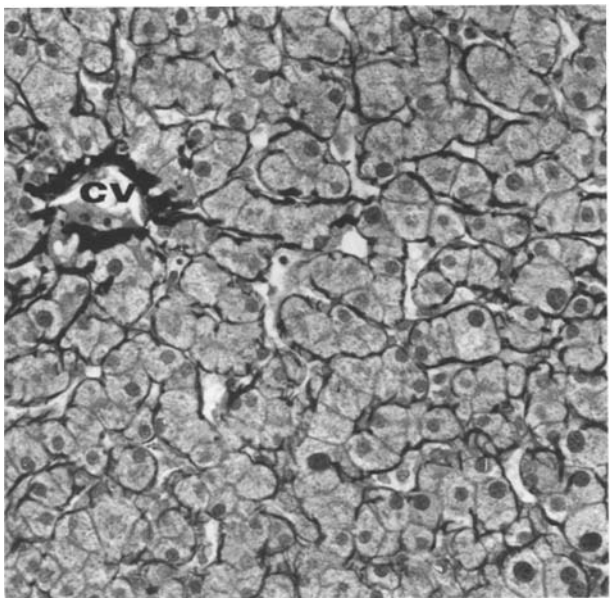

Fig. 1. Light microscopy – Sirius red (patient n° 1). The perisinusoidal network is increased with a thickened central vein (*CV*). × 280

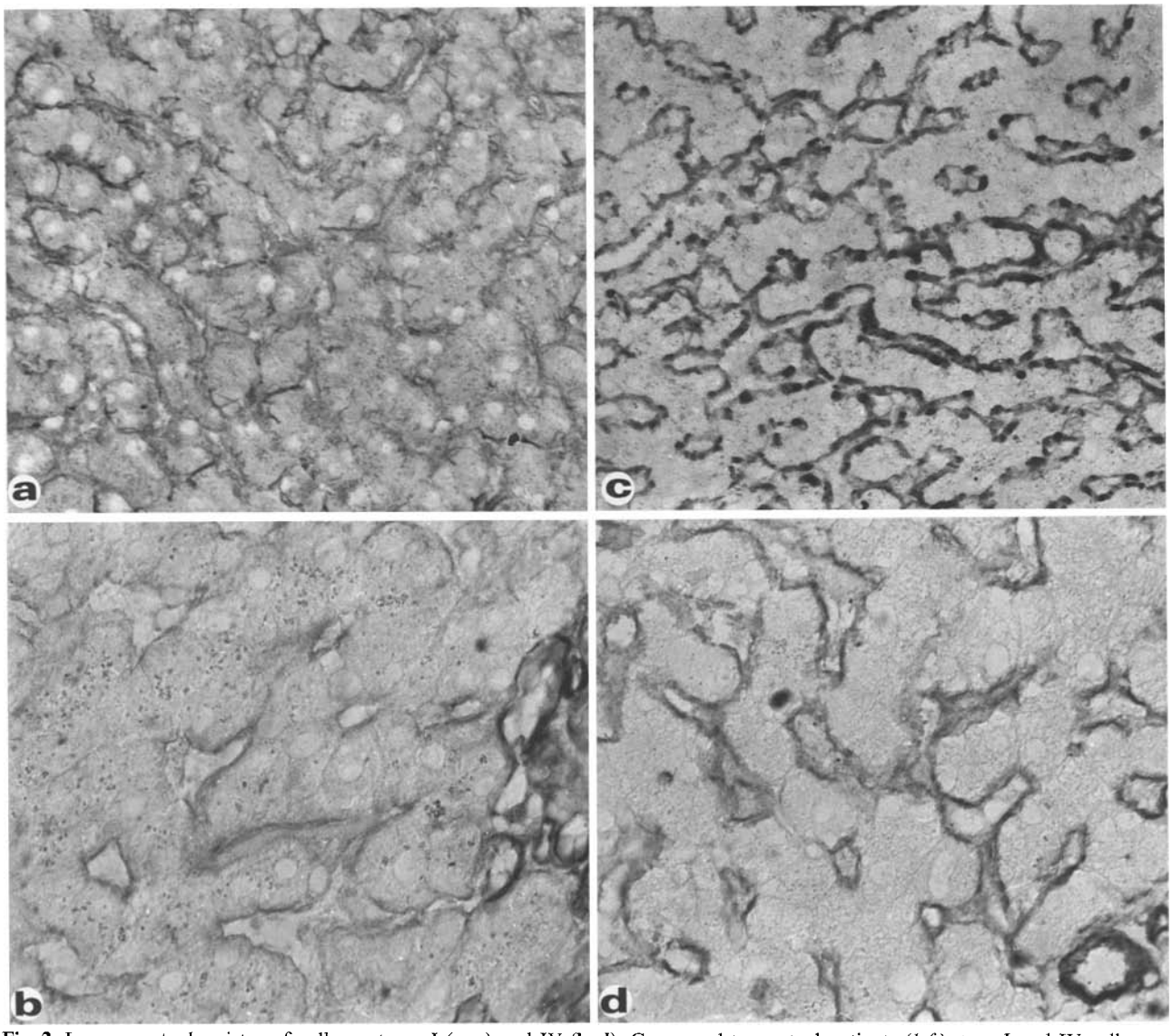

Fig. 2. Immunocytochemistry of collagen types I (**a, c**) and IV (**b, d**). Compared to control patients (*left*), type I and IV collagens are increased in thrombocytopenic purpura (*right*). × 280 (**a, c**); × 450 (**b, d**)

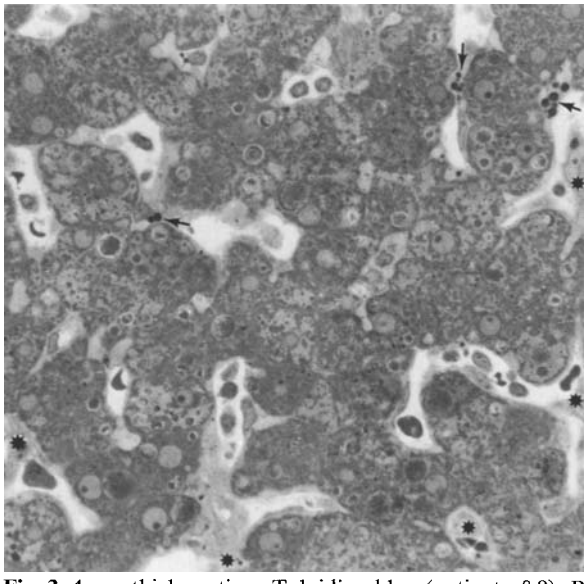

Fig. 3. 1 μm thick section. Toluidine blue (patient n° 9). Presence of numerous Kupffer cells (*asterisks*). Some perisinusoidal cells contain abundant lipid droplets (*arrows*). The Disse space appears slightly enlarged. ×428

tions were stained with toluidine blue. Ultrathin sections were double contrasted with uranyl acetate and lead citrate; grids were examined on a Philips EM 301 (Centre de Microscopie Electronique de l'Université de Bordeaux II, France).

Four wedge liver biopsies from patients undergoing cholecystectomy for asymptomatic cholelithiasis were used as controls. They had normal liver function tests and normal liver histology (light and electron microscopy).

Results

All TP patients had normal liver function tests and routine histology (H & E) with the exception of a non specific mild portal infiltration in six cases. PSF, when appreciated on Sirius red staining, was absent in 3 cases (patients 5-6-8), mild and irregular in 4 cases (patients 2-4-9-10), moderate and often irregular from one area to another in 3 cases (patients 1-3-7). PSF was often accompanied by perivenular fibrosis (Fig. 1).

Immunolocalization in the extracellular matrix showed an increase in all collagens tested, laminin and fibronectin, for the 8 patients studied. However the increase was generally slight and more pronounced for collagen types I and IV (Fig. 2).

On semi-thin sections, numerous Kupffer cells were seen in the sinusoidal lumen (Fig. 3). The Disse space was occasionally enlarged. In two cases, there was a noticeable increase in the number of perisinusoidal cells filled with abundant lipid droplets.

Under the electron microscope, the general aspect of the sinusoids was the same in the 7 cases studied: 1. Perisinusoidal fibrosis due to numerous collagen bundles in the Disse space (Figs. 4, 9). 2. Numerous Kupffer cells containing phagocytic material and situated in the sinusoidal lumen, or forming the sinusoidal barrier (Figs. 5, 6). 3. Transformation of some perisinusoidal cells (loaded with lipids in two cases) into cells with some of the characteristics of fibroblasts (increased RER) or myofibroblasts (an increase in the patches of condensation of the filamentous network below the plasma membrane) (Figs. 7, 8) 4. More frequent fragments of basement membrane-like material, generally localized between endothelial and perisinusoidal cells (Fig. 9). 5. Increased activity of some endothelial cells, which appeared thicker, with many endocytic vesicles.

It is worth mentioning that even in those cases where fibrosis appeared mild, irregular or even absent on Sirius red staining, abnormalities were visible by electron microscopy, although possibly to a minor degree.

Discussion

In all ten cases studied, we found PSF associated with perivenular fibrosis. Liver function tests were normal. Liver histology on routine H & E staining was normal or subnormal. Two patients (cases 7 and 8) were overweight (Marubbio et al. 1976). Otherwise, no aetiological factors were found for the fibrosis: no alcohol (Lieber and Leo 1986), drug abuse (Zafrani et al. 1983) (particularly drugs known to induce fibrosis, such as vitamin A), nor diabetes (Latry et al. 1987). Corticosteroids have been reported to increase the number of perisinusoidal cells (Bronfenmajer et al. 1966) and oral contraceptives can cause sinusoidal dilatation and tumours (Zafrani et al. 1983). However, neither cause fibrosis. In patients 9 and 10 ITP was associated with a disseminated lupus erythematosus (probable or certain). This disease is known to be associated in most cases with non specific hepatic lesions including steatosis and interstitial hepatitis. Frequently, the liver is histologically normal (Diebold and Camilleri 1974). The presence of fibrosis in all these cases, although of varying severity, suggests that the aetiological agent is TP itself. Some specific and individual factors might in certain cases have played an additional role. Indeed, although not seen in the present study, mild perisinusoidal fibrosis of unknown aetiology is observed in approximately one out of 10 control patients.

All the patients studied had chronic persistent thrombocytopaenia, caused by a circulating anti-

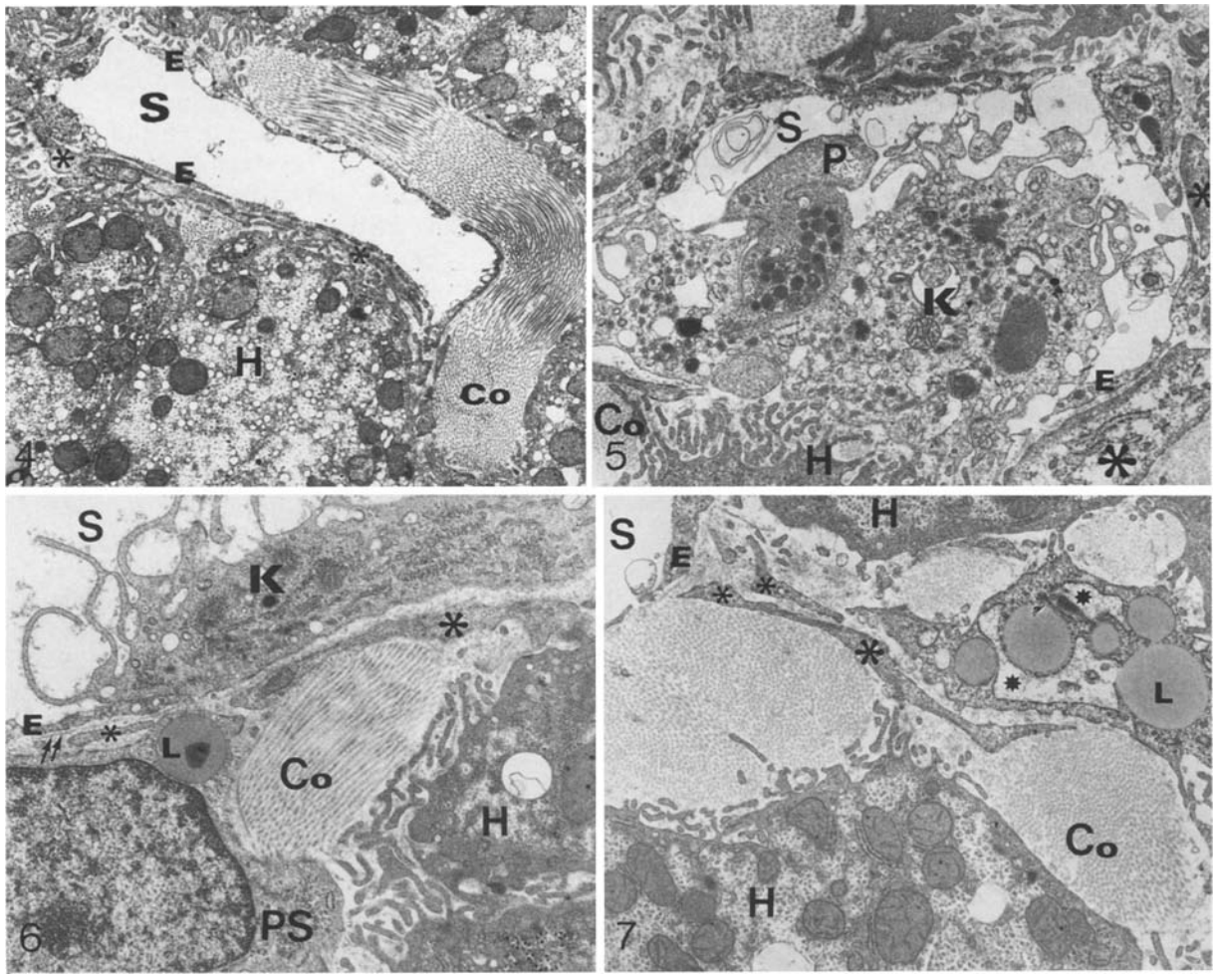

Fig. 4. Electron microscopy (patient n° 1). This empty sinusoid (*S*) is delimited by a thin endothelial barrier (*E*); thick collagen bundles (*Co*) enlarge the Disse space. *H*: hepatocyte, asterisks: processes of perisinusoidal cells. ×6300

Fig. 5. Electron microscopy (patient n° 4). This Kupffer cell containing numerous lysosomes (*K*) engulfs a platelet (*P*); the Kupffer cell forms part of the sinusoidal barrier, and is next to the microvilli of the hepatocyte (*H*). *E*: endothelial cell, asterisk: process of perisinusoidal cell, *Co*: collagen. ×8000

Fig. 6. Electron microscopy (patient n° 4). This Kupffer cell (*K*) forms part of the sinusoidal barrier and is next to a long process of a perisinusoidal cell (*PS*) (*asterisk*). A *PS* can easily be recognized with its lipid droplet (*L*). Short fragments of basement membrane-like material (*double arrow*) can be seen between the endothelial cell (*E*) and the *PS*. *S*: sinusoid, *Co*: collagen, *H*: hepatocyte. ×15400

Fig. 7. Electron microscopy (patient n° 9). Part of a perisinusoidal cell with small lipid droplets (*L*) and a dilated *RER* (*stars*). *S*: sinusoid, *E*: endothelial cell, *H*: hepatocyte, asterisk: process of perisinusoidal cell, *Co*: collagen. ×8000

platelet factor found in 8 cases out of 10. This resulted in platelet destruction by the reticulo-endothelial system (MacMillan 1981). In eight cases, the TP was idiopathic; in two cases it was secondary to a systemic lupus.

Although PSF has never yet been reported in patients with TP, we were not surprised to find it in these cases. We and others have recently reported PSF in patients with agnogenic myeloid metaplasia (Degott et al. 1985; Bioulac-Sage et al. 1986b). In this pathology, myelosclerosis is probably related to the liberation by ineffective megakaryocytosis of two proteins contained in the alpha granules (Platelet Derived Growth Factor (PDGF) and Platelet Factor 4 (PF4)) (Castro-Malaspina and Moore 1982). By analogy, we speculate that megakaryocyte components, in excessive concentration in the Disse space and the sinusoidal lumen, could also liberate PDGF and PF4. PDGF stimulates the proliferation of fibroblasts and increases

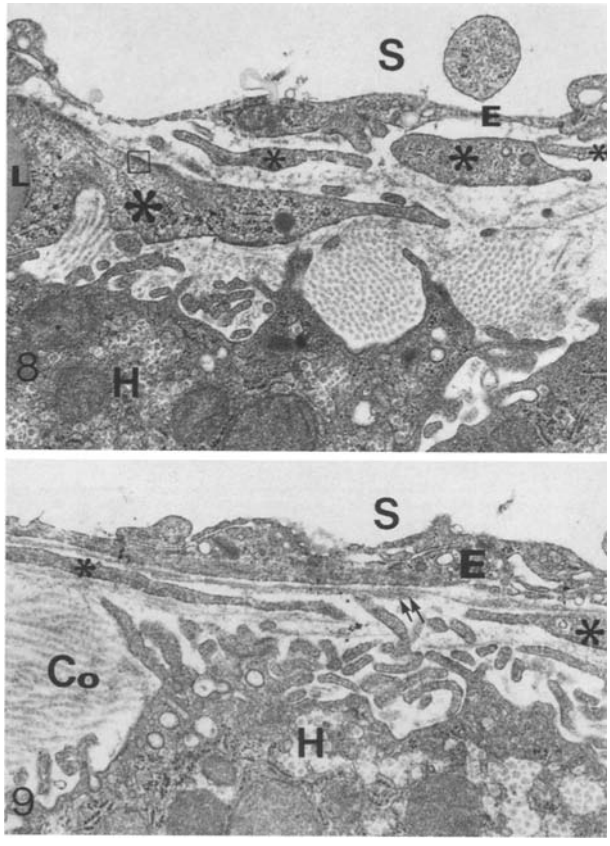

Fig. 8. Electron microscopy (patient n° 2). Beneath the endothelial wall (*E*) there are several short fragments of perisinusoidal cells (*asterisks*); one of these, containing a lipid (*L*), has numerous microtubules and microfilaments. Some filaments are condensed below the plasma membrane (*empty square*). *H*: hepatocyte, *S*: sinusoid. ×24600

Fig. 9. Electron microscopy (patient n° 2). In between the endothelial wall (*E*) and the processes of perisinusoidal cells (*asterisks*), a thick fragment of basement membrane-like material is visible (*double arrow*). *H*: hepatocyte, *Co*: collagen, *S*: sinusoid. ×15400

their collagen excretion (Castro-Malaspina and Moore 1982); PF4 is a potent inhibitor of collagenases (Hiti-Harper et al. 1978).

A TP requiring splenectomy is usually associated with massive platelet destruction in the liver (Aster and Reene 1969). It is therefore possible that, in this disease, excess fibrosis could be linked to PDGF and PF4. Other mechanisms could also be involved: a) phagocytosis of platelets activates macrophages, which then synthesize free oxygen radicals, toxic for the liver (Arthur et al. 1986). b) It has recently been shown that activated Kupffer cells control the proliferation of fibroblasts through a secretory factor (Rojkind M and Valadez 1985). c) Finally, TP, whether idiopathic or related to systemic lupus, is an immune disease; in such situations, various factors can activate collagen synthesis, such as lymphokines and monokines (Nouri-Aria et al. 1986).

If the above mechanisms are really involved in fibrogenesis, one might expect the list of diseases associated with PSF to be much longer. For the moment, preliminary results from our laboratory have shown that PSF can also be found in some cases of lymphomas (unpublished observation).

The origin of collagen deposition in TP is unknown. All hepatic cells are capable of synthesizing collagens (Clément et al. 1985). However, perisinusoidal cells are also good candidates. In the present study, there were some indications of their transformation into fibroblasts and myofibroblasts, cells known to synthesize components of the extracellular matrix.

The absence, as yet, of documented hepatic fibrosis in TP is surprising (Diebold and Camilleri 1974), but may have several hypothetical explanations: 1. fibrosis is mild, and visible only by special techniques; 2. fibrosis occurs only in the severe forms requiring splenectomy; 3. fibrosis decreases after splenectomy and could therefore not be mentioned in autopsy records. Additional studies are clearly needed to correlate the severity of the disease and the intensity of fibrosis.

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