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Split and Extremely Thin Glomerular Basement Membranes in Hereditary Nephropathy (Alport's Syndrome)

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Summary. Alterations of the glomerular basement membrane are the earliest ultrastructurally demonstrable lesions in the course of hereditary nephropathy. The basement membranes may be either focally or diffusely thickened. They may be (a) homogeneously broadened, (b) show a typically split lamina densa, or (c) show a focally or diffusely thinned lamina densa layer. The latter may be one third or one half of the normal thickness. These findings provide further evidence for the hypothesis of basement membrane alterations as the basic lesion in hereditary nephritis.

Introduction

Thickening of glomerular basement membranes (GBM) was reported as the earliest pathohistological alteration found during the course of renal deterioration in hereditary nephropathy (Kinoshita *et al.*, 1969; Kaufman *et al.*, 1970). Recently it has been demonstrated electron microscopically that the GBM thickening is due to a characteristic splitting of the lamina densa (Kinoshita *et al.*, 1969; Langer and Thoenes, 1971; Hinglais *et al.*, 1972; Spear and Slusser, 1972; Churg and Sherman, 1973). An occasional occurence of thin basement membrane segments was additionally reported by Spear and Slusser, 1972 and Grünfeld *et al.*, 1973. The significance of this finding, however, has never been investigated. This communication is concerned with the GBM ultrastructure in patients suffering from hereditary nephropathy and special attention was paid to the thin basement membranes and lamina densa layer.

Materials and Methods

Renal biopsy specimens obtained from ten patients from seven families with a family history of nephropathy (see below) were studied by light and electron microscopy, and two to four glomeruli were examined ultrastructurally in each case.

Light microscopy: Biopsy specimens were fixed in 4% formalin solution and embedded in paraffin. Stains used were H.-E., PAS-haemalum, Pearse, Goldner's trichrome.

Electron microscopy: Small tissue blocks were fixed in phosphate buffered (pH 7.2) 3% glutaraldehyde solution and embedded in Epon 812. Semithin sections were stained with silver methenamine, and ultrathin sections with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Family Data

Family LA

The probands were two brothers, 8 (Mi.) and 12 (Ma.) years of age, both of whom had constant hematuria detected by microscopy, slight or inconstant leukocyturia and moderate

proteinuria. Recurrent bouts of macroscopic haematuria were observed in patient Mi. Renal function tests were normal. Nephropathy was first detected when the boys were 4 months (Mi.) and 3 years (Ma.) old. Nerve deafness was striking clinically at the age of 4 years (Mi.) and 8 years (Ma.). Both the mother and grandmother suffered from chronic nephropathy of unknown nature.

Family MU

Patient J., a 5 year old boy, suffered from severe deafness of the neurosensory type, haematuria and albuminuria. The fundi of the eyes showed abnormal pigmentation. His sister P. and mother were both affected with persistent haematuria.

Family WO

The proband H. D. was a 16 year old boy, suffering from haematuria, proteinuria, and leukocyturia since he was 10 years old. Nerve deafness and ocular abnormalities were also present. Examination of his parents showed both had haematuria and nerve deafness. Two brothers of the proband were heatlhy.

Family GE

The probands, two sisters 5 and 7 years of age, were affected with constant microscopic haematuria, proteinuria and leukocyturia. Renal function tests, ophthalmological and audiometric examinations were normal. Their father, 35 years of age had suffered from nerve deafness since the age of 10, and from cataracts since he was 15. A clinical examination at the age of 31 revealed renal insufficiency, hypertension, microscopic haematuria, proteinuria and glycosuria. For the past year he had been on chronic intermittent home dialysis. The mother, 30 years of age, was in good health with no signs of renal disease, eye or ear disorders. The father's sister suffers from recurrent bouts of pyelonephritis, and an X-ray showed a stone in her right renal pelvis. Her audiometric examination was normal.

Family WE

The 15 year old male proband was deaf and affected with haematuria, proteinuria and leukocyturia since 10 years of age. His mother and one cousin died from renal failure.

Family DR

The 12 year old boy suffered from nerve deafness, haematuria, proteinuria, and leukocyturia. A great-grandmother, a grandmother and a sister of his mother had died from renal failure.

Family LO

The proband was a boy, 8 years of age, suffering from recurrent bouts of macroscopic haematuria since the age of 6, following an acute episode of sore throat and otitis media. Audiometric and opthalmological examinations were normal. The mother had a glomerulonephritic episode following a sore throat at the age of 22, and had slight proteinuria, oedema and persistent hypertension during both subsequent pregnancies at the age of 24 and 29. Two years previously she developed renal failure and a few weeks later she started intermittent dialysis. The father, 33 years of age, complained of diminished hearing since scarlet fever at the age of $2^1/_2$ years. A 3 year old sister of the proband showed microscopic haematuria. The maternal grandmother had pyelonephritis when she was a young girl and had recently suffered from diminished hearing and hypertension.

Results

Light Microscopy

In all patients except one, the glomeruli showed a slight degree of mesangial thickening due to increased amounts of PAS-positive matrix masses and slightly increased mesangial cell numbers (Fig. 1a). Sometimes a segmental accentuation

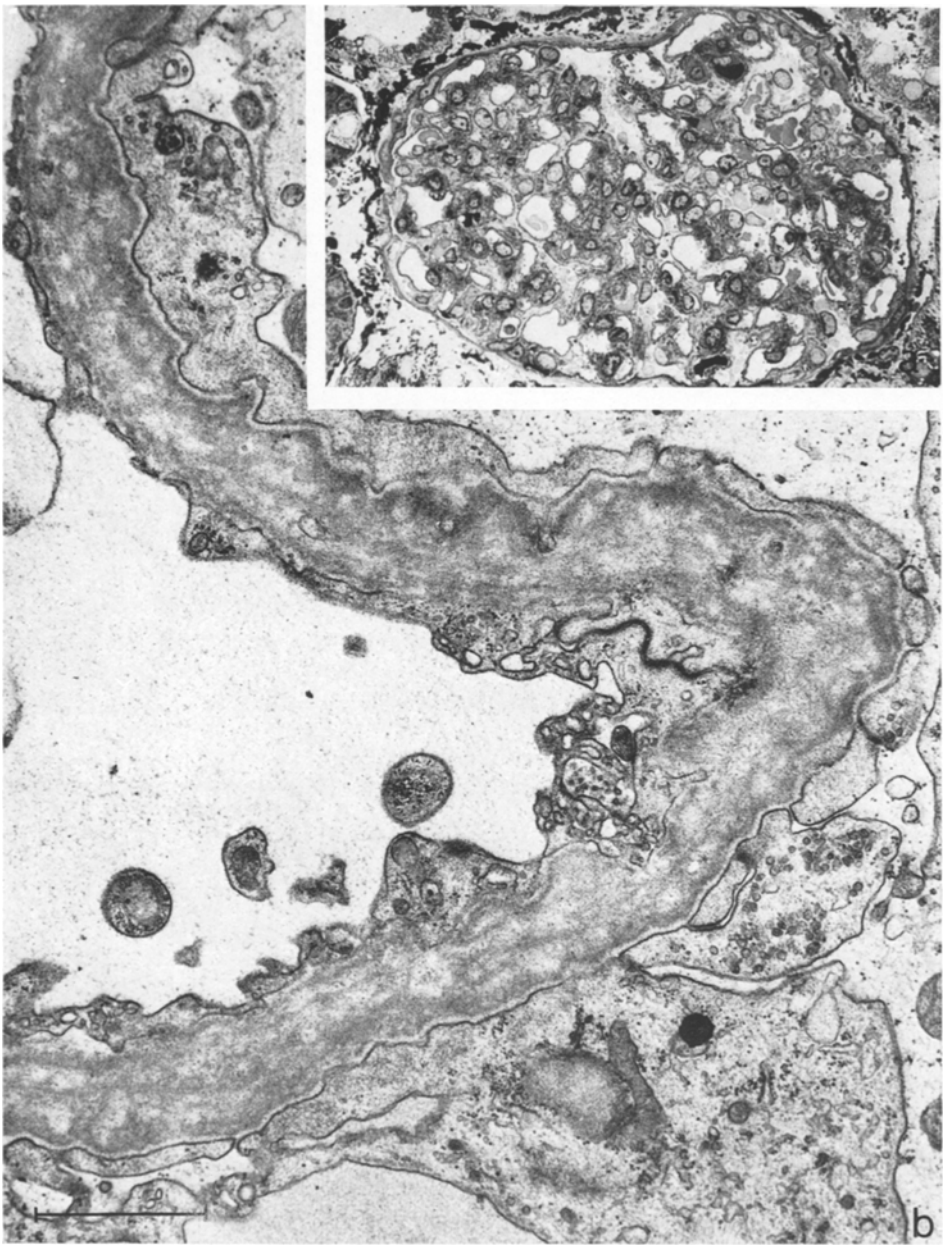

Fig. 1 a and b. Hereditary nephropathy family LA, patient Ma. (a) Glomerulus showing slight mesangial cell proliferation and slight increase in mesangial matrix. The peripheral capillary walls seem to be normal in structure. Semithin section, silver methenamine. × 430. (b) Portion of a capillary loop showing basement membrane thickening. The lamina densa is split into thin lamellae, its wavy epithelial aspect accords with the irregular course of the epithelial cell plates. × 22500

Table 1. Some clinical and pathomorphological findings in 10 cases of hereditary

Patient	Sex	Age at biopsy, ys	Clinical data			
			Hematuria	Proteinuria	Nerve deafness	Ocular abnormalities
LA. Ma.	♂	12	+	+	+	−
LA. Mi.	♂	8	+	+	+	−
MU. P.	♀	6	+	+	−	−
MU. J.	♂	5	+	+	+	−
WO. H.	♂	16	+	+	+	+
GE. K.	♀	4	+	+	+	−
GE. E.	♀	6	+	+	+	−
WE. R.	♂	15	+	+	+	−
DR. W.	♂	12	+	+	+	+
LÖ. M.	♂	8	+	−	−	−

of mesangial thickening was noticed. In sections impregnated with silver salts the GBMs seemed to be locally thickened; on the contrary, those in the patient LO (Fig. 3a) appeared thin and fragile. As shown in Table 1 atrophic tubules, interstitial fibrosis and inflammation, and foam cells were further but inconstant findings.

Electron Microscopy

The most striking ultrastructural alterations concerned the glomerular capillary walls, in particular the basement membranes. Besides GBM segments which appeared normal both in structure and thickness, two types of GBM alterations were observed in all patients: firstly thickening and, secondly, a partial extreme thinning of the lamina densa layers.

Thick segments consisted either of a homogeneously broadened basement membrane or showed typical splitting. In the latter case the lamina densa was split into multiple interwoven lamellae enclosing electron lucent spaces (Fig. 1b). Split as well as non-split basement membrane portions were partly interspersed with numerous electron dense particles 15 to 50 nm in size. The lesions involved either the basement membrane of the whole capillary loop or only portions of it. In places where the GBM was split the thickness, *i.e.* the distance from the epithelial to the endothelial covering, was increased often up to 600 nm or more.

Thin basement membrane segments occured immediately adjacent to or intercalated between split portions (Fig. 3d), and the whole length of the peripheral capillary wall was sometimes affected (Fig. 2).

The mean diameter was 100 nm, and the related lamina densa was 60–80 nm, and sometimes even less than 50 nm. The split type of lesion was clearly seen in the two brothers of family LA and in the patients WO, WE, and DR: approximately 50% of all glomerular loops were affected (compare Table 1). In the four probands of the families MU and GE this type of lesion was less pronounced (about 25% of

nephropathy. − absent; + present (+, ++, +++, ++++ = degree of change)

Light microscopy				Electron microscopy ultrastructure of GBM			
Mesangial thickening	Foam cells	Tubular atrophy	Fibrosis, inflammation	Normal segments	Thickened segments		Thin segments
					without splitting	with splitting	
+	−	+	−	+	+++	+++	+
+	−	+	−	+	+++	+++	+
+	−	−	+	+++	++	++	+
+	−	−	+	+++	++	++	+
+	+	+	−	−	+++	++++	+
+	−	−	−	++	++	++	++
+	−	−	−	+	++	+++	++
+	++	+	++	−	+++	++++	+
+	−	+	+	+	++	++++	+
−	−	−	−	++	+	+	++++

the loops), and in the patient LO only a few, short split segments could be found in spite of intensive search. Thin basement membrane segments were present in all patients especially in the sisters GE (25–30%) and in the patient LO in which GBMs showed a general thinning of peripheral as well as mesangial portions (Fig. 2).

The degree of epithelial foot process alterations differed widely. In some patients the foot processes were extensively changed into large cell plates (Fig. 1 b), while in others they were broadened, swollen or normal. The alterations correlated vaguely with the thickened portions of the GBM while thin segments often had foot processes of normal appearances (Fig. 2).

For purpose of comparison, GBM and lamina densa thicknesses were estimated in seven children affected with minimal change lesions. Ten capillary loops from two glomeruli were randomly selected from each patient. The smallest portion of the loop was considered to represent the true diameter. The calculated mean values are summarized in Table 2.

Table 2. Mean values ($n = 20$) of glomerular basement membrane (cell to cell distance) and lamina densa thickness in places with the smallest peripheral width in children affected with minimal change lesions

Patient	Sex	Age ys	Basement membrane thickness	Lamina densa thickness
V.D.	♀	3	230 (190–270)	200 (180–220)
R.H.	♂	4	205 (170–250)	180 (150–210)
S.M.	♂	5	300 (250–360)	250 (210–300)
G.B.	♀	7	300 (240–350)	210 (180–270)
A.M.	♂	7	290 (220–360)	240 (200–300)
J.R.	♂	8	205 (175–260)	160 (145–200)
S.I.	♀	10	280 (230–340)	200 (170–240)

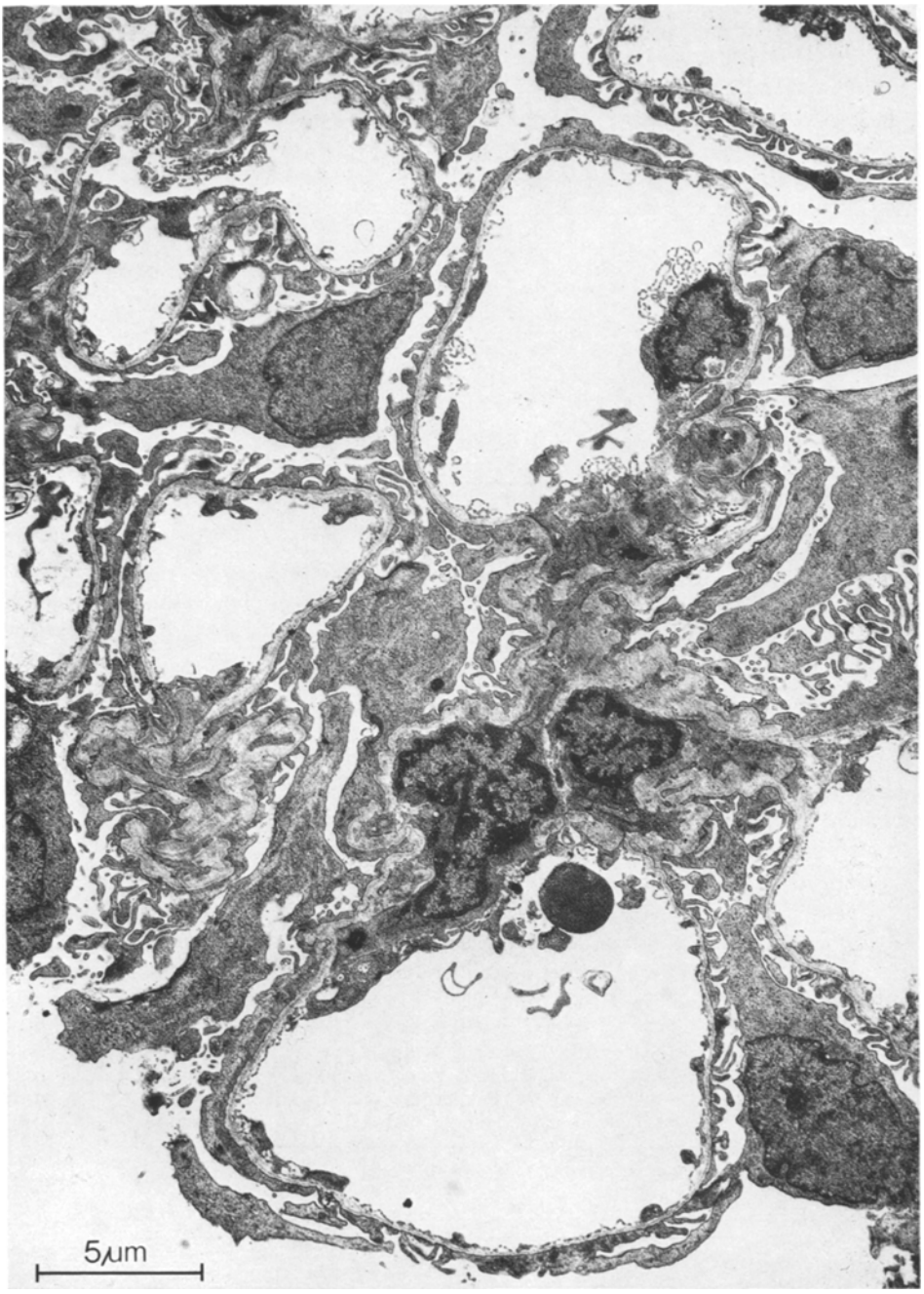

Fig. 2. Low power electron micrograph showing a portion of a glomerulus of patient LÖ. The mesangial stalk is slender, the capillary lumina are wide. There is a diffuse thinning of the basement membranes, the epithelial foot processes are partly "fused". ×4500

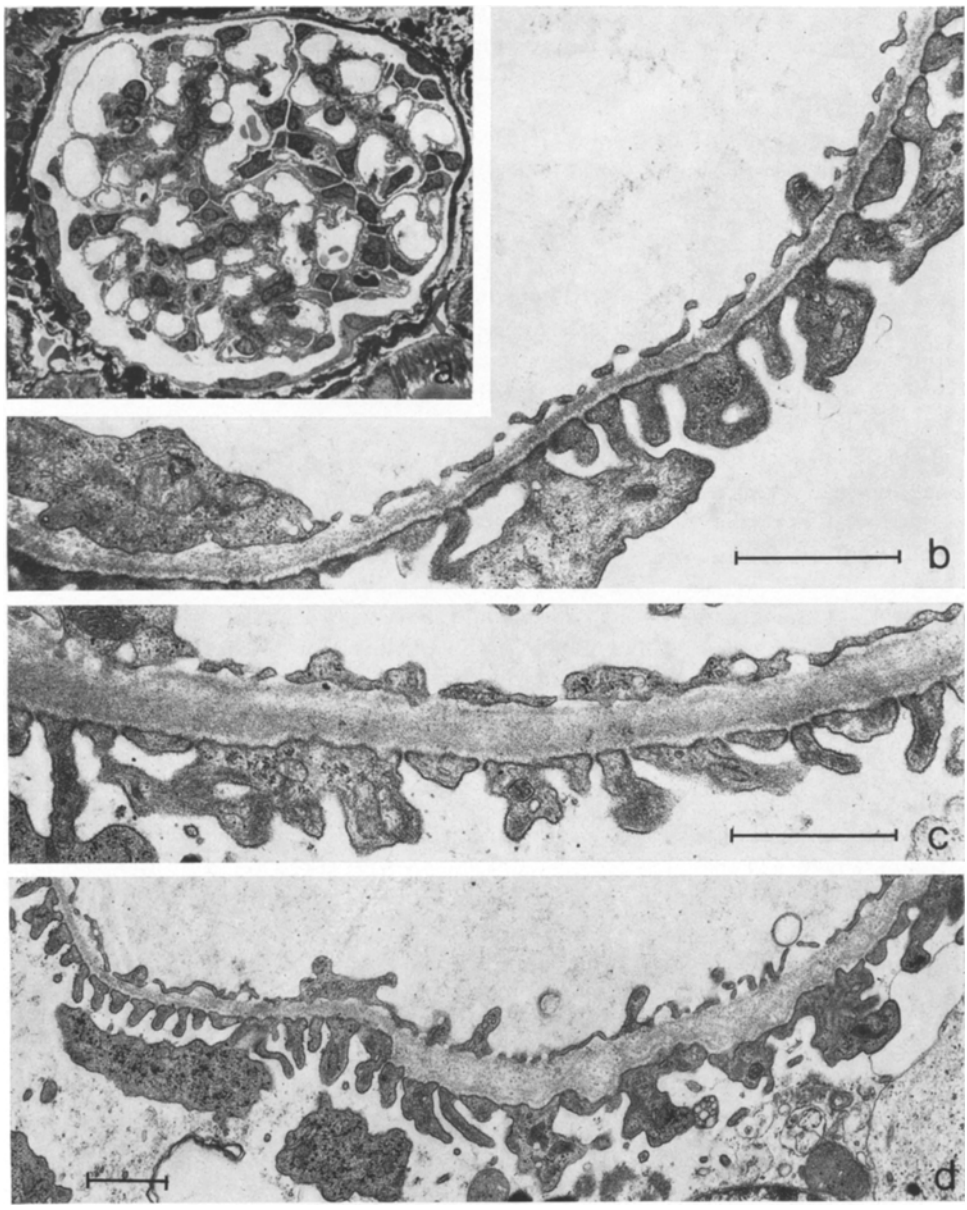

Fig. 3. (a) Glomerulus of patient LÖ showing slender capillary walls. Semithin section, silver methenamine. × 550. (b) Portion of a capillary loop from the glomerulus shown in Fig. 3a demonstrating a thin but otherwise unaltered basement membrane. × 20500. (c) Portion of a glomerular capillary wall from a patient with minimal change lesions for demonstration of the normal thickness of the GBM. × 20500. (d) Portion of a glomerular capillary wall from patient K. of family GE showing a thickened and split basement membrane portion adjacent to a thinned GBM segment which is similar in structure to that shown in Fig. 3b. × 10500

Discussion

A splitting of GBMs into many thin layers and an accumulation of small dense particles between these layers has been described as occuring exclusively in hereditary nephropathy. The lesion is therefore thought to represent a specific ultrastructural feature of this disease (Hinglais *et al.*, 1972; Churg and Sherman, 1973). Furthermore portions of the basement membrane have been observed which were obviously thinner than normal (Spear and Slusser, 1972; Grünfeld *et al.*, 1973) and, in places, even a total absence of lamina densa material was noticed (David *et al.*, 1966; Spear and Slusser, 1972). In all patients both split as well as thin and in places extremely thin GBM portions were found. Thin GBMs were mostly present as short segments intermingled between split and homogeneously broadened segments, and it was only in a few cases that the most extreme degree of this type of lesion was noticed. Thus in patient LO all the GBMs were extremely thin.

The normal width of the whole basement membrane, *i.e.* the distance from the epithelial to the endothelial covering, is well known from studies of healthy adults (Bergstrand and Bucht, 1958; Osawa *et al.*, 1966; Jørgensen and Bentzon, 1968), but there are only few data available concerning the normal GBM thickness in children. The thickness of lamina densa layers in children, however, has as far as we know never been evaluated. Bloom *et al.* (1959) found a mean basement membrane thickness of 110 nm in infants and small children. In slightly older children and adults the mean value was 270 nm. Østerby Hansen (1965) examined three healthy children the GBMs of which varied between 250 and 286 nm. Because tissue from healthy children was not available we decided to take biopsy material from children with minimal change lesions (Fig. 3C) for estimation of GBM parameters needed for the purpose of comparison. The values found for the mean basement membrane thickness at its thinnest points (Table 1) are in good agreement with those found by Bloom *et al.* (1959) and Østerby Hansen (1965) in healty children. Thus it is probable that the corresponding lamina densa thickness summarized in Table 1 were representative of those for normal children. Thus the thickness of the thin basement membranes in hereditary nephropathy reach only one third to one half of the normal values.

The fact that the GBMs of patient LO and the sisters GE who at the time of biopsy were 8, 6 and 4 years old still show a thickness normally found in infants (Bloom *et al.*, 1959), supports the concept of a developmental disturbance as the basic pathogenetic mechanism in hereditary nephropathy as was proposed by Van Buchem and Beestra (1966) and Langer and Thoenes (1971).

Thin lamina densa layers may be the result of either an impairment of synthetic processes or an increased turnover of basement membrane material. Alterations in basement membrane collagen synthesis were postulated by Spear (1973).

Functionally, thin GBMs seem to be largely intact as far as the permeability of serum proteins is concerned. Thus in patient LO there are no morphological signs of an increased tubular reabsorption of proteins nor does a "foot process fusion" (Farquhar *et al.*, 1957) indicate an increased permeability (Farquhar *et al.*, 1958; Churg *et al.*, 1965).

The purpose of this communication was to describe extremely thin GBMs as a further type of basement membrane lesion in hereditary nephropathy, adding further evidence for the concept of a basement membrane alteration as the basic lesion.

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