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Ultrastructural Lesions in Kidneys with Minimal Involvement by Systemic Lupus erythematosus*

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Summary. Six renal biopsies from patients with Systemic Lupus erythematosus were studied with the electron microscope. By light microscopy only mild endothelial and mesangial hipercellularity were present. Ultrastructurally the visceral epithelial cells were hypertrophic with microvilli formation, hypertrophy of diverse cytoplasmic organelles and fusion of the foot processes. Four cases had subepithelial deposits. All four cases were given high doses of steroids suggesting that these deposits are not affected by the treatment.

The basement membrane in all the cases had focal areas of thickening or nodularity. The endothelial cells showed proliferation. There was an increase in mesangial cells and matrix. In one case collagen fibers were present in the mesangial matrix.

The finding in a significant number of cases of subepithelial deposits not detected by light microscopy indicates the need of routine ultrastructural studies for the complete evaluation of Lupus nephritis.

Introduction

The ultrastructural features of severe renal involvement by Systemic Lupus erythematosus (SLE) are extensively described in the literature. The electron microscopic features of specimens found to have minimal lesions by light microscopy are less well known.

Several authors, (Pirani *et al.*, 1961; Grishman *et al.*, 1963; Pirani and Manaligod, 1966; Comenford and Cohen, 1967; Pollack and Pirani, 1970) of general articles on the ultrastructural findings in Lupus nephritis mention that in the milder cases the glomeruli can show focal fusion of foot processes, nodularity of the basement membrane and a few dense deposits. These deposits can be located in the basement membrane, the mesangium or in the subendothelial or subepithelial areas. Only one paper is found in the literature devoted specifically to this subject (Grishman and Porush, 1964) and they describe findings similar to those previously mentioned.

In this paper, a selected group of renal biopsies having mild lesions by light microscopy are studied in detail with the electron microscope. The high incidence of subepithelial deposits found in these cases is previously unreported and emphasizes the importance of ultrastructural studies in the evaluation of patients with renal lesions for therapy.

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Materials and Methods

Six percutaneous renal biopsies were studied. For light microscopic studies the specimens were processed in the usual fashion, cut at 4 microns, stained with Hematoxylin-eosin, Periodic-Acid-Schiff and Silver Methenamine. For electron microscopy the specimens were immediately fixed in glutaraldehyde and then post-fixed in 1% osmium tetraoxide in phosphate buffer at pH 7.2 for four hours. The samples were embedded in Epon 812. Thick sections were cut a 1 micron, stained with paraphenilendiamine and examined with a phase microscope. In that way, two to three glomeruli per specimen were localized. Multiple thin sections were cut from these blocks (golden-silver interference colours), mounted on uncoated copper grids, stained with uranyl acetate and examined with a Phillips 200 electron microscope.

The patient's charts were reviewed and pertinent clinical and laboratory data was recorded including the treatment received.

Results

In all six cases, the light microscopic study showed a Lupus glomerulitis (Muehrcke *et al.*, 1957). Criteria for this were endothelial (Fig. 1) or mesangial hypercellularity (Fig. 2) and focal thickening of the glomerular basement membrane. Focal fibrinoid, hematoxylin bodies or other morphological features of activity were absent. Tubules, interstitium and vessels were normal.

All patients had an unequivocal diagnosis of SLE on the basis of clinical and serological data (Tables 1 and 2). The kidney biopsy was performed for the evaluation of possible renal disease.

Table 1. *Clinical data*

Case	Age (years)	Sex	Duration of SLE (months)	Duration of renal involvement (months)	Rash	Arthritis	Pleuritis	Edema
1	8	♀	42	32	+	+	0	0
2	36	♀	16	12	+	+	0	0
3	14	♀	12	12	0	+	0	0
4	14	♀	10	7	0	+	+	0
5	11	♀	8	8	+	+	0	0
6	5	♀	3	1	+	0	0	0

Table 2. *Laboratory values*

Case	Hct (%)	WBC (mm^3)	ESR (mm/1st hour)	L.E. prep.	ANF	Gamma glob. (g/100 ml)	BUN (mg/100 ml)	Creat. (mg/100 ml)	24 h urinary protein (in g)	Potinuria	Sediment	Cr. Cl. (ml/min)
1	40	8600	20	+	+	26.7	16	0.77	1.0	++	ABN	72
2	39	4500–	18	+	—	19.4	18	1.20	—	—	N	86
3	37	4600–	20	+	+	16.3	14	0.50	—	+	ABN	139
4	42	8600	28–	+	—	11.0	17	0.78	—	+	N	63
5	45	7600	20	+	—	20.6	10	0.54	0.33	+	N	87
6	26	10000	25–	+	+	25.0	13	0.63	0.20	+	ABN	72

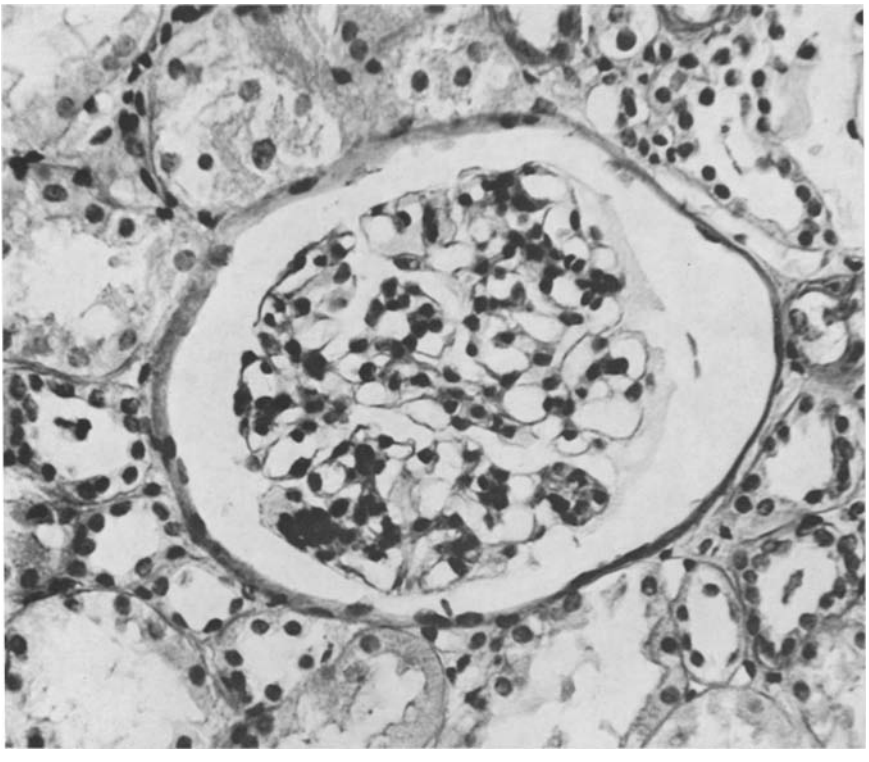

Fig. 1. Case 4. Lupus glomerulitis with focal endothelial hypercellularity. Hematoxylin-eosin, ×350

In the Tables 1 and 2, the clinical and laboratory results obtained immediately before the renal biopsy are shown.

Ultrastructural Findings

All the biopsies were abnormal under the electron microscope. There was hypercellularity of parietal and visceral glomerular epithelial cells and hypertrophy of their endoplasmic reticulum and Golgi apparatus. Cytoplasmic microfilaments and multivesicular vacuoles were seen. Bowman's capsule was normal.

In five of the six cases, there was fusion of the foot processes. This was more extensive in areas overlaying alterations of the basement membrane, and was seen in both the juxtamesangial and peripheral portions of the capillary.

In five cases, microvilli formation was seen in the epithelial cells (Fig. 3). These microvilli were found to arise from areas of cytoplasm almost devoid of organelles and with an increase in cytoplasmic matrix. Free organelles were found in Bowman's space (Fig. 3). Both changes have been named by Comenford and Cohen (1967) "disruption" and interpreted as a form of damage of the epithelial cytoplasm.

In none of the cases was the basement membrane normal. In four of the six cases, subepithelial deposits were present (Fig. 4). They were well delimited,

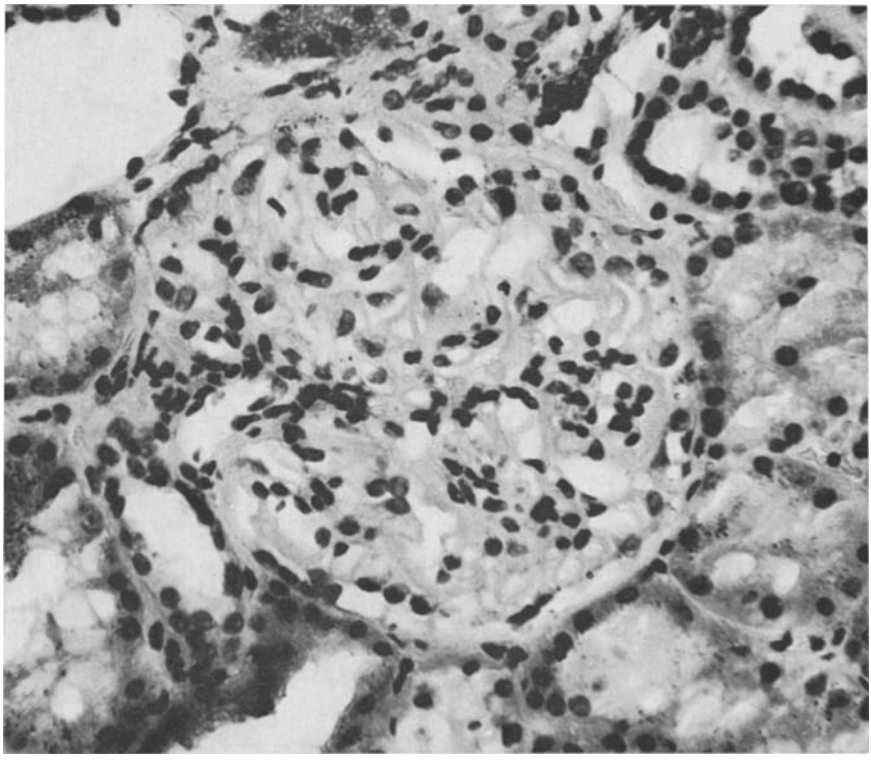

Fig. 2. Case 1. Glomerulus showing mesangial hypercellularity. Hematoxylin-eosin, ×350

measured up to 3000 Å in greatest diameter and surrounded by a clear halo. The deposits were finely granular and had an electron density similar to that of the basement membrane.

Between the deposits the basement membrane formed irregular projections or "spikes". Occasionally, the deposits were completely surrounded by the basement membrane, being intramembranous instead of subepithelial. No subendothelial deposits were found. In all cases the basement membrane had focal areas of nodularity and thickening in the peripheral as well as juxtamesangial areas. A few, ovoid membrane-bound, intramembranous inclusions, that measured up to 3000 Å in diameter, were found. They were similar to the type II inclusions described by Comenford and Cohen (1967).

The endothelial cells showed proliferation with occasional obliteration of the capillary lumen by the cell bodies. As in the epithelial cells hypertrophy of diverse

Fig. 3. Case 4. There are numerous cytoplasmic projections of visceral epithelial cells that have developed microvilli (*mv*). In the cytoplasmic projections are lysosomes (*L*) and also mitochondria (*m*) with a dense matrix and partial loss of cristae. There are also free organelles in Bowman's space (*BS*). A fragment of normal Bowman's capsule (*BC*) and capillary lumen (*CAP*) are seen. (Approx. 6000)

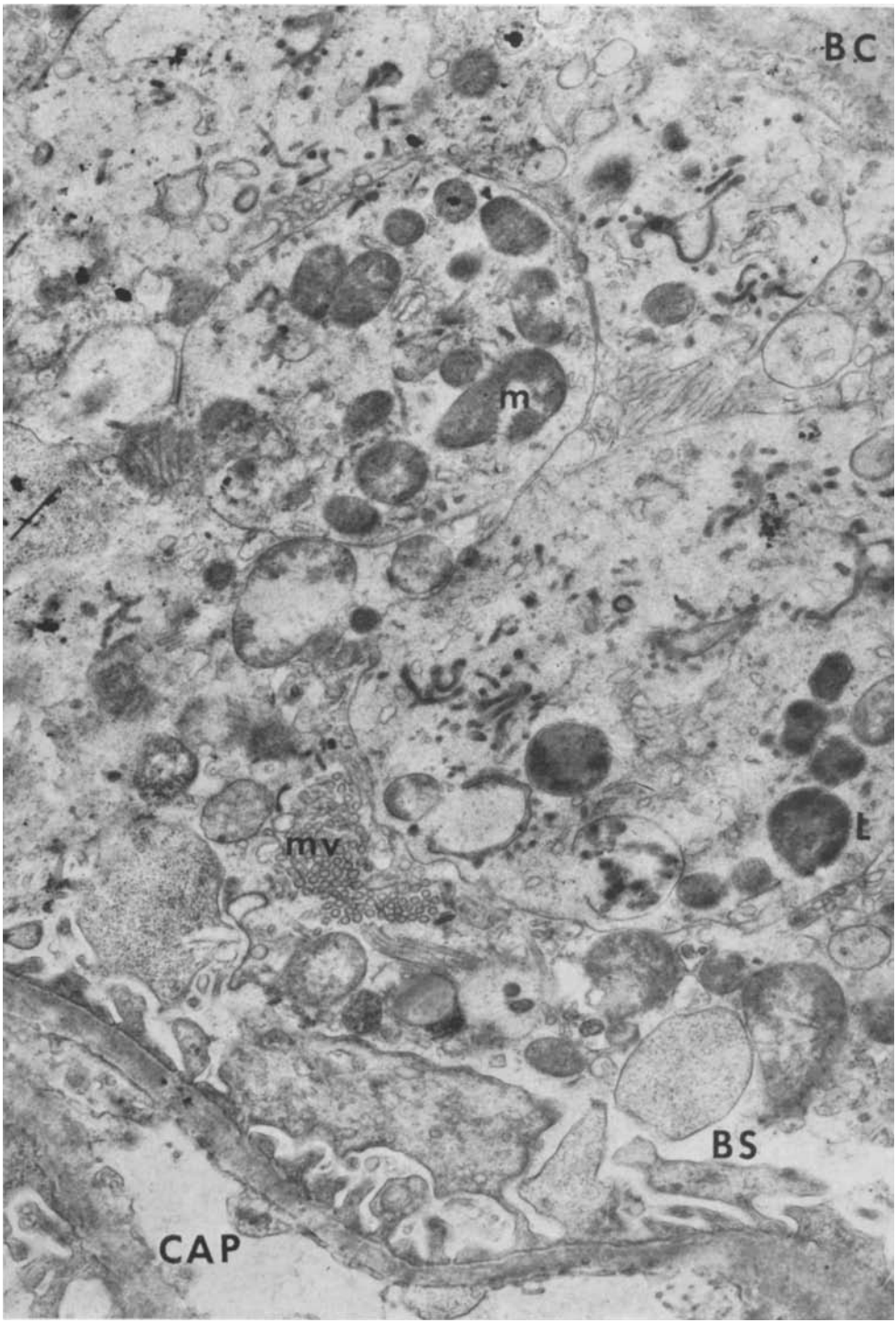

Fig. 3

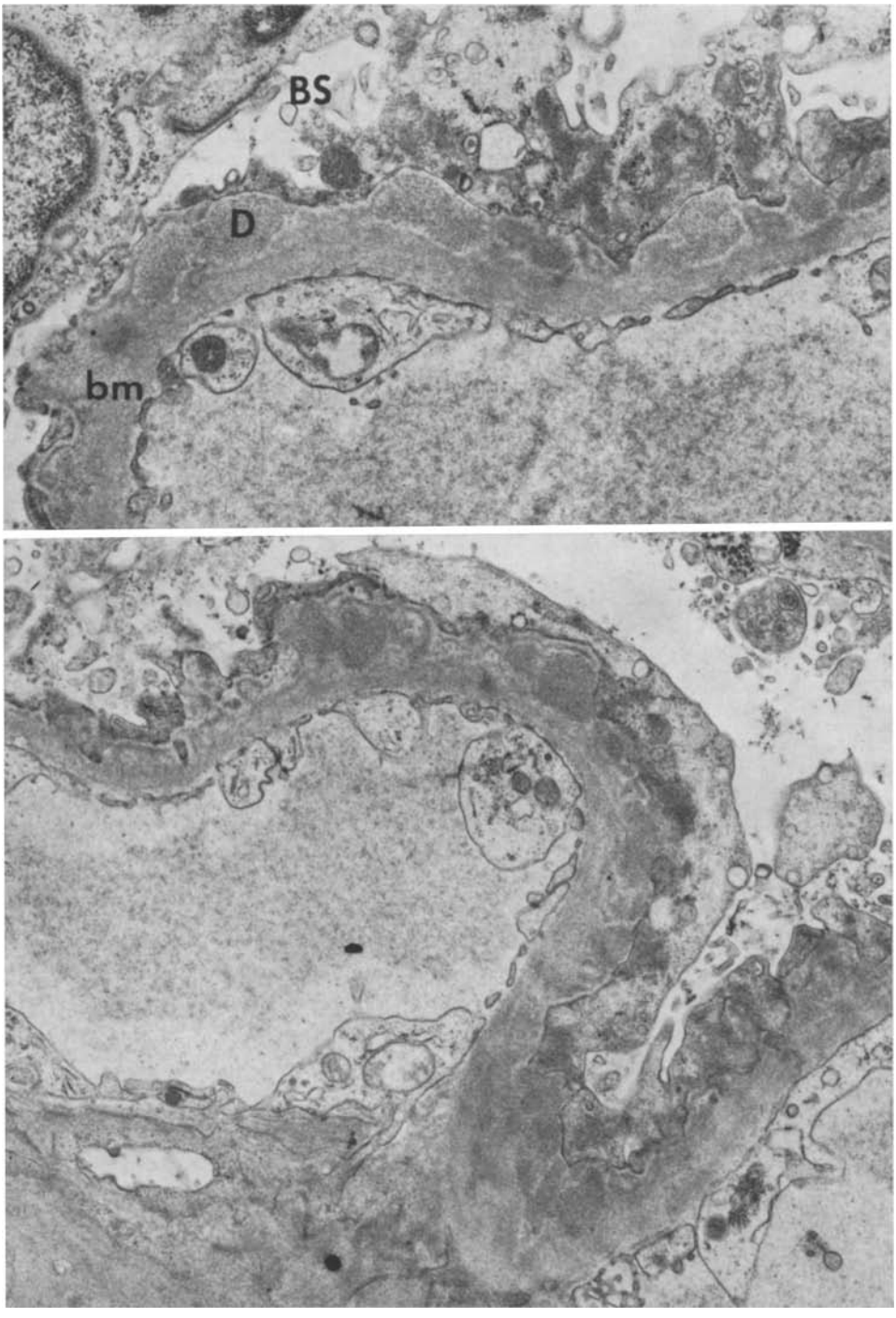

Fig. 4. Case 3. Subepithelial deposits (*D*) and a thickened basement membrane (*bm*). The deposits measure up to 3000 Å in diameter and are surrounded by a clear halo. Some of the deposits are intramembranous. In Bowman's space (*BS*) there is fusion of the foot processes of the epithelial cells. (Approx. 11250)

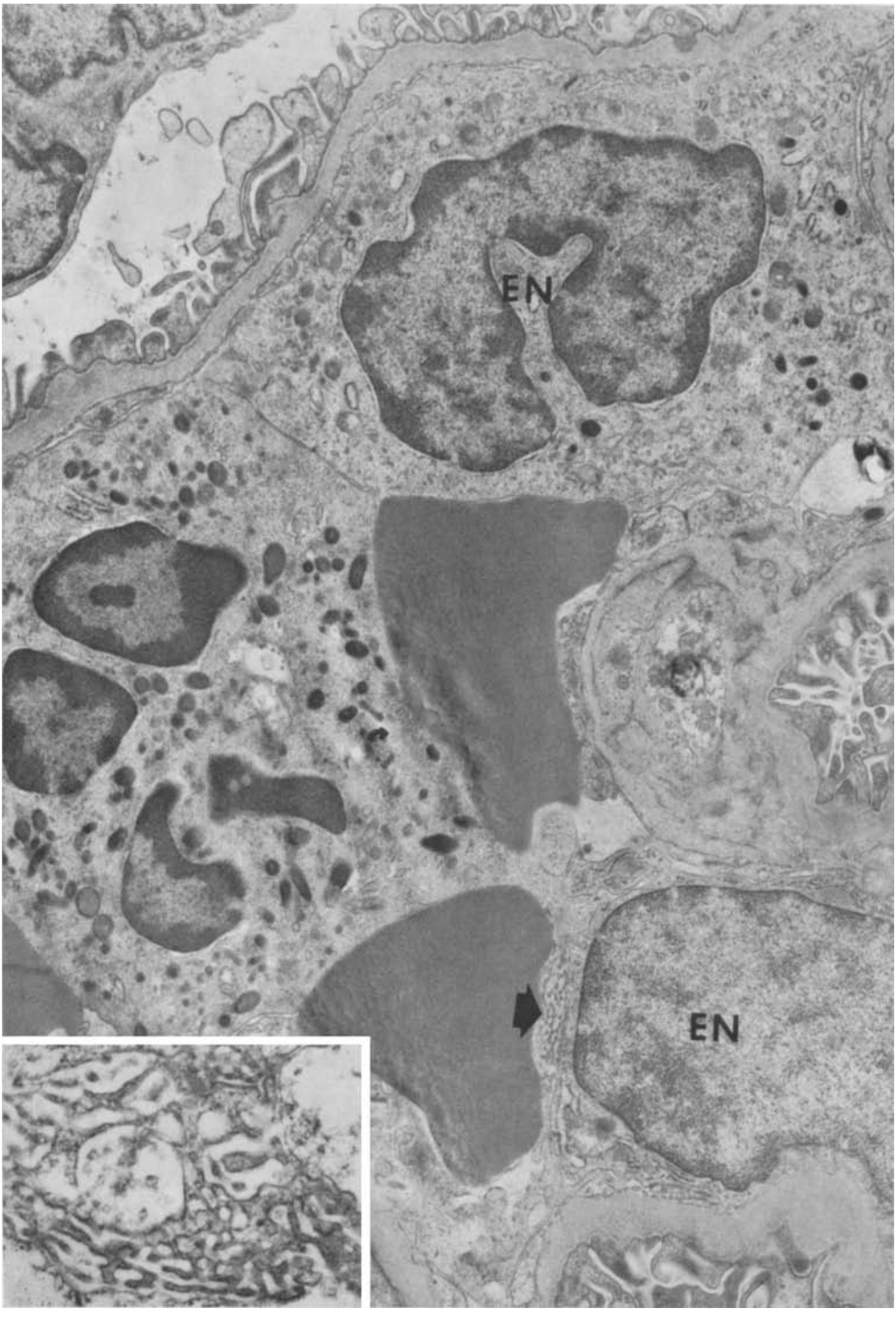

Fig. 5. Case 4. Proliferation of endothelial (*EN*) cells with hypertrophy of cytoplasmic organelles. There are areas of reticulation (arrow), better shown in the insert. In the capillary lumen there are red blood cells and a leukocyte. (Approx. 11200, insert approx. 31200)

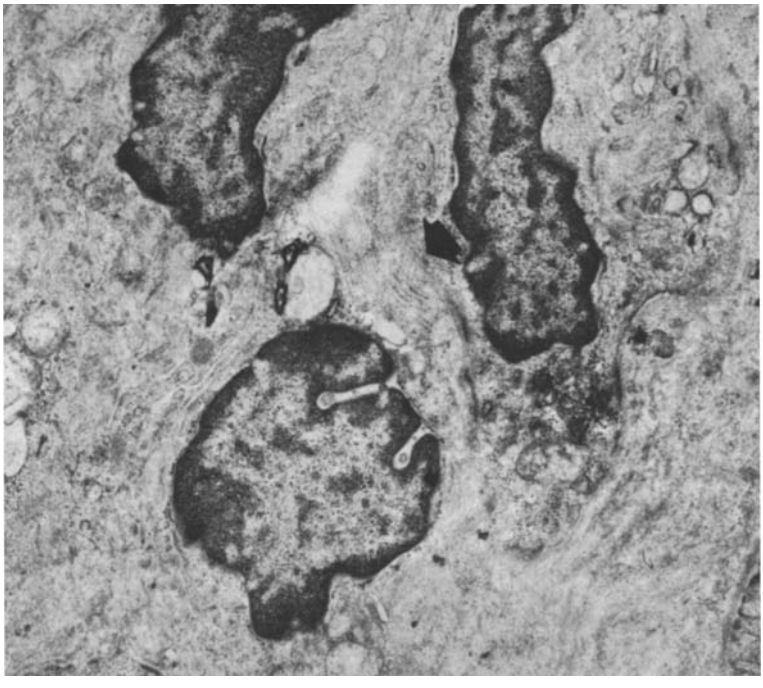

Fig. 6. Case 3. Increase in cells and mesangial matrix. Note the presence of collagen fibers (arrow). (Approx. 11200)

cytoplasmic organelles was seen (Fig. 5). A change in the endothelial cytoplasm termed reticulation (Fig. 5) was seen in three of the six cases. The appearance was that of a delicate network that involved the perinuclear or parietal cytoplasm and was caused by enlargement and distorsion of the endothelial fenestrae (Faith and Trump, 1966). In the capillary lumen red blood cells and neutrophils were seen.

In the mesangial area there was increase in the number of cells and in the amount of matrix. One case showed collagen fibers in the mesangial matrix

Table 3

Case	Steroid treatment prior to renal biopsy	Type of ultrastructural deposit present
1	High dose[a]	Subepithelial deposit
2	High dose	Subepithelial deposit
3	High dose	No deposit present
4	High dose	Subepithelial deposit
5	High dose	Subepithelial deposit
6	No treatment	No deposit present

[a] High dose is defined as no less than 40 mg of Prednisone daily for a continued period of at least six months.

(Fig. 6). No dense deposits were seen in the mesangium. As seen in Table 3, five patients received treatment with high doses of steroids and in four of these subepithelial dense deposits were seen.

Discussion

None of the changes described above is specific for SLE. Among the abnormalities found in the epithelial cells, microvilli formation can be seen in the various glomerulonephritides, amyloidosis and diabetic glomerulosclerosis. Together with the hypertrophy of the Golgi apparatus and an abundant rough endoplasmic reticulum, it suggests a non-specific form of damage of a labile active and highly specialized cell (Trump and Benditt, 1962).

The appearance of the subepithelial deposits is what Ehrenreich and Churg (1968) have named "membranous transformation", and when it affects the glomerulus diffusely it is characteristic of membranous glomerulonephritis. As described by these authors, these deposits are first subepithelial, and then are gradually surrounded by spikes. In a later stage, they became incorporated in the basement membrane substance and disappear leaving a thickened and nodular basement membrane.

Areas of membranous transformation can be found also in the kidneys of patients with renal vein thrombosis, diabetes and SLE. They were found in six of forty-six cases of SLE studied ultrastructurally by Grishman (1968).

By immunofluorescence these deposits were shown to contain gamma globulin and complement. According to Dixon (1968), the subepithelial location of the deposits indicates that circulating immune complexes are trapped in the glomerular walls. When the antibody involved is antibasement membrane, the deposits are located in a linear pattern along the inner aspect the glomerular basement membrane.

The proliferation of the endothelial cells and hypertrophy of diverse cytoplasmic organelles can be considered as a non-specific reaction to injury. The presence of reticulation is described in many glomerular lesions such as glomerulonephritis, eclampsia, nephrotoxic serum nephritis and SLE (Faith and Trump, 1966). It can be found isolated or associated with subendothelial deposits and is related to endothelial phagocytosis or other mechanisms of transendothelial transport.

The presence of collagen mesangial fibers occurs infrequently in SLE (Comenford and Cohen, 1967) and in other chronic glomerular diseases. Jones (1963) showed that the initial structural event in glomerular obsolescense is not the production of collagen, but the increase in mesangial matrix. Collagen fibers appear in a later stage and trend to accumulate particularly in the urinary space (Nagle *et al.*, 1969).

Pirani *et al.* (1961); Grishman *et al.* (1963); Grishman and Porush (1964); Pirani and Manaligod (1964); Pollak and Pirani (1970), mention in their reports that in kidneys from patients with SLE that appear either normal or have mildly involved by light microscopy, diverse ultrastructural lesions can be found. They describe focal fusion of foot processes of the epithelial cells, thickening and nodularity of the basement membrane and focal subendothelial or mesangial dense deposits.

Comenford and Cohen (1967) examined in great detail 13 kidney biopsies from 12 patients with SLE. They divided their cases ultrastructurally in three groups. The first group had no dense deposits, but had diverse abnormalities in the epithelial and endothelial cells and in the glomerular basement membrane. The second group had group I abnormalities and in addition subepithelial deposits. In the third group there were group I abnormalities and subendothelial deposits. Seven of their patients had clinically mild renal disease and under the light microscope showed slight proliferative or membranous changes. Five of these patients had ultrastructural group I lesions, i.e. no dense deposits. Two cases had subepithelial deposits.

Compared to the studies previously mentioned, the cases described here are different in that they show that in four out of six cases subepithelial deposits were found with the electron microscope in patients with minimal renal disease by light microscopy.

As all four patients had been treated with high doses of steroids it can be speculated that the subepithelial deposits may be not affected by the treatment.

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