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Electron Microscopic(EM) Application on Renal Biopsy

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INTRODUCTION

EM has not replaced the light microscope(LM) in the evaluation of renal biopsy specimen. A correct interpretation of renal biopsy specimens requires the correlation of LM, EM and immunofluorescence(IF) microscopic informations with the patients clinical findings. EM examination provided a more satisfactory evaluation with a workable detailed classification of renal biopsy lesion permitting an accurate prognostic evaluation.

EM provides a mechanism for more detailed assessment of the various morphological lesions of renal disease thus facilitating:

1. A better understanding of the changes seen by LM,
2. More precise categorization of disease and, therefore, improved prognosis
3. Pathogenetic insight.

EM is the sole method of specific diagnosis: e.g. hereditary glomerulopathy, minimal change disease & often adds crucial information in lupus nephritis, early amyloidosis, MGN, diabetes, light chain disease, MPGN, type II, etc.

NORMAL ULTRASTRUCTURE

The glomerulus is composed of 4 cell types with specific different functions, a collagenous matrix around mesangial cells, a basement membrane between endothelial and visceral epithelial cells and basal lamina beneath the parietal epithelial cells.

* Endothelial cells－Paramesangial cell body, peripheral cytoplasm, tightly adherent to GBM, Fenetrae (－70 nm in diameter)

* Mesangial cells－Star shaped, microfibrils, mechanical support, contractility and glomerular hemodynamics, phagocytic activity

* Mesangial matrix－fibrils, collagen(type IV), fibronectin mechanical support, transport, removal of macromolecules.

* GBM and LRI & LRE; Thickness(320nm±100)

* Podocytes－villous, interdigitating, foot processes, filtration slits, FS diaphragm(7nm in diameter, 7－14nm in length, zipperlike configuration), fibrils, pinocytotic vesicles.

* Parietal epithelial cells & Basal lamina: flat, overlapping, junctions, fibrils. laminated basal lamina(1,200 nm)

Juxtaglomerular apparatus

Tubule

Interstitium

Blood vessels

GLOMERULAR DISEASE

The ultrastructural expression of glomerular disease results from:

1. Alteration of intrinsic components
2. The infiltration of circulating inflammatory cells
3. The formation of deposition in the glomerulus of materials not normally present, such as immune complexes, amyloid or fibrin

CAPILLARY LUMINAL REACTIONS

〈Inflammatory cells〉:

* PIGN－neutrophil infiltration, endothelial ulceration and intimate apposition between neutrophils and GBM(Fig. 1 & 2)

＊Transplant rejection－luminal narrowing or occlusion by mononuclear cells

〈Thrombosis with formation of platelet/fibrin masses〉: systemic intravascular coagulation, hemolytic uremic syndrome, accelerated hypertension, hyperacute rejection, severe GN with necrotizing features

〈Massive or 'giant' immune deposits〉='hyaline thrombi' of LM: SLE, macroglobulinemia, cryoglobuli nemia－subendothelial deposits

〈Hematoxyphil bodies〉: pathognomonic structures of SLE, consists of altered nuclear material 〈Fat vacuoles, bacteria, fungi, tumor cells〉

CAPILLARY WALL

Some condition with capillary wall alterations which can be diagnosed only by EM-MCD, benign essential hematuria(thin BM disease), Thickening of GBM-MPGN, MGN

Membranous glomerulonephropathy

Idiopathic MGN: Stage I-IV by Ehrenreich and Churg(Fig. 3)

Secondary MGN

SLE: the presence of deposits in subendothelial and mesangial sites(Fig. 4), fingerprint or tubular organi zation of deposits and endothelial tubuloreticular arrays(Fig. 5).

Gold therapy－MGN with aurosomes(gold particles) in proximal tubular cells.

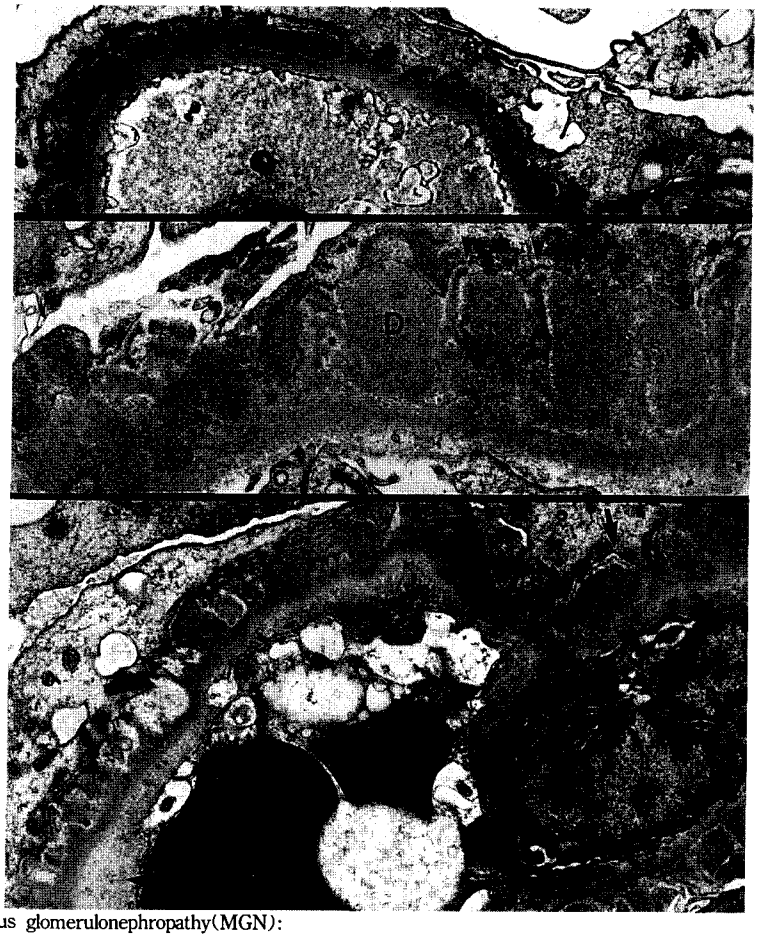

Fig. 3. Membranous glomerulonephropathy(MGN):
a) MGN, stage I-II: Small dome-shaped subepithelial deposits with minute projection of the GBM(arrow)(×5,000). The remaining GBM show the projection of BM(arrowheads) between deposits are responsible for the spikes seen in silver stain([a]×10,000, [b]×5,000, [c]×500).
b) MGN, stage II: Large subepithelial electron dense deposits are surrounded by tall projections(arrowheads)(×100,000).
c) MGN, stage III: the deposits are surrounded by new basement membrane(arrow) with varying degrees of resolution clearly thickened GBM shows an intramembranous lucent zone(arrowhead).

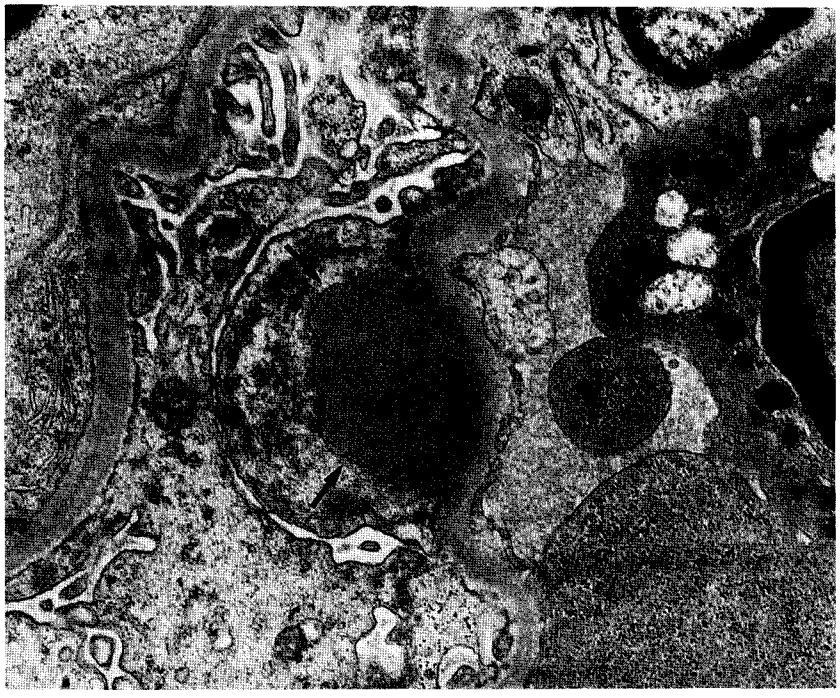

Fig. 1. PSGN: A hump(arrow) along the GBM(×12,000).

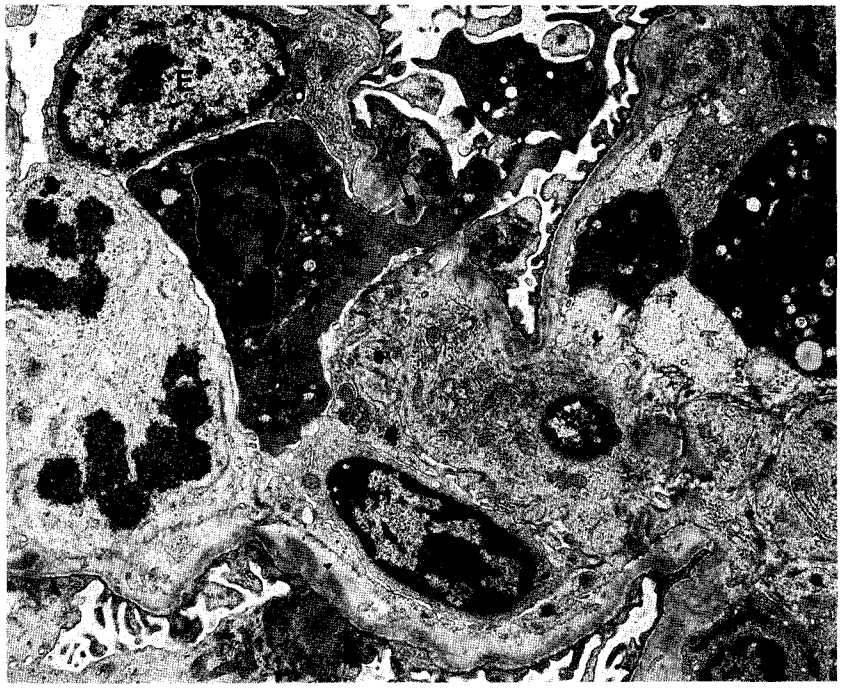

Fig. 2. Endocapillary cell proliferation and neutrophils(N). Cytoplasm of neutrophil passing through small gap in GBM(arrow)(×4,000)

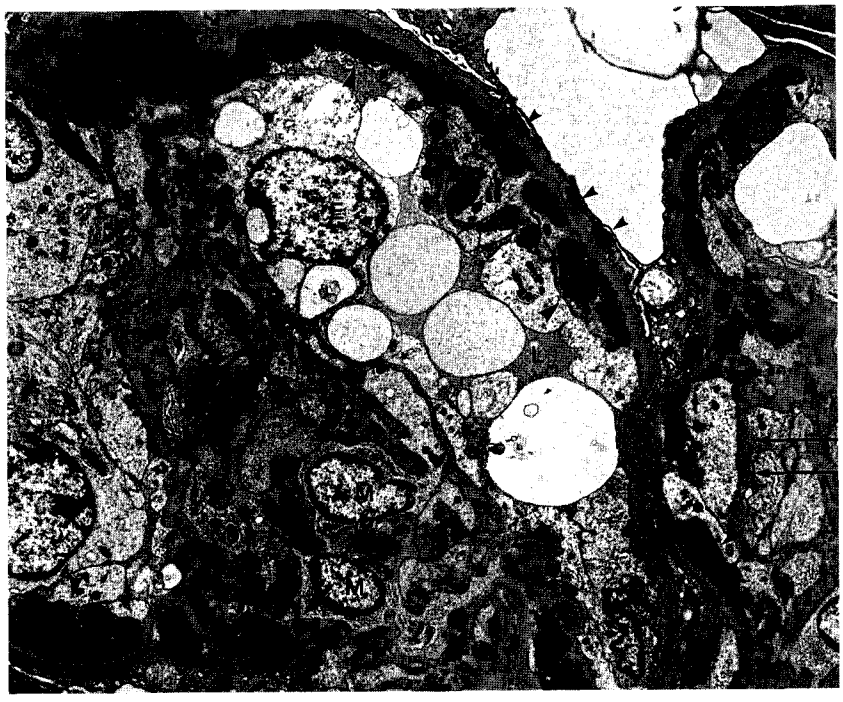

Fig. 4. Active lupus glomerulonephritis, WHO class IV: Large amount of mesangial(arrows), subendothelial(arrowheads), subepithelial(arrrowhead) deposits with segmental mesangial interposition(arrow)(×2,500).

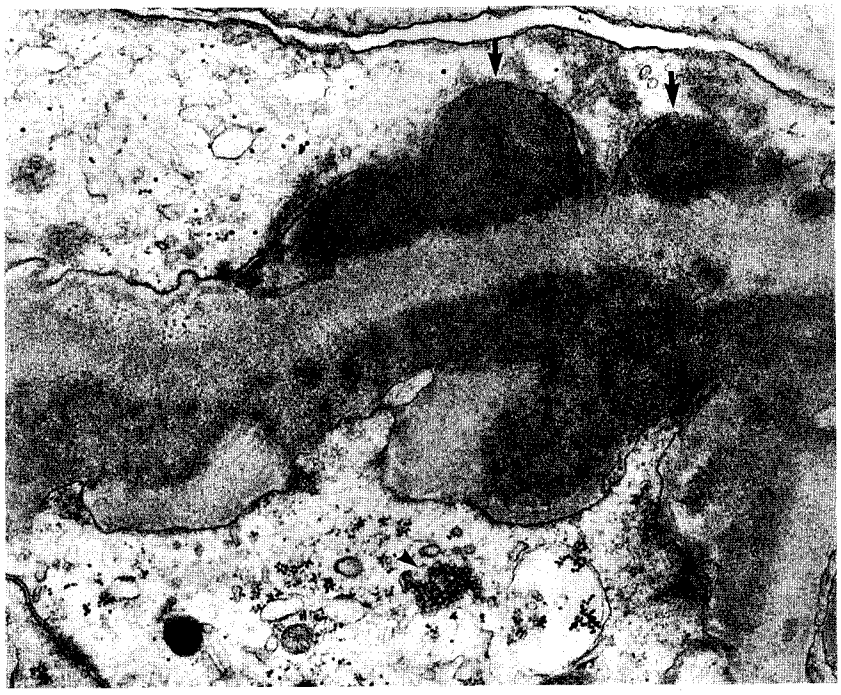

Fig. 5. Lupus nephritis: Subepithelial deposits with fingerprint configuration(arrow) and tubuloreticular structure in endothelial cell(arrowhead)(×17,000).

Diabetic glomerulosclerosis

1) GBM thickening－uniform
2) Mesangial matrix increase
3) Characteristic mesangial nodules develop

＊In most cases the severity of glomerulosclerosis correlates approximately with duration and severity of disease－variable

－Microproteinuria early in the course of DM

－Later－structural abnormalities become overt. Proteinuria becomes persistent, more severe and clinically significant.

－Nodular exaggeration of matrix accumulation(Fig. 6)

Mesangiolysis & organization of capillary aneurysms

＊Microaneurysm formation

－>simple loop dilation mesangiolysis

herniation of mesangial content through ruptures of the paramesangial BM.

Mesangial/subendothelial interposition(mesangiocapillary change)

The CW thickening and GBM 'double contour':

outer: original GMB

inner: new matrix laid down by the mesangial cells invading the subendothelial potent space

Mesangial interposition－secondary to mesangial proliferation. partial or circumferential.

1. MPGN

Type I: MPGN with subendothelial deposits

Type II: Intramembranous dense deposit disease (DDD)

Type III: massive accumulation of intramembranous deposits with extension into subendothelial & subepithelial areas

＊IDDD: unique ribbon-like pattern of linear dense transformation of the GBM.

The dense material lacks the granularity, is centrally located in GBM in ribbon-like fashion.

In areas, the material may be discontinuous or may show great variation in thickness.

Similar deposits in mesangium, subepithelial hump, or Bowman's capsule and TBM

2. Transplant glomerulopathy. circumferential mesangial interposition, subendothelial widening, electron-lucent deposit.

'Accelerated obsolescence'

Some cases of accelerated hypertension, HUS, Preeclampsia, TTP, DIC, PSS, Radiation injury, Transplant glomerulopathy

EM: Subendothelial widening—>luminal stenosis

Fibrin, RBCs, red cell fragments and other formed elements are seen in the subendothelial zone.

Microfibrils may be found.

An irregular layer of GBM-like material

Mesangial disruption by the lucent substance may be associated with mesangiolysis

＊Pathogenesis－secondary to endothelial injury

Lighty－chain nephropathy

－Amyloidosis

－Nodular nonamyloid glomerulopathy(kappa light chain nephropathy) finely granular, non-fibrillar deposits often as continuous bands or clusters within BM and mesangium.

Kappa light chain characterize most cases(demonstrable by IH) but lambda light chain & heavy-chain deposition have been reported occasionally.

Fibrillary deposit formation

1. Amyloidosis: nonbranching fibrils(10nm in diameter)(Fig. 7)

DDx;

a) Congo red-negative amyloidosis-like glomerulopathy or so called immunotactoid glomerulopathy－fibril

b) Light chain disease－fibrillar deposits

c) Cryoglobulinemic GN－organized deposits

2. Microfibrils(＊unit collagen fibrils－30nm in diameter or greater)(Fig. 8)

'large' fibrils(18－20nm in diameter),

'small' fibrils(10nm in diameter),

'thin filaments'(3－5nm in diameter).

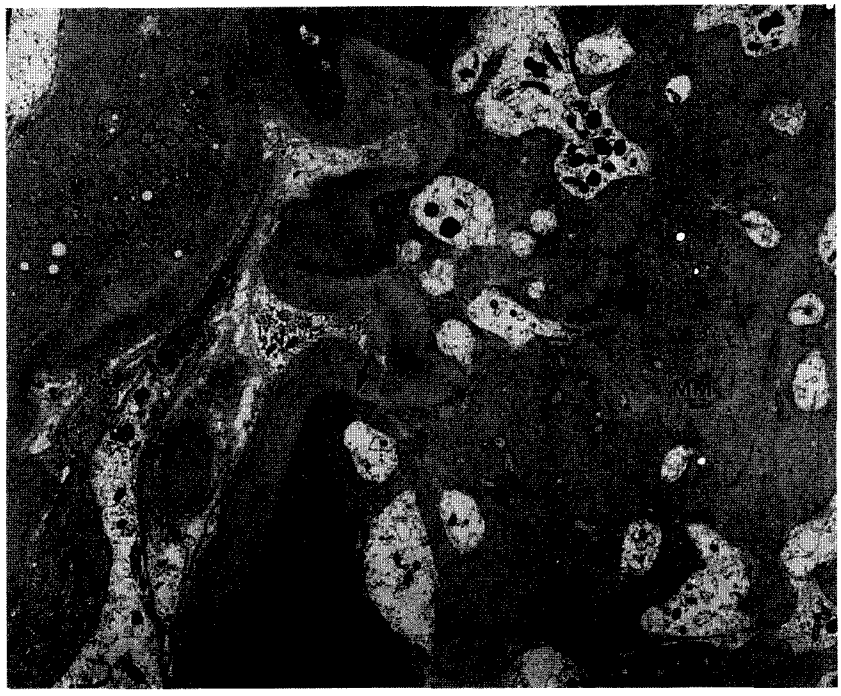

Fig. 6. Diabetic glomerulosclerosis: Marked increase of coarse mesangial matrix, insudative lesion(fibrin-cap)(arrow) that lies immediately adjacent to the lamina rara interna of GBM(×3,500)

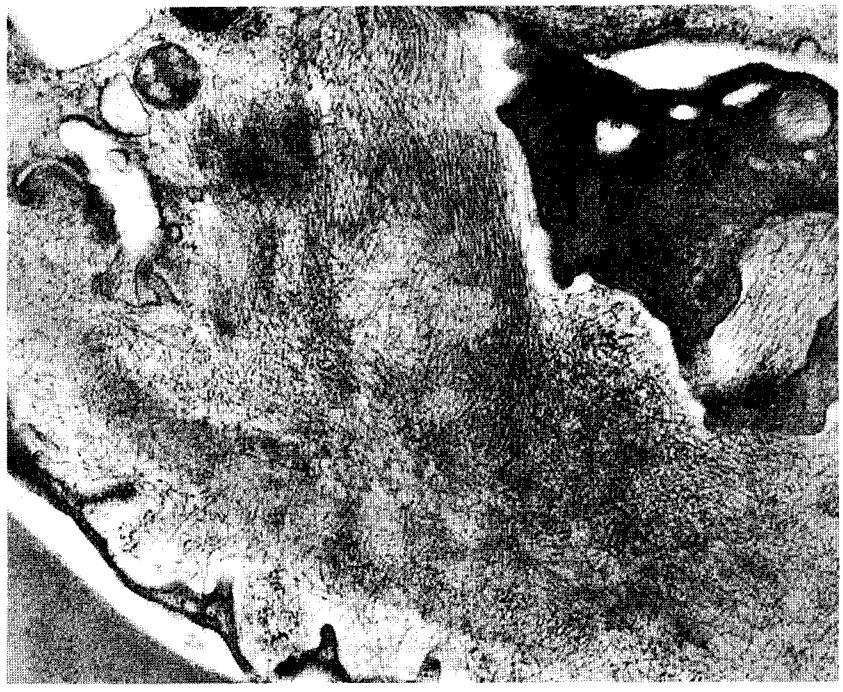

Fig. 7. Intramembranous amyloid deposits: The fibrils appear as randomly arranged and rigid, nonbranching rods, measuring 50~10nm in diameter and 30~1,000nm in length. Some fibrils are arranged in bundles perpendicular to the lamina densa(×20,000).

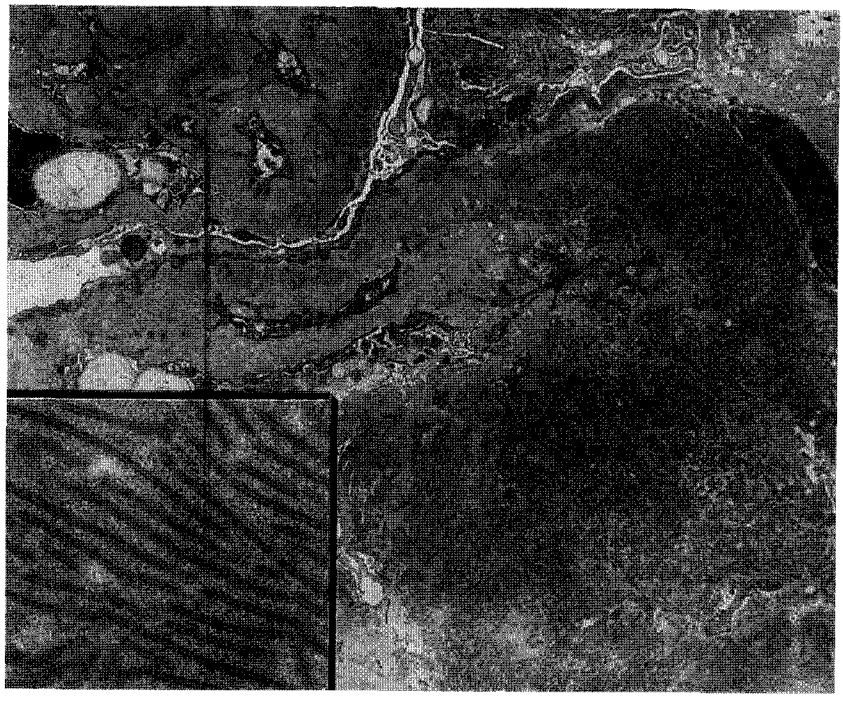

Fig. 8. Microtubular fibrillary deposits(×3,000, [inset] ×20,000).

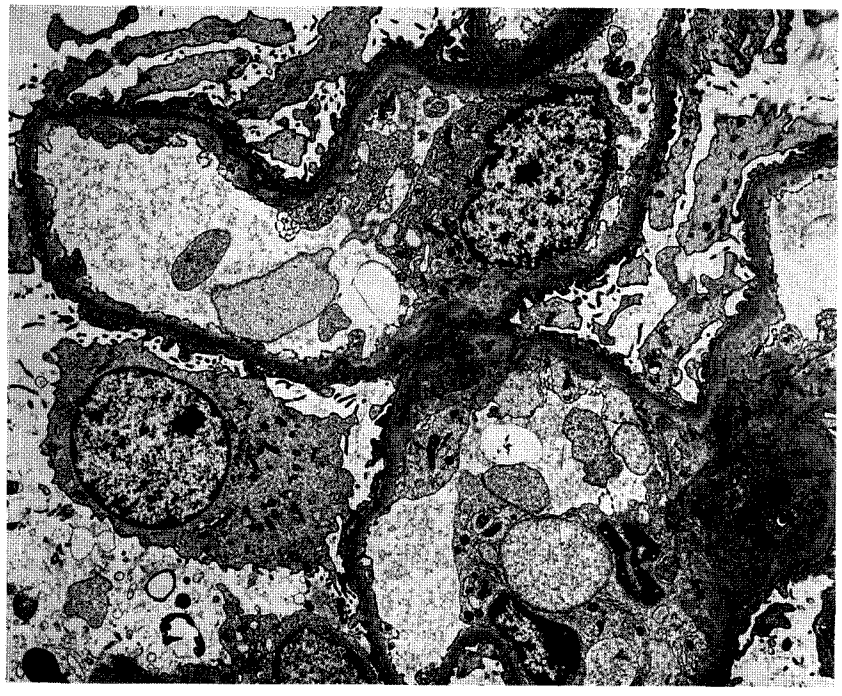

Fig. 9. Minimal change disease: Diffuse effacement of foot processes and microvillous transformation of the visceral epithelial cells(×3,000).

3. 'Immunotactoid glomerulopathy' —highly organized crystalloidal structure of immune deposits
4. Organization of immune deposits
   a) lupus nephritis—fingerprint
   b) cryoglobulinemia—aggregates of cylindrical structures, individual tubules—20—30nm or more
   c) Macroglobulinemia—granular and fibrillar—crystalline deposits

Minimal change disease(MCD)(Epithelial cell disease)

EM: —Total or wide spread foot process effacement,(is due to extensive cell swelling and retraction of the foot processes into the parental visceral cell bodies and not to actual fusion)

—The epithelial cell cytoplasm appears hyperactive, with an increase in cytoplasmic organelles and frequently cysts and numerous microvilli(Fig. 9)

—A zone of increased cytoplasmic density immediately adjacent to the GBM.

Nephrotic focal segmental glomerulosclerosis

EM:—MCD findings plus

—sclerotic lesion—capillary collapse & increase in mesangial matrix, accumulation of granular ED material often containing lipid vacuoles, foam cells and fibrillar collagen

—focal epithelial cell ulceration or detachment, multilayered new BM formation

—frequent podocyte vacuolation(Fig. 10)

Hereditary glomerulopathies

1. Alport's syndrome

—The GBM is irregular in contour, width and texture

—areas of marked thickening alternate with zones of attenuation,

—Lucencies and longitudinal lamellation—'basket weave' pattern(Fig. 11)

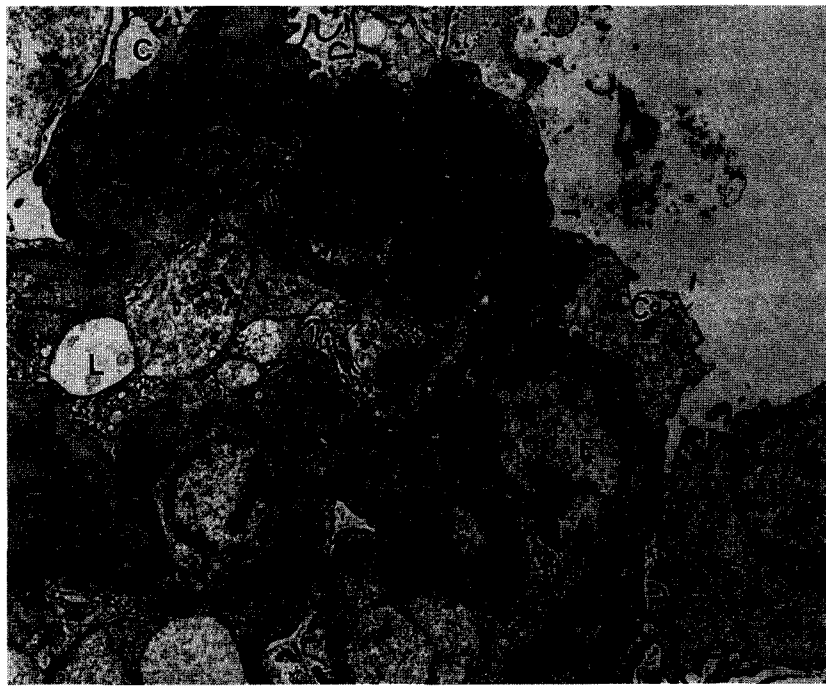

Fig. 10. Focal segmental glomerulosclerosis, The epithelial cells are hypertrophied with prominent pseudocysts(C). The GBM is folded with capillary collapse and reducing the capillary lumina(×4,000).

2. Benign essential(familial) hematuria

-Diffuse attenuation of the GBM without major textural disturbances

-Morphometric study-variable

3. Nail-patella syndrome

-presence of collagen-like fibers within the BM

-thickening of the BM and focal areas of electron lucency

4. Fabry's disease(Fig. 12)

-Numerous membrane-bound lamellated cytoplasmic inclusions round with a concentric myelin-like structure ovoid with parallel arrangement of layers

*Dx-confirmed by specific enzyme defect(in serum, urine or leukocytes) α-galactosidase

MESANGIAL REACTIONS

Mesangial expansion is the commonest manifestation of glomerular disease, -due to increases in cellular or matrix components, inflammatory cell infiltration, or the deposition or formation of materials foreign to the normal glomerulus.

1) Acute GN(PIGN)

2) Mesangial proliferative GN-IgA nephropathy

3) Mesangiolysis

4) Cirrhotic glomerulosclerosis

EXTRACAPILLARY REACTIONS

Epithelial hypertrophy

Epithelial hyperplasia and crescent formation

TUBULOINTERSTITIAL DISEASE

1. TBM deposits-lupus nephritis
cryoglobulinemic GN,
DDD

granular dense transformation-light chain nephropathy

2. Cast nephropathy(Fig. 13)

a) Finely granular ED homogeneous casts

b) Coarsely granular-globular cast with or without associated finely granular material and cellular debris

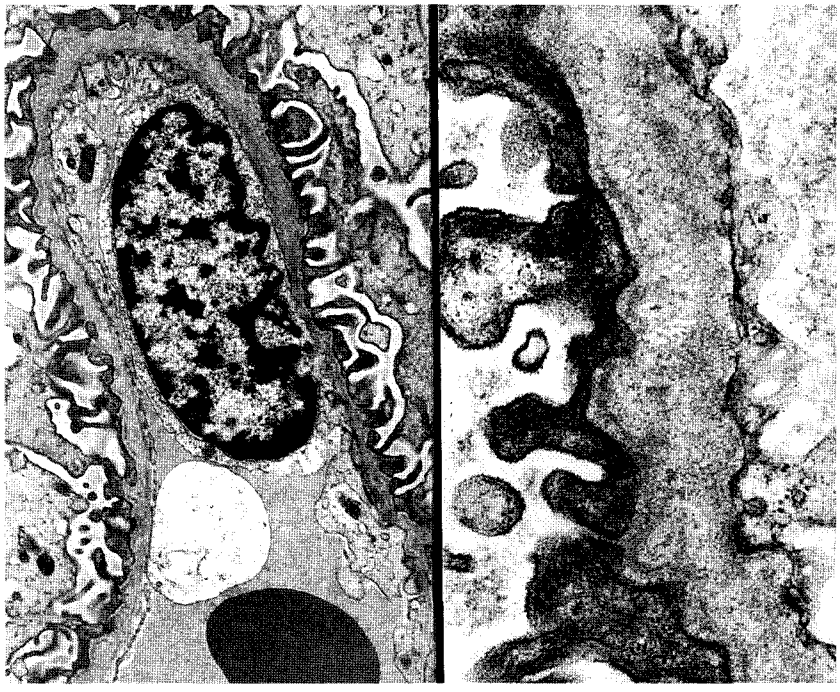

Fig. 11. Alport's syndrome: Characteristic lamina densa thickening with basket-weaving and lucent zone containing fine granular material([a]×5,000, [b]×20,000)

Fig. 12. Fabry's disease: Cytoplasmic membrane bound laminated inclusions(×20,000).

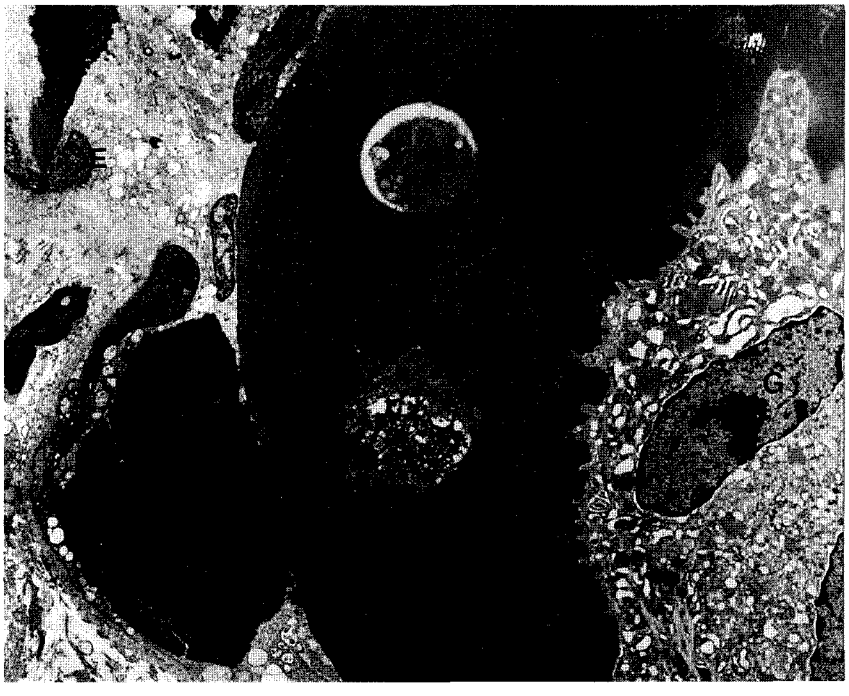

Fig. 13. Myeloma casts within tubular lumina are surrounded by multinucleated giant cells(G). Tubular epithelial cells(E) are compressed(×2,500).

c) Casts consisting primarily of microspherical particles(MSP) measuring 100 to 200 nm, usually surrounded by a finely granular matrix, and associated. with elongated thin or needle shaped crystal

d) Casts consisting of crystalline structures often surrounded by finely granular material, cellular debris or MSP.

* Crystals－elongated(400-500nm to 5-15 μm in length & 1-200nm to 1-2μm in cross), rectangular, pentagonal, or hexagonal shape

At high magnification－a lattice substructure with linearly and regularly arranged ED components measuring 5-10nm in diameter.

Giant cells－resembling cells of monocytes-macrophage system than tubular epithelial cells.

3. Aurisomes－chrysotherapy－MGN

4. Vacuolar tubulopathy－hypertonic agent, hypokalemia

5. Nuclear inclusions－lead poisoning

6. Lysosomal inclusions－lysosomal storage disease

VASCULAR DISEASE

Hyaline & Hyperplastic arteriosclerosis, PSS, HUS

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