# Morphologic Changes in the Peritoneal Membrane of Patients with Renal Disease

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Abstract. This study examined the morphologic features of the parietal peritoneal membranes of 130 patients undergoing peritoneal dialysis (PD) and compared them with the features of the peritoneal membranes of normal individuals, uremic predialysis patients, and patients undergoing hemodialysis. The median thickness of the submesothelial compact collagenous zone was 50  $\mu$ m for normal subjects, 140  $\mu$ m for uremic patients, 150 µm for patients undergoing hemodialysis, and 270  $\mu$ m for patients undergoing PD (P < 0.001 for all versus normal subjects). Compact zone thickness increased significantly with the duration of PD therapy [0 to 24 mo, 180  $\mu$ m (n = 58); 25 to 48 mo, 240  $\mu$ m (n = 24); 49 to 72 mo, 300  $\mu$ m (n = 13); 73 to 96 mo, 750  $\mu$ m (n = 16); >97 mo, 700  $\mu$ m (n= 19)]. Vascular changes included progressive subendothelial hyalinization, with luminal narrowing or obliteration. These changes were absent in samples from normal subjects but were

Loss of peritoneal function is a major factor leading to treatment failure in peritoneal dialysis (PD) (1-3). Although the precise biologic mechanisms responsible for these changes have not been defined, it is widely assumed that alterations in peritoneal function are related to structural changes in the peritoneal membrane. There is accumulating, albeit indirect, evidence that continuous exposure to bioincompatible dialysis solution components and repeated episodes of bacterial peritonitis play major roles in the observed long-term changes in peritoneal function (ultrafiltration loss and increased solute clearance) (2,4-6). To date, however, the structure-function relationship has not been fully defined. Although a number of studies have identified various mesothelial, vascular, and interstitial changes in peritoneal morphologic features during PD, neither the factors responsible for these changes nor the time during which they develop has been identified (7-9). The changes observed include loss or degeneration of the mesothe-

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present in 28% of samples from uremic patients and 56% of biopsies from patients undergoing PD. In the PD group, the prevalence of vasculopathy increased significantly with therapy duration (P = 0.0001). The density of blood vessels per unit length of peritoneum was significantly higher for patients with membrane failure and was correlated with the degree of fibrosis (P = 0.01). For the first time, a comprehensive crosssectional analysis of the morphologic changes in the peritoneal membranes of patients undergoing PD is provided. The infrequency of fibrosis in the absence of vasculopathy suggests that vasculopathy may predispose patients to the development of fibrosis. This study provides a sufficiently large cohort of samples to allow structure-function relationships to be established, as well as providing a repository of tissue for further studies.

lium, submesothelial thickening (variously described as fibrosis or sclerosis), changes in the structure and number of blood vessels, and vascular basement membrane reduplication (10-16).

Previously published data suggested a strong causal relationship between peritonitis (its frequency and severity) and longterm loss of peritoneal membrane function (2,4). Those studies did not examine the relationship between functional changes and possible morphologic changes in the peritoneal membrane, however. In addition, a number of articles attempted to address the relationship between morphologic changes and specific clinical events. In an autopsy study, Rubin *et al.* (17) demonstrated that chronic changes in the peritoneal "serosa" were correlated with the number of episodes of peritonitis.

More recently, investigators have focused on changes within the peritoneal vascular bed, because it is presumed that changes in vessel density or morphologic features might directly affect membrane function (18). Honda *et al.* (7,8) observed (in a small number of samples) structural changes in venular walls and related those changes to ultrafiltration changes in the same patients. There was a correlation between decreased ultrafiltration and the appearance of vasculopathy, with the development of submesothelial fibrosis. Mateijsen *et al.* (9) demonstrated an increase in blood vessel density in the submesothelial zone among patients with peritoneal "sclero-

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sis," compared with control subjects. Vessel wall thickening and capillary dilation were also observed. More recently, increased expression of nitric oxide synthase (a putative surrogate marker of blood vessel density) was observed to be directly correlated with the duration of PD therapy (19).

There is thus significant evidence that changes occur in both the interstitial and vascular compartments of the dialyzed peritoneal membrane. Although it is likely that these changes are related to the duration of dialysis, to peritonitis, and perhaps to dialysis solution components, the exact relationships are poorly understood, as is the potential contribution of uremia.

The aim of this study was to establish a system whereby peritoneal biopsies could be collected in a standard reproducible manner, specifically to avoid collection- or fixation-induced artifacts. In addition, peritoneal function data were simultaneously collected from the same individuals. These samples allowed the precise structural changes in dialyzed peritoneal membranes to be characterized in a large number of well preserved samples, as well as allowing initial evaluation of some of the potential factors responsible for the observed structural alterations.

Our data provide evidence of both fibrotic and vascular changes being related to uremia and the duration of therapy. These changes are exacerbated among patients who experience PD complications while undergoing dialysis.

# **Materials and Methods**

#### Patients

Twenty centers from Europe and Japan participated in the study, with 63% of the samples being collected from Cardiff. Preliminary work was performed to establish the optimal conditions for collection, fixation, and specimen transportation. Detailed instructions were provided to each participating center, to ensure uniformity of sampling and to minimize fixation-related or artifactual changes in the specimens.

Biopsies were collected, during abdominal surgery, from four patient groups, as follows: (1) kidney donors (biopsies obtained during donor nephrectomies were classified as normal if there was no history of abdominal pathologic conditions or previous surgery); (2) uremic patients, defined as patients who underwent biopsies either at the time of insertion of the PD catheter or during kidney transplantation but who had never previously undergone dialysis; (3) patients undergoing hemodialysis (who had never undergone PD); and (4) patients undergoing PD. Surgery was undertaken for renal transplantation, because of some incidental abdominal condition, or because of a PD-related problem (excluding recent peritonitis), e.g., catheter repositioning, catheter replacement, or catheter removal because of membrane failure. Patients with membrane failure were defined as patients who could no longer continue PD because of altered solute transport. Approval for the study was obtained from local ethics committees, and all patients gave written informed consent.

#### Biopsy Collection and Processing

Samples of the parietal peritoneum were obtained in a standardized manner. Briefly, the peritoneum was exposed and a suture loop was inserted through the part of the peritoneum to be sampled. A loose knot was tied, to facilitate orientation during fixation. By using the suture to lift the peritoneum, an ellipse (approximately 2 cm in length and up to 5 mm in depth) that included the knot at one end was

excised. The sample was placed in Sorensen's phosphate buffer with 2% sucrose (SPBS), immediately pinned onto a silicone elastomer surface (Sylgard 184; Dow Corning, Barry, UK) with the mesothelial surface uppermost, and fixed with 0.2% gluteraldehyde/4% formal-dehyde in SPBS. After 24 h of fixation at room temperature, samples were washed and stored at 4°C in SPBS before processing.

Fixed samples were examined and dissected by using a stereomicroscope. Two pieces (5  $\times$  5 mm) were routinely processed for scanning electron microscopy by full dehydration through graded ethanol mixtures, critical-point drying, and sputter coating with gold. Specimens were viewed at 10 kV with a JEOL 840A scanning electron microscope (JEOL, Tokyo, Japan). Four pieces (5  $\times$  1 mm) were routinely processed for light microscopy and/or transmission electron microscopy by postfixation in uranyl acetate, partial dehydration through graded ethanol mixtures to 70%, infiltration with hard-grade LR White acrylic resin (Light Resin Co., Reading, UK), embedding in acrylic resin, and cold catalytic polymerization at 4°C (20). Semithin  $(0.35-\mu m)$  sections for light microscopy were stained with 0.5% toluidine blue for morphologic assessments and were stained by using a modification of the periodic acid thiocarbohydrazide-silver proteinate-silver enhancement (PATCH-SP-SE) method to facilitate observation of small blood vessels and capillaries (21).

#### Sample Analyses

Samples were assessed by light microscopy, using a standardized method, by an experienced histopathologist who was unaware of patient characteristics and clinical details. Normal human parietal peritoneum (Figure 1) is composed of a sheet of flat mesothelial cells, separated by a basement membrane from a thin compact zone of mature fibrous tissue containing collagen and scattered elastin fibers. Deep to this is looser connective tissue containing widely spaced collagen fibers, occasional spindle-shaped fibroblast-like cells, scattered mononuclear phagocytes, mature lymphocytes, and adipose tissue. Small blood vessels, lymphatic vessels, and nerves are present in this loose connective tissue zone, and some extend into the compact zone.

In assessments of the biopsies, attention was paid to the morphologic features of the mesothelial surface and the underlying interstitium. The integrity of the mesothelial cells was assessed, and any inflammation (acute or chronic) or foreign material was noted. The maximal thickness of the submesothelial compact zone (in micrometers) was measured in sections oriented perpendicular to the serosal surface (Figure 1). Fibrosis was defined as a submesothelial compact zone measuring >150  $\mu$ m (the highest value recorded for normal subjects).

Early experience indicated that many of the biopsies exhibited vascular abnormalities, manifested by varying degrees of subendothelial hyalinization affecting predominantly venules and small veins but sometimes arterioles. The hyaline material was periodic acid-Schiff stain positive, and in florid cases led to distortion and narrowing of the vascular lumen or even complete luminal obliteration (in which cases the hyaline material was sometimes finely calcified). The degree of this "hyalinizing vasculopathy" was subjectively graded according to the criteria presented in Table 1, as illustrated in Figure 2, and data for the worst lesion in each biopsy were recorded.

The reproducibility of grading was evaluated by two observers using 40 samples that had been selected by a third member of the study group to include the entire spectrum of vasculopathy. Interobserver agreement was moderate for weighted analyses ( $\kappa$  for agreement = 0.45) and good for unweighted analyses ( $\kappa$  for agreement = 0.64).



*Figure 1.* Morphologic features of the parietal peritoneal membrane. Well oriented parietal peritoneal biopsies from a normal individual (a) and a patient who had undergone peritoneal dialysis (PD) for 9 yr (b) are presented. Two zones can be observed, *i.e.*, a compact submesothelial collagenous band and a deeper loose adipose connective tissue that contains scattered collagen fibers, mononuclear cells, and blood vessels. The compact zone is markedly thickened in the sample from the patient undergoing long-term dialysis. Toluidine blue. Scale bar, 500  $\mu$ m.

Tab	ole i	l. Grad	ling of	vascul	lopati	hy

Grade	Description
1	Subendothelial hyaline material $<7 \ \mu m$ in thickness
2	Subendothelial hyaline material $>7 \ \mu m$ in
	thickness, without luminal distortion or narrowing
3	Luminal distortion or narrowing
4	Luminal obliteration

Because a significant number of obliterated blood vessels were observed in preliminary analyses, it was clear that the use of specific endothelial cell markers would not accurately establish the number of blood vessels. The PATCH-SP-SE method was therefore used, which allowed the identification of all vessels on the basis of their basement membranes or (when obliterated) their mural hyalinization. The density of blood vessels (including obliterated vessels) in PATCH-SP-SE-stained sections oriented perpendicular to the peritoneal surface was thus quantified and expressed as vessel numbers per length (in millimeters) of surface peritoneum.

#### Statistical Analyses

Data are expressed as the median and interquartile range (IQR) (25 to 75%). Data were analyzed by using SPSS 10 for Macintosh (SPSS Inc., Chicago, IL). Because the data were not normally distributed, nonparametric analyses were performed throughout.

#### Results

#### Patients

Peritoneal biopsies were collected from 212 individuals, including nine normal individuals, 25 uremic patients, 48 patients undergoing hemodialysis, and 130 patients undergoing PD. Age, gender, and the incidence of diabetes mellitus for these patient groups are detailed in Table 2.

The PD patients were further subdivided into group 4a (patients presenting at random for transplantation or incidental surgery) and group 4b (patients presenting for surgery for a PD-related problem or catheter replacement/repositioning and patients experiencing membrane failure). Patients with membrane failure exhibited loss of solute clearance (a number were diagnosed during laparotomy as having sclerosing encapsulating peritonitis).

#### Thickness of the Submesothelial Compact Zone

Figure 3 presents the thickness (median and IQR) of the submesothelial compact zone in the four groups of subjects (ranging from normal subjects to patients who had undergone PD for >8 yr). The median thickness ranged from 50  $\mu$ m (IQR, 25 to 135  $\mu$ m) in the normal group to 700  $\mu$ m (IQR, 460 to 1060  $\mu$ m) in the group that had undergone PD for >8 yr.

The median thickness values were 140  $\mu$ m (IQR, 60 to 220  $\mu$ m) for the uremic group and 150  $\mu$ m (IQR, 100 to 200  $\mu$ m) for hemodialysis patients who underwent biopsies either during the insertion of a PD cannula or at transplantation.

Because the data were not normally distributed, nonparametric statistical analysis was performed. Mann-Whitney analysis revealed significant thickening of the submesothelial compact zone in uremic patients (P = 0.011) and patients undergoing hemodialysis (P = 0.013), compared with normal subjects. Analysis of biopsies from PD patients demonstrated a significantly progressive increase in thickness up to 96 mo of PD (Kruskal-Wallis one-way ANOVA, P = 0.0001).

Further analysis of data for PD patients (Figure 4) demonstrated that the thickness of the submesothelial compact zone in group 4a (patients who underwent biopsies during transplantation or incidental surgery) was significantly less than that in group 4b (patients who underwent catheter-related surgery or exhibited membrane failure). The median thickness ranged from 180  $\mu$ m (IQR, 100 to 270  $\mu$ m) in the transplant group (n= 59) to 650  $\mu$ m (IQR, 400 to 1100  $\mu$ m) in the membrane failure group (n = 21). Mann-Whitney analysis demonstrated significant differences between the transplant group and the catheter problem group (P < 0.000), as well as between the incidental surgery group and the membrane failure group (P <0.000). No difference could be demonstrated between the submesothelial compact zone thickness of the catheter problem group and that of the membrane failure group (P = 0.13).

#### Vasculopathy

No vasculopathy was observed in biopsies from normal subjects. The overall prevalence of vasculopathy, irrespective of grade, among non-PD patients (n = 73) was 28%, which was similar to the value for the group that had undergone PD for up to 24 mo (29%). The prevalence increased as the duration of PD increased, to a value of 89% for patients who had undergone PD for >72 mo (Figure 5).

Vasculopathy was graded from 1 to 4 (complete obliteration of the vessel lumen) (Figure 2). Table 3 presents the relationship between the duration of PD and the grade of vasculopathy. It is clear that not only the prevalence of vasculopathy but also the severity of the vascular changes increased with the duration of PD. Among patients who had undergone PD for <24 mo, 71% exhibited no vasculopathy, 27% exhibited grade 1 or 2 vasculopathy, none exhibited grade 3 vasculopathy, and 2% exhibited grade 4 vasculopathy. In contrast, of the patients who had undergone dialysis for >72 mo, 71% exhibited grade 3 or 4 vasculopathy.

The data were also analyzed on the basis of sample origin (Table 4). Of the biopsies obtained during surgery performed

Figure 2. Morphologic features of blood vessels in the parietal peritoneum. Transverse sections of small peritoneal blood vessels, showing grades of hyalinizing vasculopathy (Table 1), are presented. (a) The subendothelial hyaline zone is slender (up to 7  $\mu$ m), corresponding to grade 1. (b) The subendothelial zone is > 7  $\mu$ m, but the lumen is not distorted or narrowed, corresponding to grade 2. (c) The lumen is distorted or narrowed, corresponding to grade 3. (d) The vascular lumen is occluded by connective tissue containing fine calcific stippling, corresponding to grade 4. Toluidine blue. Scale bar, 10  $\mu$ m.

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	Donor	Uremic	HD	PD	
Age (yr) <sup>b</sup> Diabetes mellitus (Y:N) Gender (M:F)	50 (44 to 57) 0:9 5:4	43 (38 to 58) 5:20 19:6	48 (40 to 58) 5:43 37:11	50 (40 to 60) 18:112 87:41 <sup>c</sup>	

Table 2. Demographic characteristics of individuals from whom biopsy samples were collected<sup>a</sup>

<sup>a</sup> HD, hemodialysis; PD, peritoneal dialysis.

<sup>b</sup> Median and interquartile range.

<sup>c</sup> Two values missing.

for transplantation or some incidental reason, 52% exhibited no vasculopathy and only 7% exhibited grade 3 or 4 changes. In contrast, of the biopsies obtained from patients with membrane failure, 74% exhibited either grade 3 or 4 vasculopathy.

The relationship between fibrosis (submesothelial compact zone, >150  $\mu$ m; >2 SD) and vasculopathy was assessed by  $\chi^2$  analysis of data for all biopsies (P < 0.0001) (Table 5). Of the patients with vasculopathy, 77 (84%) exhibited significant fibrosis and only 14 did not. Of those without vasculopathy, only 54 (44%) exhibited a similar degree of fibrosis.

#### General Morphologic Features

Surface mesothelium was present in 134 biopsies and absent in 78. Among PD patients, 64 biopsies (49%) exhibited no mesothelium; among non-PD patients, surface mesothelium was absent in 14 of 82 biopsies (17%). For biopsies from PD patients, there were correlations between the loss of surface mesothelium, thickening of the submesothelial compact zone (P = 0.01, Spearman's test), and the presence of vasculopathy (P = 0.01). Twenty-five of 66 biopsies from PD patients with an intact mesothelium exhibited "reactive" changes (cellular





*Figure 3.* Changes in the submesothelial compact zone with biopsy origin and with PD duration. The thickness of the submesothelial compact zone (in micrometers) was measured in biopsies from normal individuals, uremic patients, patients undergoing hemodialysis (HD), and patients undergoing PD, grouped according to the duration of dialysis. Data are presented as box plots, with the boxes representing the interquartile range (IQR). Lines extend from the box to the highest and lowest values, excluding outliers. The median value is represented by the thick line across each box. \*, Statistical comparisons made using the Mann-Whitney U test. \*\*, Statistical comparison made using Kruskal-Wallis one-way ANOVA.  $\circ$ , outliers;  $\Box$ , extremes.

*Figure 4.* Changes in the submesothelial compact zone with biopsy origin. Patients undergoing PD were subdivided into two groups according to the surgical origin of the biopsy. Patients whose biopsies were obtained at the time of renal transplantation or during incidental abdominal surgery (group 4a) were compared with individuals whose biopsies were obtained either during surgery related to PD or at the time of catheter removal because of membrane failure (group 4b). Data are presented as box plots, with the boxes representing the IQR. Lines extend from the box to the highest and lowest values, excluding outliers. The median value is represented by the thick line across each box. \*, Statistical comparisons made using the Mann-Whitney *U* test.  $\circ$ , outliers.



*Figure 5.* Prevalence of vasculopathy according to the duration of PD. The presence of vasculopathy was identified in biopsies from predialysis patients, and values were compared with those for PD patients subgrouped according to the duration of PD.

enlargement with epithelioid morphologic features, increased nuclear/cytoplasmic ratios, cytoplasmic basophilia, or vacuolation) in the mesothelial cells. Inflammation was present in only 16 biopsies from the PD patient groups (12%). This inflammation was characterized as acute (defined as the presence of neutrophils, eosinophils, or mast cells) in four biopsies and chronic (defined as increased numbers of lymphocytes or mononuclear cells or the presence of plasma cells or lymphoid aggregates) in 12. There was no relationship between the presence of inflammatory cells and increased thickness of the compact zone. No foreign material or granulomatous inflammation was identified in any of the biopsies examined.

#### Diabetes Mellitus and Vasculopathy

In view of the morphologic similarity between the observed vasculopathy in the peritoneal membrane and diabetic vasculopathy, the data were analyzed with respect to clinical diabetes mellitus. The number of samples obtained from diabetic patients was small (n = 28). Of the biopsies obtained from predialysis diabetic patients, four of 10 exhibited vasculopathy. In the nondiabetic predialysis group, 14 of 63 patients (22%) exhibited vasculopathy. Among diabetic PD patients, 39% (seven of 18 patients) exhibited vasculopathy. Of 112 nondiabetic PD patients, 66 (59%) exhibited vasculopathy.

## Vessel Density

One hundred two biopsies were analyzed for vessel density per length of surface peritoneum. There were no significant differences in vessel density for any of the patient groups and, in particular, no significant change with the duration of PD (Figure 6A). When the samples were grouped according to the biopsy origin (Figure 6B), samples obtained from patients with membrane failure demonstrated significantly more vessels per length than did samples from normal individuals (P = 0.018) or from non-PD patients (P = 0.035). When samples were analyzed according to whether they demonstrated fibrosis (>150 µm), the biopsies with fibrosis exhibited significantly greater vessel density, compared with the samples without fibrosis (P = 0.01).

# Discussion

The median thickness of the submesothelial compact zone of the parietal peritoneal membrane in normal biopsies was 50  $\mu$ m. Our findings differ from the results of an earlier small study in which "simple sclerosis" was said to be present if the submesothelial layer was >20  $\mu$ m in thickness (17). More recently, a comprehensive review of studies in this field (22) emphasized that the thickness of the submesothelial tissue in simple sclerosis did not exceed 40  $\mu$ m. Indeed, the concluding sentence of that article indicated that PD should be suspended if the submesothelial layer was >40  $\mu$ m in thickness. In complete contrast was a separate study in which the thickness of the normal peritoneal membrane was recorded as 327  $\mu$ m (23). Those biopsies were, however, obtained from patients undergoing elective surgery, and it is impossible to rule out the possibility of coexistent intraperitoneal inflammation. This wide variation in observed thicknesses also highlights possible variability in sampling and the need to obtain biopsies in a standardized way.

Predialysis uremic patients demonstrated a significantly thicker submesothelial compact zone, compared with that observed in parietal peritoneal membranes from normal individuals. The thickness was similar to that in biopsies obtained from patients who had undergone hemodialysis for varying periods before the initiation of PD. These findings indicate that uremia itself may induce changes in the peritoneal membranes of patients before they commence PD, which may represent changes related to chronic humoral inflammation (24).

The thickness of the compact zone in biopsy samples obtained from patients undergoing PD demonstrated a progressive significant increase in thickness with duration of PD. Our results are in contrast to those of a recent smaller series, in which the thickening of the submesothelial zone in PD patients (492 to 266  $\mu$ m) was not different from that in uremic predialysis patients (492 ± 207  $\mu$ m) (23).

The major limitation in the collection of peritoneal biopsy samples has been access to the peritoneum. Almost all previous studies have been limited to (1) biopsies obtained during catheter placement, (2) biopsies obtained during catheter removal, or (3) biopsies obtained during incidental laparotomy (applicable to hemodialysis and predialysis patients). Although this approach would result in the gradual acquisition of large numbers of biopsies, it would tend to skew the results away from "nonproblematic" peritoneal samples. We overcame this problem by collecting biopsies at the time of kidney transplantation (random collection).

When the data in this study were subdivided according to biopsy origin, submesothelial compact zone thickness was

	No. of Patients				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Donor $(n = 9)$	9 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Uremic $(n = 25)$	17 (68%)	1 (4%)	5 (20%)	2 (8%)	0 (0%)
HD $(n = 48)$	38 (79%)	8 (17%)	1 (2%)	1 (2%)	0 (0%)
PD, 0 to 24 mo $(n = 58)$	41 (71%)	10 (17%)	6 (10%)	0(0%)	1 (2%)
PD, 25 to 48 mo $(n = 24)$	9 (38%)	1 (4%)	11 (46%)	1 (4%)	2 (8%)
PD, 49 to 72 mo $(n = 13)$	3 (23%)	1 (8%)	2 (16%)	4 (31%)	3 (23%)
PD, >72 mo $(n = 35)$	4 (11%)	2 (6%)	3 (9%)	3 (9%)	23 (66%)

Table 3. Degree of vasculopathy in different biopsy groups<sup>a</sup>

<sup>a</sup> HD, hemodialysis.

Table 4. Degree of vasculopathy in biopsies according to surgical origin

	No. of Patients				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Renal transplantation $(n = 59)$	32 (53%)	11 (19%)	12 (22%)	1 (2%)	3 (5%)
Incidental abdominal surgery $(n = 12)$	6 (50%)	1 (8%)	3 (25%)	0 (0%)	2 (17%)
Surgery related to dialysis problems $(n = 38)$	15 (41%)	2 (6%)	6 (18%)	4 (8%)	11 (26%)
Membrane failure ( $n = 21$ )	4 (21%)	0 (0%)	1 (5%)	3 (16%)	13 (58%)

significantly greater among patients who had undergone surgery to treat PD-related problems or who had experienced membrane failure, compared with PD patients whose biopsies had been obtained at random. This finding was irrespective of therapy duration. We also examined the relationship between the duration of dialysis and membrane thickness in these same patient groups during the first 7 yr of dialysis. Patients whose biopsies were obtained at random demonstrated no correlation between thickness and the duration of dialysis. In contrast, among patients with a history of PD-related problems or membrane failure, there was a direct significant relationship between thickness and the duration of PD. This indicates that thickening of the membrane is not inevitable for all individuals undergoing PD.

A variety of vascular changes have been observed in the peritoneal membranes of patients undergoing PD, resembling changes observed in diabetic microvascular disease. These changes include reduplication of capillary basement membranes (25,26), expansion of extracellular matrix within the

*Table 5.*  $\chi^2$  analysis of the relationship between vasculopathy and fibrosis<sup>a</sup>

	Incidence (%)		
	Fibrosis	No Fibrosis	
Vasculopathy No vasculopathy	77 (56.2) 54 (74.8)	14 (34.8) 67 (46.2)	

<sup>a</sup> Numbers in parentheses indicate expected values.

media of arterioles (27), and deposition of type IV collagen within the arterial wall (28). In addition, most of these changes are accompanied by the deposition of advanced glycosylation end products in the vessel wall (7,29,30). In this study, overt vasculopathy was present in 20% of the biopsies obtained from uremic non-PD patients. The proportion of patients with vasculopathy then increased with the duration of PD, so that, after 6 yr of therapy, 87% of biopsies exhibited evidence of vasculopathy. When the results were grouped according to biopsy origin, the severity of vasculopathy was significantly less in the random samples. Previous studies identified a link between the loss of ultrafiltration and the degree of vasculopathy and also proposed that the vascular changes might be related to the deposition of advanced glycosylation end products in the vessel wall (7).

The presence or absence of mesothelium on the surface of a peritoneal biopsy has often been taken to indicate underlying pathologic conditions (31). In view of the fragility of the mesothelial layer, we were meticulous in the development of our techniques, to minimize the possibility of trauma to the specimens. Thirty-six percent of the biopsies in this study were devoid of surface mesothelium. This finding was more common among the PD population (49%) than among patients who had never been exposed to PD (17%). Importantly, the absence of the mesothelium among PD patients was correlated with the presence of fibrosis, as well as with vasculopathy, although there were a number of specimens with fibrosis/vasculopathy and an intact mesothelium. It is therefore likely that submesothelial changes precede the loss of the mesothelium and that the loss of the mesothelium may be related to local ischemia.



*Figure 6.* (A) Blood vessel density according to the duration of PD therapy. Blood vessels were counted per surface length (in millimeters) of biopsy. Biopsies from normal individuals, from uremic and hemodialysis patients who had not undergone PD, and from patients undergoing PD (grouped into 24-mo bands) were compared. Data are presented as box plots, with the boxes representing the IQR. Lines extend from the box to the highest and lowest values, excluding outliers. The median value is represented by the thick line across each box. None of the groups demonstrated a significant increase in vessel number.  $\circ$ , outliers. (B) Blood vessel density according to the biopsy collection origin. Data are presented as box plots, with the boxes representing the IQR. Lines extend from the box to the highest and lowest values, excluding outliers. The median value is represented as box plots, with the boxes representing the IQR. Lines extend from the box to the highest and lowest values, excluding outliers. The median value is represented by the thick line across each box. \*, Statistical comparisons made using the Mann-Whitney U test.  $\circ$ , outliers.

Interestingly, however, there was little evidence of cellular inflammation (acute or chronic) in any of the biopsies. In particular, there did not seem to be any evidence of chronic cellular inflammation in samples with fibrosis.

It is likely that peritoneal fibrosis is of multifactorial pathogenesis and that both inflammation and dialysis solution exposure contribute to it. Recent studies demonstrated that effluents from PD patients contained increased levels of latent transforming growth factor- $\beta$ 1 and that these increases were related to increased local production, possibly from mesothelial cells, after glucose exposure (32). Transforming growth factor- $\beta$ 1 has been identified as a key growth factor in the development of interstitial fibrosis in diabetic nephropathy (33). In our study, analysis of the relationship between vasculopathy and fibrosis demonstrated an overwhelming positive correlation between the two, suggesting a causal relationship. The results suggest that fibrosis is at least partly dependent on the presence of vasculopathy. The vasculopathy may lead to relative ischemia, exacerbating the development of fibrosis (34).

Among the small number of biopsies from diabetic patients, there did not seem to be an increased incidence of vasculopathy. This finding is supported by previous reports indicating that vascular changes in the gastrointestinal tract of diabetic patients are unusual (35). That observation suggests that vasculopathy among diabetic patients is organ related (35) and that the extent of the changes observed in the membranes of PD patients may be a unique development specifically driven by the process of PD and/or uremia.

Recent studies suggested that, in addition to the development of vasculopathy, changes in the vascular bed of the peritoneal membrane included the growth of new blood vessels or neoangiogenesis (9), which was thought to be particularly marked among patients described as having peritoneal sclerosis. The mechanism of such a change could be the increased deposition of advanced glycosylation end products in the membrane, resulting in increased release of vascular endothelial growth factor (36). This would, in turn, result in angiogenesis and increased vessel permeability. Much of the current data on angiogenesis, however, are derived from animal studies, in which there is rapid development of new blood vessels in the visceral peritoneum after exposure to glucose (37). Such results should be treated with caution when human structurefunction relationships are being considered.

In this study, we used a variation of the periodic acid-Schiff staining technique to identify vessels. Because endothelial markers do not identify obliterated vessels or vessels with damaged endothelium, we used a technique that would identify and thus allow enumeration of all vessels on the basis of their basement membranes. An additional problem was how to analyze vessel numbers. Most studies have recorded numbers per high-power field (9,19,23). With the variability in membrane thickness (20 to 1200 µm), however, it would be impossible to make valid comparisons between specimens. We therefore elected to express vessel numbers per millimeter of peritoneal surface, which would take into account the total number of vessels irrespective of the thickness of the tissue. With this form of analysis, a significant increase in vessel number was observed only in biopsies obtained from patients with membrane failure and not in biopsies obtained at random. Furthermore, when samples with fibrosis were compared with those without fibrosis, the former samples exhibited a significantly greater number of vessels.

This study provides, for the first time, a comprehensive

analysis of the morphologic changes that occur in the parietal peritoneal membranes of patients undergoing PD. It is, by necessity, cross-sectional, because a longitudinal study would be impractical with current biopsy methods. Nevertheless, our study clearly illustrates the wide variation in peritoneal morphologic features among patients and emphasizes the need to base conclusions on large sample numbers. It demonstrates that some of the changes predate PD and are present among uremic patients. It also demonstrates that, in the first 5 yr of dialysis, patients who do not experience problems with PD do not develop increased thickening of the submesothelial compact zone and do not develop significant vasculopathy. This study provides a sufficiently large cohort of samples to allow structure-function relationships to be established, as well as providing a repository of tissue for a variety of additional studies.

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