30% had normal renal structure. 40% had more advanced tubulo-interstitial and/or vascular lesions and strating the heterogeneity of renal structure in Type 2 diabetic patients. In electron microscopy were similar to those by light microscopy, demon-
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pansion is the main variable, whereas further interstitial expansion does not occur. A large number of Type 2 patients were also studied. Early
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fact, only 30% of MA patients had typical diabetic glomerulopathy, while 40% had more advanced tubulo-interstitial and/or vascular lesions and 30% had normal renal structure.

**SUMMARY** - Structural changes underlying diabetic nephropathy in Type 1 diabetes are predominant in the glomerulus (thickening of glom-
erular basement membrane (GBM) and mesangial expansion), but also include arteriolar, tubular and interstitial lesions. The structural measure that correlates best with all renal functional parameters in Type 1 diabe-
tes is mesangial fractional volume [Vv(mes/glom)], an estimate of mesan-
gial expansion. Structural-functional relationships in Type 2 diabetes are much less known. These studies investigated renal structure in the early
stages of nephropathy [microalbuminuria (MA)] in patients with Type 1 and Type 2 diabetes. Diabetic glomerulopathy was quite advanced in
Type 1 diabetic patients with MA, and both Vv (mes/glom) and GBM width were increased as compared to normoalbuminuric (NA) patients when the albumin excretion rate (AER) was > 30 µg/min. Serialrenal biopsies were performed 5 years apart in 11 Type 1 diabetic patients to evaluate
whether glomerular and interstitial lesions progress jointly. AER in-
creased significantly in 5 years, while the glomerular filtration rate re-
mained unchanged. All structural parameters were initially abnormal. Vv(mes/glom) and mean glomerular volume increased significantly, whereas GBM width and the interstitial volume fraction were un-
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**RÉSUMÉ** - Progression de l’atteinte anatomique dans la néphropa-
tie du diabète de type 1 et de type 2. Les anomalies structurales soutendant la néphropathie du diabète de type 1 impliquent surtout le glomérule (épaississement de la membrane basale (MBG) et expansion mésangiale) mais aussi des lésions artério-
laire, tubulaires, et interstitielles. La mesure structurale la mieux corré-
lée avec tous les paramètres fonctionnels rénaux est le volume fraction-
nel mésangial [Vv (mes/glom)] qui estime l’expansion mésangiale. Les relations structure/fonction sont moins bien connues pour le diabète de
type2. Nous avons analysé la structure rénale aux stades précoces mi-
croalbuminurniques de la néphropathie chez des diabétiques de type 1 ou
2. La glomérulopathie était assez évoluée chez les diabétiques de type microalbuminurniques, et le Vv (mes/glom) et l’épaississement de la MBG
étaient augmentés par rapport à des patients normoalbuminuriques avec
albuminurie (ERA) >30 µg/min. Des biopsies rénales séquentielles à 5 ans
d’écart ont été réalisées chez des diabétiques de type 1 pour savoir si les
lésions glomérulaires et interstitielles évoluaient ensemble. L’ERA, mais
pas la FGR, augmentait significativement en 5 ans. Tous les paramètres
structuraux étaient anormaux lors de la première biopsie. Le Vv (mes/ glom) et le volume glomérulaire moyen augmentaient significativement.
En revanche, la MBG et le volume interstitiel ne changeaient pas en 5
ans . De plus, le changement du Vv (mes/glom) était corrélaté à celui de
l’ERA (r = 0,64, p < 0,05). Donc, au stade d’évolution vers une microalbumi-
minurie ou une protéinurie, l’expansion mésangiale continue est la varia-
bles principale, alors qu’il ne se produit pas d’expansion interstitielle. Chez
des diabétiques de type 2, une glomérulopathie précoce était détectable
en microscopie électronique (ME) chez les patients normoalbuminurni-
ques, et était plus évoluée chez les patients microalbuminurniques ou pro-
téinuriques (ces lésions étant plus faibles que dans le diabète de type 1). Ces
études en ME, en cohérence avec les études en microscopie opti-
què, démontrent l’hétérogénéité de la structure rénale au cours du dia-
bète de type 2: seuls 30 % des patients microalbuminurniques ont une
glomérulopathie typique, 40 % ayant des lésions tubulo-interstitielles
et/ou vasculaires plus avancées et 30 % présentant une structure nor-
male.

**Mots-clés :** structure rénale, diabète, microscopie, membrane basale glomérulaire, volume fractionnel mésangial.
D isabilities and mortality caused by end-stage renal disease (ESRD) are important health problems in Western countries. Diabetic nephropathy is currently the most common cause of ESRD in the United States, Japan and Europe, and there has been a dramatic increase in the proportion of ESRD patients affected by diabetes in recent decades [1-4]. Between 1982 and 1992, the number of patients with diabetic ESRD rose from 27% to 36% in the United States and from 11% to 17% in Europe. The rate of increase of ESRD in patients with diabetes has been much more rapid than that of ESRD from other causes. The majority of diabetic patients with ESRD have Type 2 diabetes. In the past, there was confusion regarding the classification of diabetes among ESRD patients because many on insulin treatment were also classified as Type 1 when they were affected by Type 2 diabetes, and 40% of Type 2 diabetic patients received insulin. Thus, the proportion of ESRD patients with Type 1 diabetes was overestimated. In Europe, Type 2 diabetes accounts for 43% of diabetic ESRD, and it has been estimated that 67% of Italian diabetic patients with ESRD have Type 2 diabetes [1, 4]. In the USA over 80% of diabetic patients with ESRD have Type 2 diabetes. Although Type 2 diabetes is the most common cause of ESRD, the majority of studies on renal morphology have been performed in patients with Type 1 diabetes. The clinical manifestations of diabetic nephropathy, proteinuria, decreased glomerular filtration rate and increasing blood pressure are similar in Type 1 and Type 2 diabetes [5-7], but the spectrum of renal lesions is not the same, and some differences in the clinical course of renal disease are apparent. For example, proteinuria or microalbuminuria is rarely present at the onset of Type 1 diabetes, whereas several patients have abnormal albumin excretion rates at the time of diagnosis of Type 2 diabetes [8, 9]. Although this may be explained by a long period of undiagnosed hyperglycaemia, it is also possible that the structural changes underlying abnormalities in albumin excretion rate differ in Type 1 and Type 2 diabetes. Some time ago, the risk of progression to proteinuria in microalbuminuric Type 1 diabetic patients was estimated to be about 80% [10-13]. More recently, some prospective studies have suggested that the risk of progression from microalbuminuria to proteinuria was about 40% after 5 to 6 years of follow-up [17-22]. The risk of overt nephropathy over a longer period of follow-up is not known, but presumably greater.

RENAL STRUCTURE IN TYPE 1 DIABETES

Glomerulopathy is the most important structural change in Type 1 diabetic patients, characterised by thickening of the glomerular basement membrane (GBM) and mesangial expansion, leading to progressive reduction in the filtration surface of the glomerulus [23-26]. Although the most important structural changes occur in the glomeruli [23-26], concomitantly and proportionally to the degree of glomerulopathy, the arterioles [27], tubules [28] and interstitium [29] also develop morphological lesions. Although all the pathologic findings of diabetes can be observed in other renal diseases, a constellation of lesions such as thickening of glomerular (GBM) and tubular basement membrane (TBM), mesangial expansion, Kimmelstiel-Wilson nodules, and arteriolar hyalinosis is unique to this disease and separable from all other renal disorders [23-25]. The first change that can be quantified is thickening of the GBM [30]. There is also thickening of the TBM [22] and Bowman’s capsule. Within a few years after onset of diabetes, afferent and efferent arteriolar hyalinosis can be noted. This can progress to the replacement of the smooth muscle cells of these vessels by hyaline material. This lesion and other “exudative” ones (hyaline deposits) in the subendothelial space (hyaline caps) and along the parietal surface of Bowman’s capsule (capsular drops) contain a variety of plasma proteins, especially immunoglobulins, complement, fibrinogen, albumin, etc. [31, 32]. Increases in the volume of cellular and matrix components of the mesangium can be detected in some patients as early as 5 to 7 years after onset of diabetes [25, 30]. Diffuse and generalised mesangial expansion is termed “diffuse diabetic glomerulosclerosis”. Nodular glomerulosclerosis consists of areas of marked mesangial expansion forming large round fibrillary mesangial zones with palisading of mesangial nuclei around the periphery of the nodules and extreme compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules). In most but not all instances, nodular glomerulosclerosis is seen with advanced diffuse mesangial changes. Many patients with clinical diabetic nephropathy (DN) have few or no nodular lesions. Additional abnormalities include global glomerular sclerosis (GS) [33] and interstitial expansion, especially in areas of tubular atrophy or GS [25]. Currently, our hypothesis is that the interstitial changes in DN follow from progressive glomerulopathy [26]. The critical lesion of DN that leads primarily to renal insufficiency in Type 1 diabetic patients is expansion of the glomerular...
mesangial [25]. The morphometric determination of mesangial expansion is mesangial fractional volume \([Vv (\text{mes/glom})]\) (the percentage of the glomerular tuft made up by mesangium); this is the structural parameter that best correlates with all functional parameters in Type 1 diabetes. A highly significant inverse correlation exists between glomerular filtration rate (GFR) and \(Vv(\text{mes/glom})\) [25]. As the mesangium expands, it ultimately restricts glomerular capillary luminal volume and diminishes filtration surface, which is closely related to GFR [34]. Although GS and capillary closure can also influence this course of events, GFR can be reduced in patients with little or no glomerular scarring [33]. \(Vv(\text{mes/glom})\) is also related to the presence of proteinuria and hypertension [25]. Thus, proteinuria, hypertension, and declining GFR (all manifestations of clinical DN) are related to mesangial expansion and necessarily to distortions in glomerular capillary architecture. Percent of global sclerosis [33] and interstitial expansion [29] are also correlated with the clinical manifestations of DN, and are somewhat independent variables of mesangial expansion in relation to proteinuria, hypertension, and declining GFR. However, the exact contribution of each of these lesions to the renal dysfunction of DN is difficult to determine. Current studies in Type 1 diabetes suggest that progression from normoalbuminuria (NA) to microalbuminuria (MA), and from MA to overt DN, is related to the development of glomerular rather than interstitial pathology. Arteriolar lesions also progress, but there is no significant relationship between this progression and the emergence of early clinical diabetic renal disease [26].

The thickness of GBM is not related to GFR or to the presence of hypertension, although there is a direct relationship between albumin excretion rate (AER) and GBM thickness. AER is related to all glomerular parameters. Nonalbuminuric patients (NA) may have diabetic glomerulopathy of variable severity: those with low levels of microalbuminuria (MA) are comparable to NA patients for renal structure, while those with an AER > 45 mg/24 h generally have more serious diabetic renal injury, more commonly associated with features of overt DN including hypertension and reduced GFR [25]. In fact, MA in the predictive range is a marker of advanced glomerular injury in Type 1 diabetes, often bordering on the severity usually associated with overt DN. Clinical DN is always associated with advanced diabetic glomerular pathology in Type 1 diabetic patients [25]. Nonetheless, patients with long-standing Type 1 diabetes and normal AER can have quite advanced diabetic renal lesions. Finally, studies of serial biopsies and renal function conducted 5 years apart showed that NA patients with established lesions tend to progress to MA in association with progressive mesangial expansion and glomerular enlargement [26]. These studies, which found no progressive thickening of GBM or any increase in interstitial expansion during the same time period, suggest that continuing mesangial expansion is the crucial structural change at the disease stage in which some patients are in transition from NA to MA.

Changes in podocytes have also been considered important in determining altered glomerular permeability to proteins. Bjornt et al. found that the foot process width (FPW) of podocytes on peripheral basement membrane was increased in MA Type 1 diabetic patients as compared to NA patients, and that the width of filtration slits in NA- and MA diabetic patients was greater than in non-diabetic control subjects. Filtration slits were correlated with GFR in all diabetic patients. The authors concluded that changes in podocytes and filtration slits in Type 1 diabetic patients with nephropathy already occur at the microalbuminuria stage [35]. In contrast, Ellis et al. found that significant disturbances of epithelial cell structure occurred only in patients with Type 1 diabetes and overt proteinuria, and that there were no differences between patients with microalbuminuria and those with normal AER for foot process width and filtration slit length density. However, when all diabetic patients were considered, these parameters were correlated with AER [36].

Another possible mechanism accounting for albuminuria in diabetic glomerulopathy is the loss of the charge-selective barrier. In fact, the passage of plasma proteins larger than 70 kd across the GBM is believed to be normally restricted not only by the size-selective barrier but also by a charge-selective barrier. This is thought to be mainly due to polyanionic glycans in the GBM, which restrict the passage of small polyanionic plasma proteins (primarily albumin). For example, studies have revealed that the defect in minimal-change glomerulopathy results mainly from a loss of charge selectivity [37]. Vernier et al. found that heavy proteinuria in Type 1 diabetes was associated with a decrease in the number of anionic charge sites in the lamina rara externa due to a decrease of heparan sulphate proteoglycans. As the number of anionic charge sites was similar in NA and MA patients, there was no evidence that loss of heparan sulphate proteoglycans is a mechanism for early microalbuminuria [38].

## Renal Structure in Type 2 Diabetes

The pioneer paper by Kimmelstiel and Wilson in the field of renal pathology in diabetes clearly indicates that patients with Type 2 diabetes may develop glomerulopathy [39]. In Type 1 diabetes, non-diabetic renal diseases are rare, whereas the prevalence of non-diabetic renal lesions has been reported to be high.
Les données quantitatives morphométriques sont rares. Dans un groupe de patients Type 2, les auteurs ont observé des variations morphométriques similaires à celles observées dans le groupe Type 1 [46]. Cependant, plus récentes études suggèrent un léger excès d’atteinte glomérulaire normale chez les patients Type 2 [47]. Chez les patients Type 2 avec microalbuminurie, Ostertby et al. [48] ont trouvé que toutes les paramètres morphométriques étaient sur le plan normal, bien que certains patients aient eu une structure glomérulaire dans la gamme normale. De l’autre côté, dans le groupe Type 1, les patients ayant une néphropathie, la structure glomérulaire était toujours fortement altérée. Une nouvelle étude a montré que chez un groupe de patients Type 2, les patients avec microalbuminurie avaient une structure glomérulaire présente qui s’étendait tout au long de la normale. Les podocytes étaient plus nombreux dans les patients Type 2 que dans les patients Type 1, sans atteinte rénale stable. Le nombre de podocytes par glomérule était le plus élevé dans les patients Type 2, mais était inférieur dans les patients Type 1 [49].

Dans un groupe de patients Type 2 avec microalbuminurie, les auteurs ont observé une structure glomérulaire normale, bien que la structure mesangiale ait été plus altérée. Les patients Type 2 avaient aussi une structure glomérulaire donnant des résultats plus nets que les patients Type 1. Les podocytes étaient moins présents que dans les patients Type 1, mais étaient plus présents que dans les patients Type 2. Les podocytes étaient plus nombreux dans les patients Type 2 que dans les patients Type 1, sans atteinte rénale stable. Le nombre de podocytes par glomérule était le plus élevé dans les patients Type 2, mais était inférieur dans les patients Type 1 [49].

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pansion (as revealed by light microscopy), with or without tubulo-interstitial and arteriolar changes. On this basis, we proposed a classification system including 3 major groups:

**Category CI: Normal or near-normal renal structure.** These patients (41%) had biopsies which were normal or showed mild mesangial expansion, tubulo-interstitial changes, or arteriolar hyalinosis.

**Category CII: Typical diabetic nephropathy.** These patients (26%) had established diabetic lesions with an approximately balanced severity of glomerular, tubulo-interstitial and arteriolar changes. This picture was typical of that seen in Type 1 diabetic patients with obvious light microscopy DN changes.

**Category CIII: Atypical patterns of renal injury.** These patients (33%) had relatively mild glomerular diabetic changes despite disproportionately severe renal structural changes: (a) tubular atrophy, tubular basement membrane thickening and reduplication and interstitial fibrosis (tubulo-interstitial lesions); (b) advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels; and (c) global glomerular sclerosis. In the CIII group, these patterns were present in all possible combinations.

No cases of definable non-diabetic renal disease were found in this series of 53 patients.

Hyperglycaemia may cause different patterns of renal injury in older Type 2 as compared to younger Type 1 diabetic patients. Tubulo-interstitial and vascular changes could also be related to ageing, atherosclerosis, and systemic hypertension. However, hypertension, which was present in almost all patients in all three structural categories, cannot account per se for the different lesions observed in category III. Moreover, mean age was similar in patients in categories II and III (60 years), despite the different patterns of renal injury in both groups. Our observations in a large number of age-matched normal controls suggest that ageing is not a sufficient factor to account for most of the renal structural changes observed in CIII patients. Thus, it can be supposed that heterogeneity in renal structure may reflect the heterogeneous nature of Type 2 diabetes. However patients with “typical” DN lesions had longer known diabetes duration, worse metabolic control, and diabetic retinopathy in all cases (50% background, 50% proliferative). Conversely, none of the patients in categories CI and CIII had proliferative retinopathy, and background retinopathy was observed only in 50% of CI and 57% of CIII patients. In Type 1 diabetes, 28% of patients with MA had proliferative retinopathy, and 58% background retinopathy. This suggests that the different underlying pathophysiologic mechanisms responsible for Type 2 diabetes in these groups of patients may also underlie different renal pathophysiologic mechanisms or responses.

As MA is not associated with renal structural changes in a substantial subset of Type 2 diabetic patients, we considered the possibility that it might be due to *endothelial dysfunction*. Thus, plasma levels of von Willebrand factor (vWF), an endothelial-derived protein indicative of endothelial function, were measured in a group of MA patients undergoing kidney biopsy [54]. Contrary to our assumptions, vWF plasma levels in the 32 patients studied were significantly increased only in those with renal structural abnormalities (both allocated in category II-typical and category III-atypical patterns), but normal in those with normal renal structure (Category I) [54]. These studies suggest that there are two types of MA in Type 2 diabetes: one associated with increased vWF plasma levels, established renal structural lesions, and (frequently) diabetic retinopathy; and the other characterised by normal vWF plasma levels, normal renal structure, and no or mild diabetic retinopathy. Whether these two types of MA have a different prognostic impact in terms of end-stage renal disease and cardiovascular events deserves to be investigated in longitudinal studies.

Preliminary results on the course of renal function in these patients, involving repeated measurements of GFR over a follow-up of 4 years, suggest that glomerular structure has a strong impact on the course of kidney function. Those patients with more advanced diabetic glomerulopathy show a decline in GFR over time, whereas no significant changes in GFR occur in patients with no or mild glomerulopathy. Among 108 Type 2 diabetic patients studied (74 with MA and 34 with proteinuria), the number who showed a decline in GFR (“Progressor”) increased across quartiles of GBM width and Vv(mes/glom) [55].

This study also showed that AER and GFR at baseline, as well as mean blood pressure levels during follow-up, did not influence the change in GFR, whereas patients termed “Progressor” had the worst metabolic control and the odds ratio of being a “Progressor” increased across the quartiles of HbA1c [55].

Larger studies over a longer term are required to understand the clinical implications of these complex processes on renal structure and function in Type 2 diabetes.

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