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Electron Microscopic Observations on the Participation of Blood Leukocytes in the Production of Some Renal Glomerular Lesions

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Summary. A study was carried out on the electron microscopic aspects of percutaneous renal biopsy samples from a case of periarteritis nodosa.

Apart from deposits of fibrinoid and protein material, the presence of a large number of leukocytes, with the predominance of mononuclear leukocytes, was found in some of the glomeruli with hypercellularity revealed by light microscopy. The mononuclear leukocytes as well as the polynuclears present exhibited margination, with incomplete diapedesis processes and their fixation under the capillary endothelium, as well as sequestration within the thickness of the capillary wall owing to the formation of a new lamina densa layer. Subsequently, the capillary wall became thickened and deformed, producing the hypercellularity features observed in light microscopy.

These alterations are explained as being caused by hypersensitivity processes in the course of periarteritis nodosa.

Following immunological investigations, most authors agree that the vascular lesions in periarteritis nodosa are produced by hypersensitivity processes (Dörste, 1968; Cabanel *et al.*, 1965; Paronetto and Strauss, 1962; Dimitriu, 1968).

Glomerular alterations are constantly encountered in periarteritis nodosa cases with renal involvement. The onset of these alterations is related either to affection of the arterioles or to the mechanisms generally involved in the production of periarteritis, the immunopathologic mechanisms accounting for at least some of these alterations (Paronetto and Strauss, 1962). Light microscopy investigations showed, moreover, that these alterations differ from necrozing glomerulitis, lobular or total glomerulonephritis with the presence of fibrinoid material up to sclerosis of the glomeruli with or without obliteration of Bowman's space. Of particular interest appeared to be the glomerular alterations with hypercellularity and accumulation of a material of the basement membrane type, as described by Baldwin and McCluskey (1968) and also observed by us in some cases (Lenkei and Nicolescu, 1970). The present paper describes the ultrastructural character of such lesions and their mechanism of production in a case of periarteritis, studied in the electron microscope.

Material and Methods

This material studied was collected by renal biopsy puncture from a 42 year-old patient with a diagnosis of periarteritis nodosa. The material was fixed by immersion in 4% glutaraldehyde buffered at pH 7.4 with 0.1 M phosphate buffer, for 2 hours at +4° C. The samples were postfixed in 1% osmic acid solution and embedded in Epon 812 (Luft, 1961). The ultrathin sections obtained with an L.K.B. ultramicrotome were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-9A electron microscope.

Observations

Light Microscopy. Several renal glomeruli exhibiting constant hypercellularity, variable thickening of the capillary basement membranes, narrowing and sometimes obstruction of the capillary lumen, were examined on semithin sections. Other glomerules with fibrinoid damage or completely obstructed capillaries also presented sclerosis lesions.

Electron Microscopy. Study in the electron microscope included several glomeruli, especially those which exhibited less advanced lesions on examination in the light microscope, i.e. hypercellularity and discrete thickening of the capillary walls.

In these glomeruli, electron microscopy revealed several changes in the endothelial cells, which were slightly enlarged with developed, irregular cytoplasmic processes and abundant intracytoplasmic organelles: numerous mitochondria, well developed granular reticulum and many free ribosomes. In some of the capillaries protein material depositions were noted with a fibrinoid character, related to the endothelial cells and the lamina densa (Fig. 1).

The lamina densa exhibited thickenings that took on a wavy aspect over the endothelial surface, it contained more or less electron-dense deposits and was often found to undergo reduplication.

In the lumina of many of the capillaries examined a large number of circulating leukocytes, sometimes exceeding the number of erythrocytes, are found. These alterations were more evident in the vicinity of the glomerular vascular pole where the capillary lumen is larger. The leukocytes in these capillaries were of different types: polymorphonuclears, lymphocytes and monocytes, the latter two being predominant. Plasma cells were more seldom observed. The leukocytic margination phenomenon was frequently noted, the leukocytes becoming flat and the plasma membrane sticking to the capillary endothelium. The relationship between the endothelium and these leukocytes is very close yet a clearly defined space is maintained between their cytoplasmic membranes. At times, interdigitations may be noted between the two undulated plasma membranes.

Similarly, evidence was also found of leukocytes in the course of diapedesis. Certain particular aspects are worthy of note. At times, but the event is rare, next to the adjoining leukocyte, and endothelial veil-like process could be seen, tending to surround the white blood cell. At this level the lamina densa bulged towards the epithelium. Moreover, leukocytic elements, especially lymphocytes and monocytes could be discerned under the endothelial cells, between them and the lamina densa (Fig. 2). Continuing to follow up this process of cellular migration, it was found that after extravasation the cells stopped in the lamina densa, since no aspect revealed passage of the cells through it, nor the presence of any blood cell within the urinary space. Linked to this extravasation tendency, some similar cells or cell profiles were noted within the thickness of the glomerular capillary walls, without any connection with the mesangium (Fig. 3). These cells were fixed

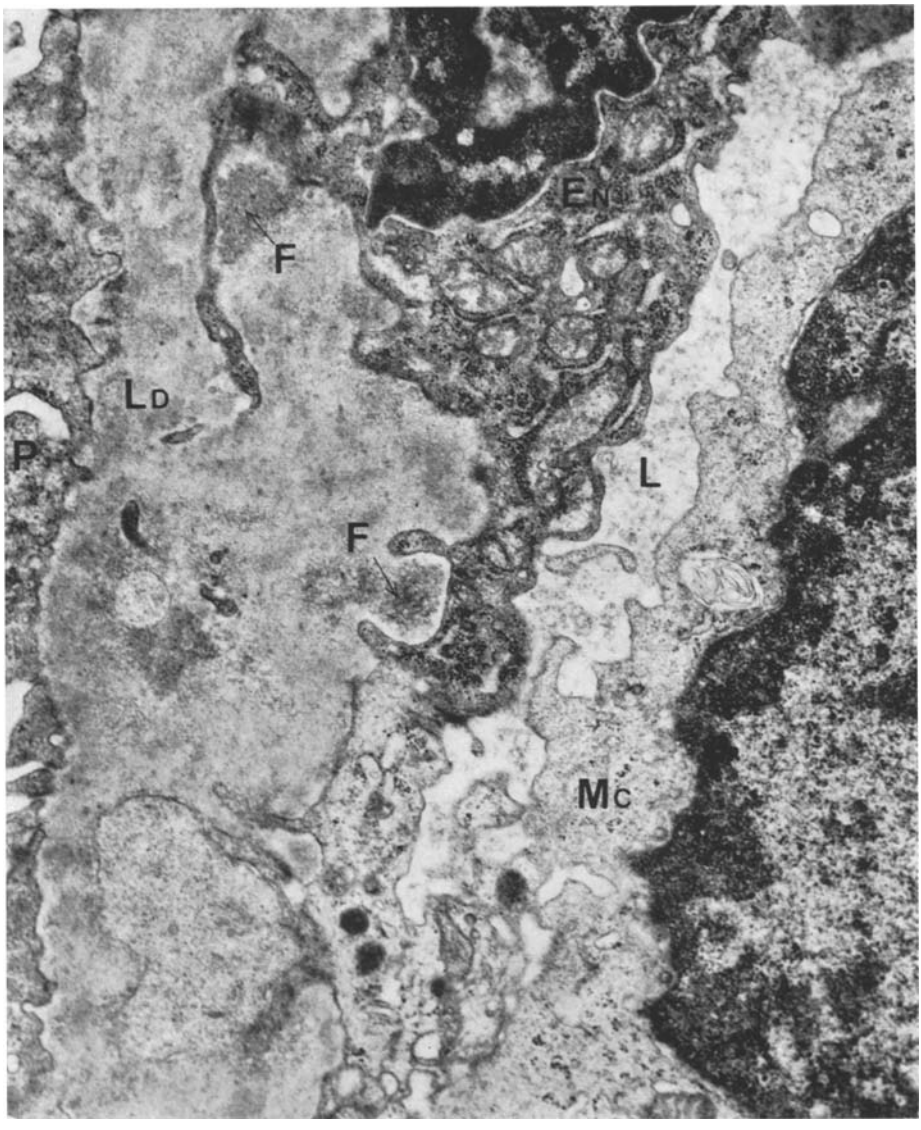

Fig. 1. Glomerular capillary wall with subendothelial deposits of fibrinoprotein material (*F*) and endothelium (*EN*) rich in mitochondria and ribosomes. Podocyte (*P*). Lamina densa (*LD*). Capillary lumen (*L*). Blood mononuclear cell (*MC*). ×17580

between two layers of lamina densa, giving the aspect of "sequestered cells". At the level of these cells, the external layer of the lamina densa is as a rule uniform, of the usual thickness and covered by the foot processes of the frequently fusioned epithelial cells. This layer may bulge towards the urinary space representing, at least apparatently, continuation of the lamina densa. Here and there this layer exhibited irregularities or electron dense deposits. However, of particular interest

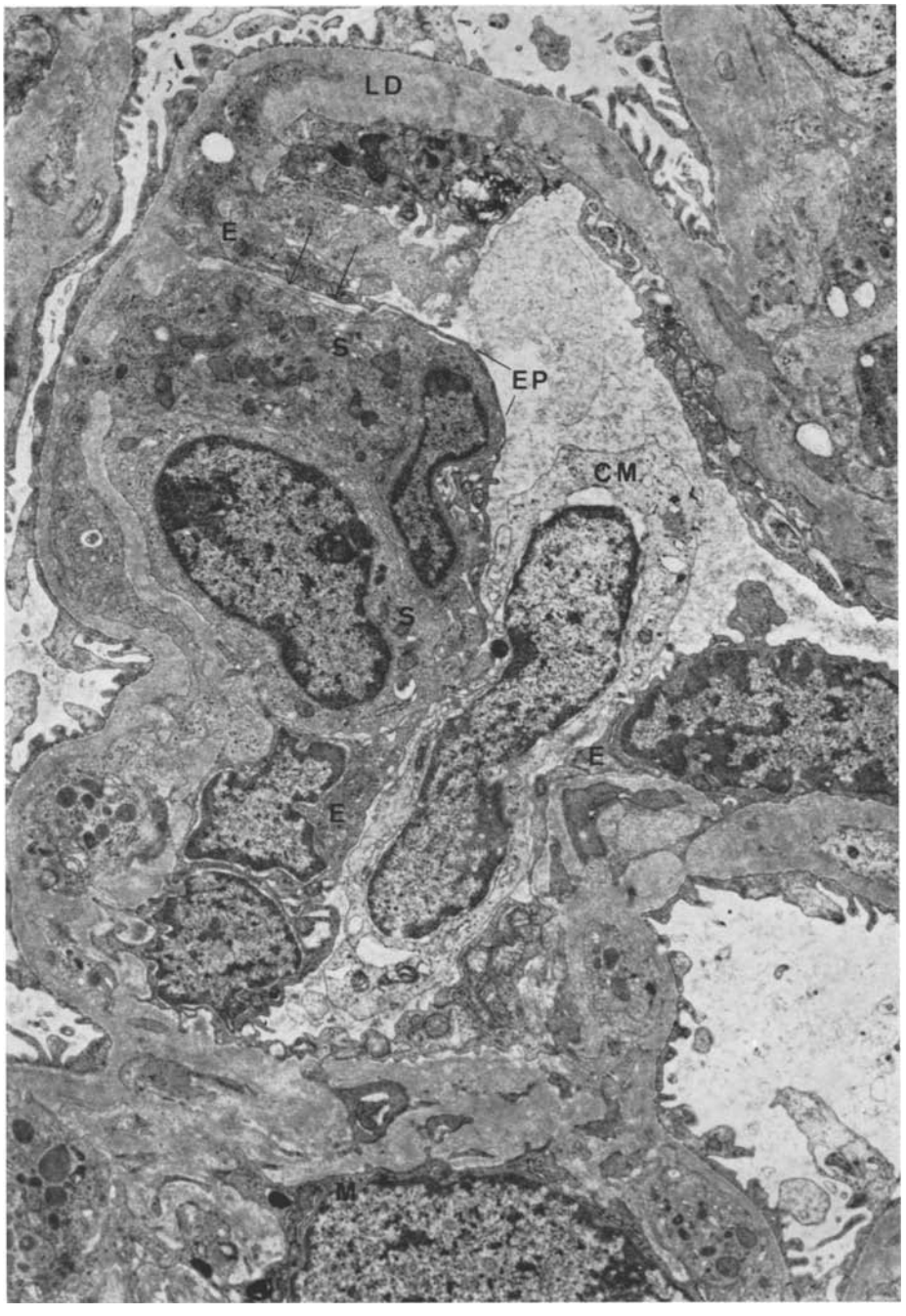

Fig. 2. The presence of subendothelially fixed mononuclear leukocytes (*S*). The arrows indicate basement membrane material in the course of subendothelial formation. Monocyte in the lumen (*CM*). Lamina densa (*LD*). Endothelium (*E*). Mesangium (*M*). Endothelial coating (*EP*). ×7200

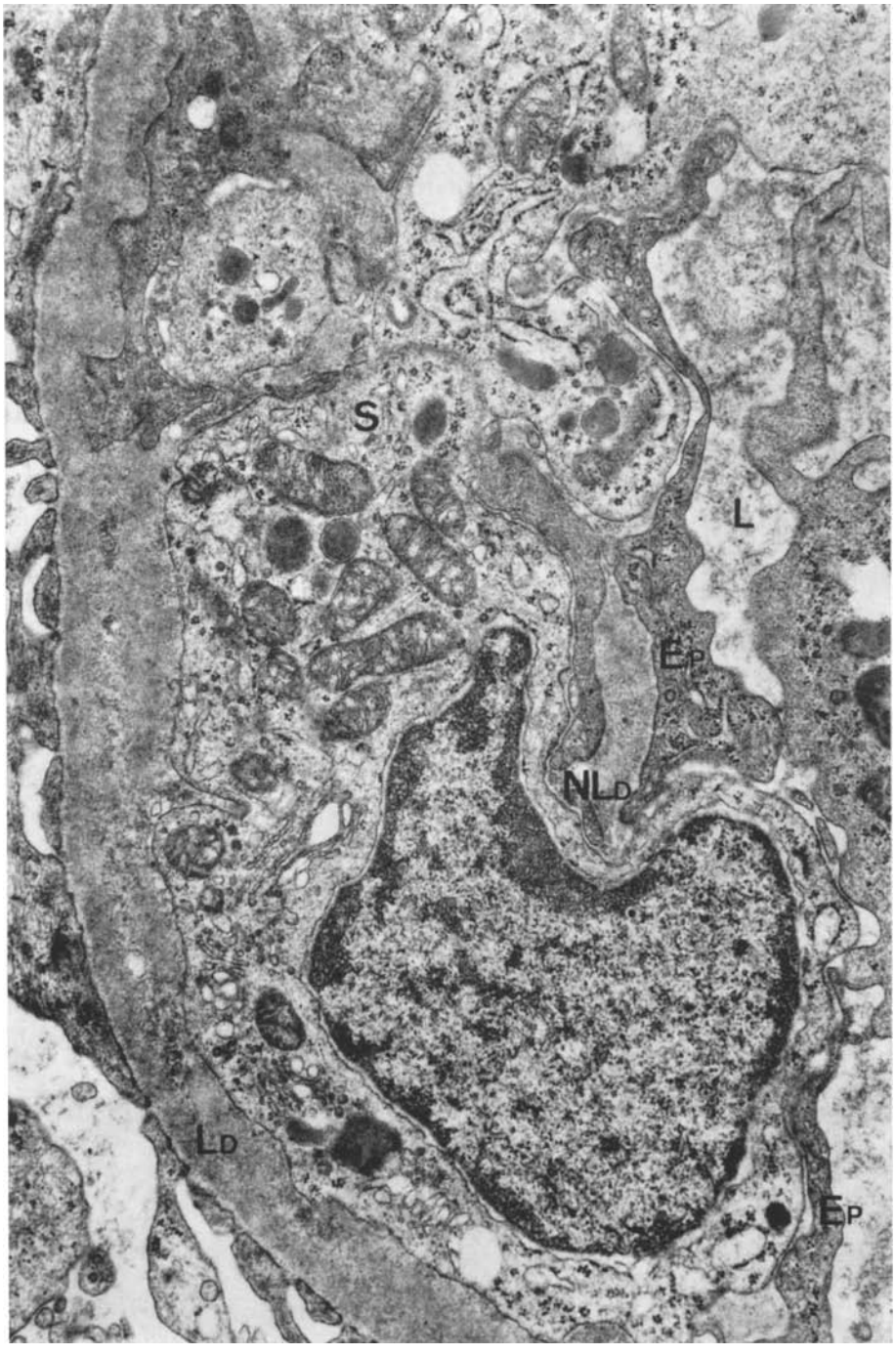

Fig. 3. Subendothelially fixed monocyte (*S*) with a material of the lamina densa type (*NLD*) forming between the endothelium and monocyte. Lamina densa (*LD*). Lumen (*L*). Endothelium (*Ep*). ×19500

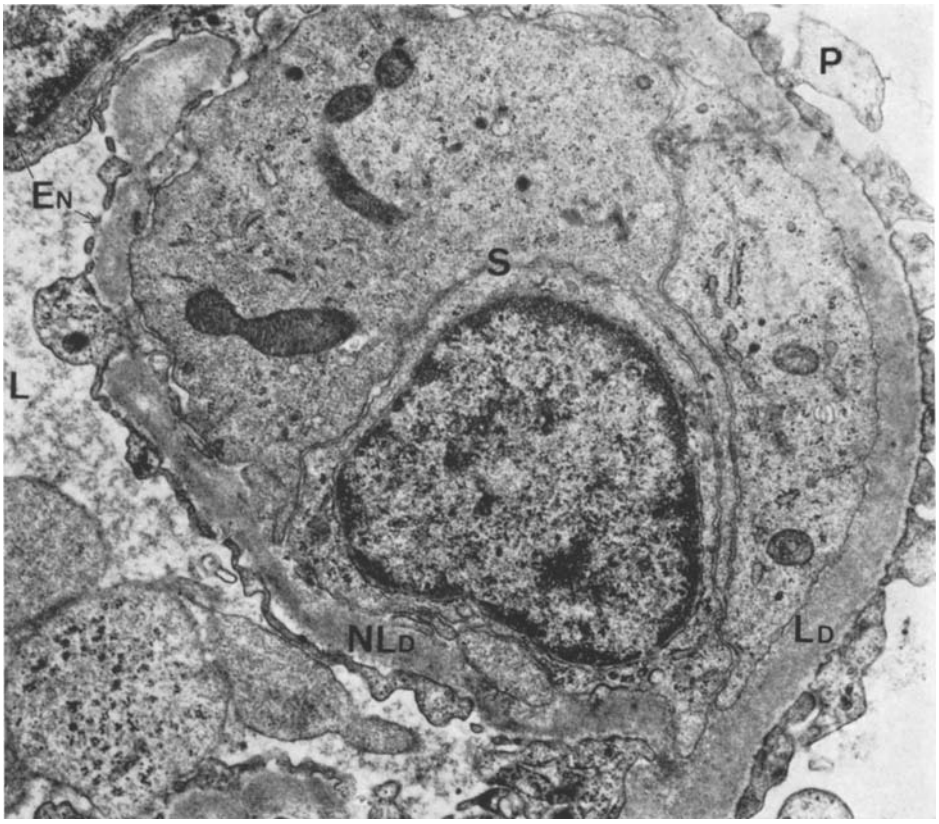

Fig. 4. Blood mononuclear cells (*S*) fixed within the capillary wall and neoformation of subendothelial material of the lamina densa type (*NLD*). Lumen (*L*). Foot processes (*P*). Lamina densa (*LD*). Endothelium (*EN*). ×17460

was that the second internal layer of the lamina densa which completely surrounded or with certain interruptions, the body of the blood cells fixed in the subendothelium (Fig. 4). It bulged into the capillary lumen narrowing it, was somewhat thinner and of more irregular thickness and was entirely covered by the endothelium. This internal layer sometimes exhibited small discontinuities through which the processes of the sequestered cells passed into the lumen. The nucleus and cytoplasm of the "sequestered cells" maintained their general architecture, presenting numerous clustered cytoplasmic organelles. The cytoplasm often contained filamentous structures or vacuolations.

In some areas in which the cellular sequestration phenomenon had occurred on a wide scale, clusters of these cells showing signs of degeneration: pyknosis, vacuolization, crystalline inclusions, etc., could be seen (Fig. 5). Due to damage of the capillary walls, the lumina gradually narrowed up to disappearance.

It is of interest to mention that the interstitial inflammatory infiltrate, present in the renal biopsy samples examined, were predominantly formed of mononuclear cells (lymphocytes, monocytes).

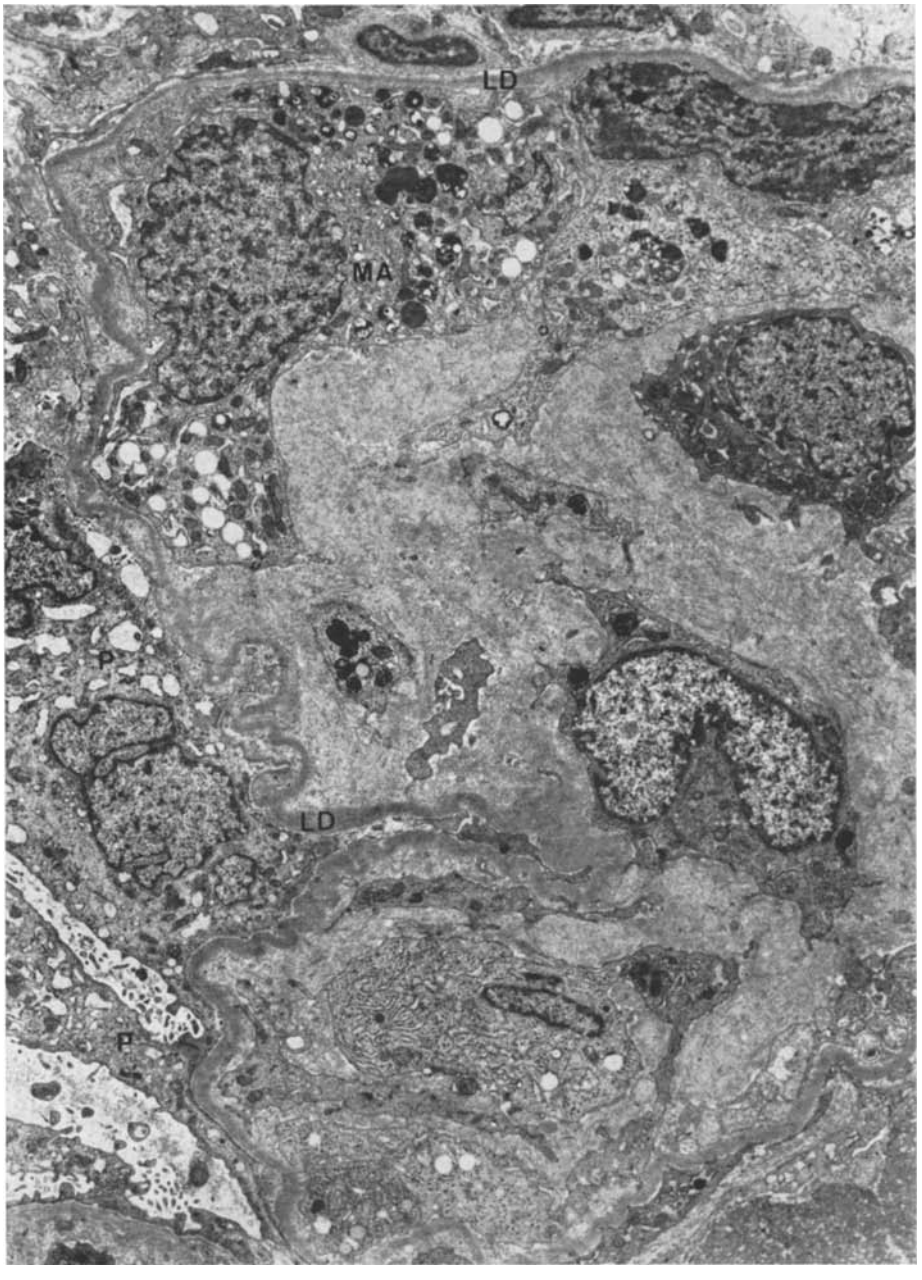

Fig. 5. Sclerosis of the glomerular loops with cells of the macrophage type (*MA*), vacuolization and droplets of fat within the cytoplasm. Podocytes (*P*). Lamina densa (*LD*). ×4980

Discussion

The particularities of the case studied consist in evidence of the essential participation of the blood mononuclear cells—lymphoid cells, monocytes and plasma cells—in the production of glomerular damage, described in light microscopy as proliferative glomerulitis, and in the evolution towards sclerosis of such lesions. The lesions appear to evolve according to a certain sequence: deposition of fibrinoid material—which may also encompass gammaglobulins (Paronetto and Strauss, 1962)—accompanied by circulatory disturbances; the development in some capillaries of endothelial cells giving the aspect of an active endothelium; selective sticking to this endothelium of a great number of leukocytes (mainly of the lymphoid and monocytic type); passage through the capillary endothelium of these cells and their fixation below the endothelium; the formation of a new lamina densa by the endothelial cells and sequestration of the blood cells within the thickness of the capillary wall, concomitant to thickening of the latter; the evolution of some of these cells towards histiocytic-like cells and the onset of degenerative alterations in others. Initiation of the glomerular lesions may be explained as consecutive to the deposition of immune complexes Dixon *et al.*, (1965) or fibrinoid material (Vassalli, Simon and Rouiller, 1963; McCluskey, Vassalli, Gallo and Baldwin, 1966; Simon and Chatelanat, 1963; Browne, Hutt, Reger and Smith, 1963) thus accounting for both local leukocytosis and leukocytic margination. The numerous areas examined in which leukocytes were frequently found, especially mononuclears in close contact with the capillary endothelium, shows that this is a constant phenomenon in the lesions studied by us. Kirsch (1957) noted the normal existence of certain fine processes on the surface of the vascular endothelium, to which the circulating leukocytes may become attached, and connected this aspect to leukocytic margination. Lockwood and Allison (1966), studying the leukocytic clusters formed after phagocytosis, showed that their finger-like projections formed interdigitations, maintaining a distance of approximately 1000 Å between the cells, never less than 250 Å. These data are agreement with our findings concerning interdigitations between leukocytic elements and the capillary endothelia. In the lesions studied the phenomenon of adhesion of the leukocytes to the endothelia must be attributed to the inflammatory process of the respective glomeruli. However, in particular, the adhesion of a great number of mononuclear leukocytes to the endothelia may be correlated to the observations of Ebert (1952), who noted similar phenomena in the hypersensitivity process. Inflammation at the level of the glomeruli also explains why extravasation of the leukocytes is favoured, and particularly their arrest between the endothelium and lamina densa and, probably, their fixation in large numbers at this site. When these cells migrate through the capillary endothelium, the latter often manifests an active role, expressed by cytoplasmic processes which surround the cells in the course of extravasation. The high incidence of mononuclear blood cells implicated in these alterations, suggest the intervention of the hypersensitivity phenomenon in their formation (Kosunen *et al.*, 1963). Similarly, from the works of Gell (1959) and Flax and Caulfield (1963) it results that in the course of hypersensitivity processes, the endothelia acquire new proper-

ties, such as the phagocytosis of carbon particles. Our findings also suggest the possibility of a new behaviour of the endothelia to leukocytic extravasation.

Fixation of some of the cells, after extravasation, between the endothelia and basement lamina is in agreement with the observations of Marchesi and Florey (1964), according to whom part of the migrating leukocytes at the level of the venules in the inflammatory lymph node tissue stop between the endothelium and the periendothelial sheath. Both this sheath in the postcapillary venules (Marchesi and Gowans, 1964) and the lamina densa in the renal glomeruli, form a barrier for the leukocytes.

Penetration of the leukocytes within the capillary walls, accompanied by separation of the endothelium from the lamina densa brings about, as results from our findings, the formation of a new lamina densa by the endothelial cells. Evidence of the participation of the endothelial cells to the formation of the lamina densa was also supplied by Pierce and Midgley (1963) by immunohistochemical studies. This synthesis role might become more accentuated under pathologic hypersensitivity conditions. Moreover, alteration of the endothelial cells and the great number of cytoplasmic organelles (ribosomes) suggest a more active cellular metabolism.

Repeated extravasation of the mononuclear leukocytes with their subendothelial fixation, followed by the formation of new structures of the lamina densa type, thus appears to represent one of the mechanisms of thickening of the glomerular capillary walls. This mechanism of thickening of the capillary walls, evolving towards sclerosis, differs from that described by Vassalli, Simon and Rouiller (1963), who consider it to be connected with proliferation of the intercapillary cells or with that described by Steiner (1962a, b) who incriminates a double endothelial and mesangeal proliferation. The mechanism described above may be probably associate with those described by Vassalli *et al.* (1963) and Steiner (1962a, b).

At the same time, processes such as that described, determine sequestration of a large number of leukocytes within the thickness of the basement membranes, thus enlarging them and accounting for the degenerative alterations they undergo probably due to the new, accentuated hypoxia conditions.

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