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Extracellular Roughly Granular Material (ERGM) in Human Glomerular Tufts

An Ultrastructural Study

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Summary. Extracellular roughly granular material different from membranoid material or collagen, amyloid, fibrin and immune deposits was observed in 19 out of 237 biopsy specimens. The nuclear origin of this material is discussed.

In the present paper we will describe an extracellular roughly granular material (ERGM) present in the glomerular tufts, and study its significance, occurrence and prevalence in various nephropathies. This material was briefly mentioned in earlier papers in relation to membranoproliferative glomerulonephritis (Bariéty *et al.*, 1971; Berger *et al.*, 1971). It is quite distinct from the extracellular materials ordinarily encountered in glomerular pathology: membranoid material or collagen, amyloid, fibrin and immune deposits.

Material

237 renal biopsies performed on 225 patients were studied by light and electron microscopy and 219 of these were studied by immunohistochemistry.

The 237 biopsies showed a broad spectrum of diagnoses: idiopathic proteinuria or nephrotic syndrome with minimal glomerular changes, membranous nephropathy (Ehrenreich *et al.*, 1968; Bariéty *et al.*, 1970), membranoproliferative glomerulonephritis (Burkholder *et al.*, 1970; Bariéty *et al.*, 1971), acute glomerulonephritis, glomerulonephritis with IgA mesangial deposits (Berger, 1969; Druet *et al.*, 1970a), focal hyalinosis (Habib *et al.*, 1971), systemic lupus erythematosus (SLE), immediate renal graft biopsies, kidney transplants more than one year after transplantation, amyloidosis, diabetic nephropathy, glomerulonephritis with extracapillary proliferation (rapidly progressive glomerulonephritis) (Habib *et al.*, 1971), rheumatoid arthritis without proteinuria, toxemia of pregnancy, primary arterial hypertension, miscellaneous and unclassified lesions.

Methods

The techniques for light microscopy and for immunohistochemistry have been previously described (Bariéty *et al.*, 1970; Druet *et al.*, 1970b). For electron microscopy, portions of renal cortex were fixed by immersion at 4°C for $1^1/_2$ hours in 1.55% glutaraldehyde and were post-fixed for 1 hour in 2% osmium tetroxide. Both solutions were buffered at pH 7.35 with Millonig's buffer. Dehydratation was effected through a graded series of alcohols and propylene oxide. Specimens were embedded in epoxy resin, stained with aqueous uranyl acetate and with lead citrate, and were examined in a Zeiss EM 9 electron microscope.

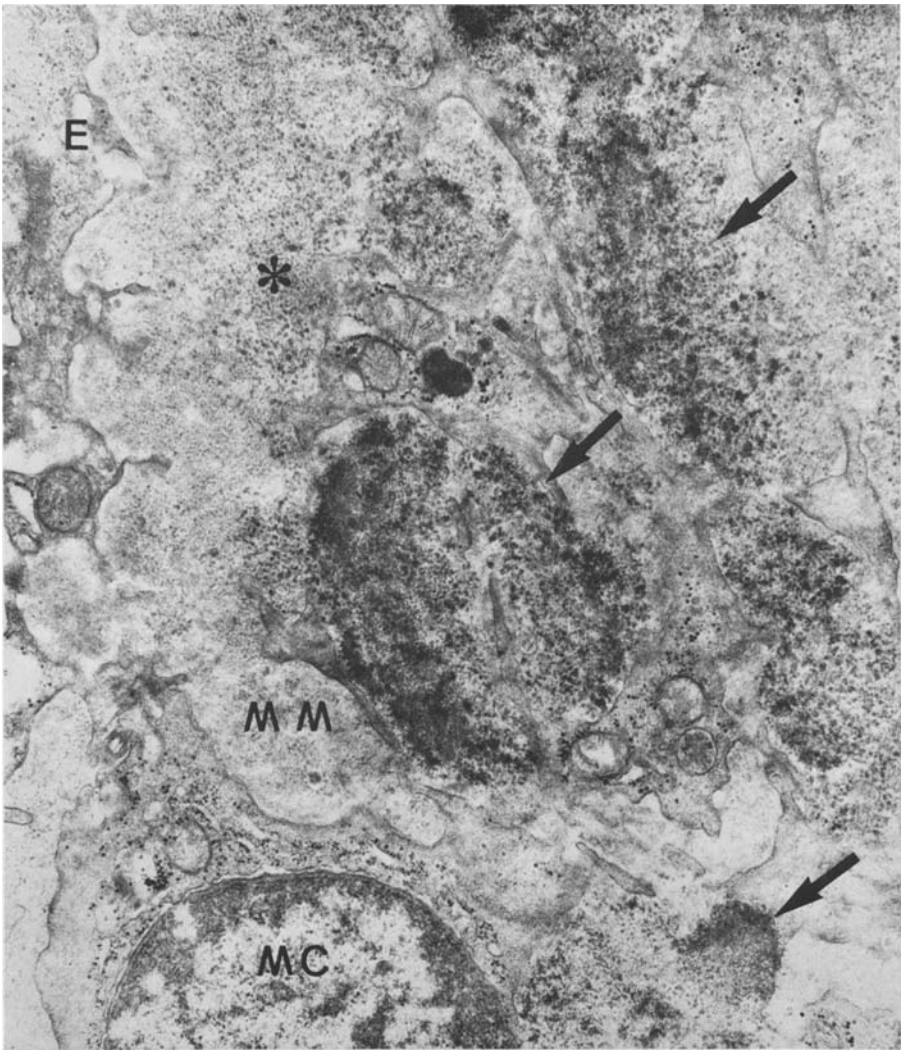

Fig. 1. Deposits of extracellular roughly granular material (ERGM), dense (arrow) or sparse (asterisk), in the mesangial matrix. *MC* mesangial cell, *MM* mesangial matrix, *E* endothelial cell. ×21900

Results

Electron Microscopy. This material was observed in 21 out of 237 renal biopsies studied by electron microscopy. It was found in certain segments of some glomeruli. It was generally detected in the membranoid material of the glomerular tufts: in the basement membrane and/or in the mesangial matrix centrolobular or interposed (Arakawa *et al.*, 1969) between the basement membrane and the endothelial cell. Sometimes ERGM was seen between the membranoid material and the plasma membrane of an adjacent cell (generally mesangial or endothelial cell).

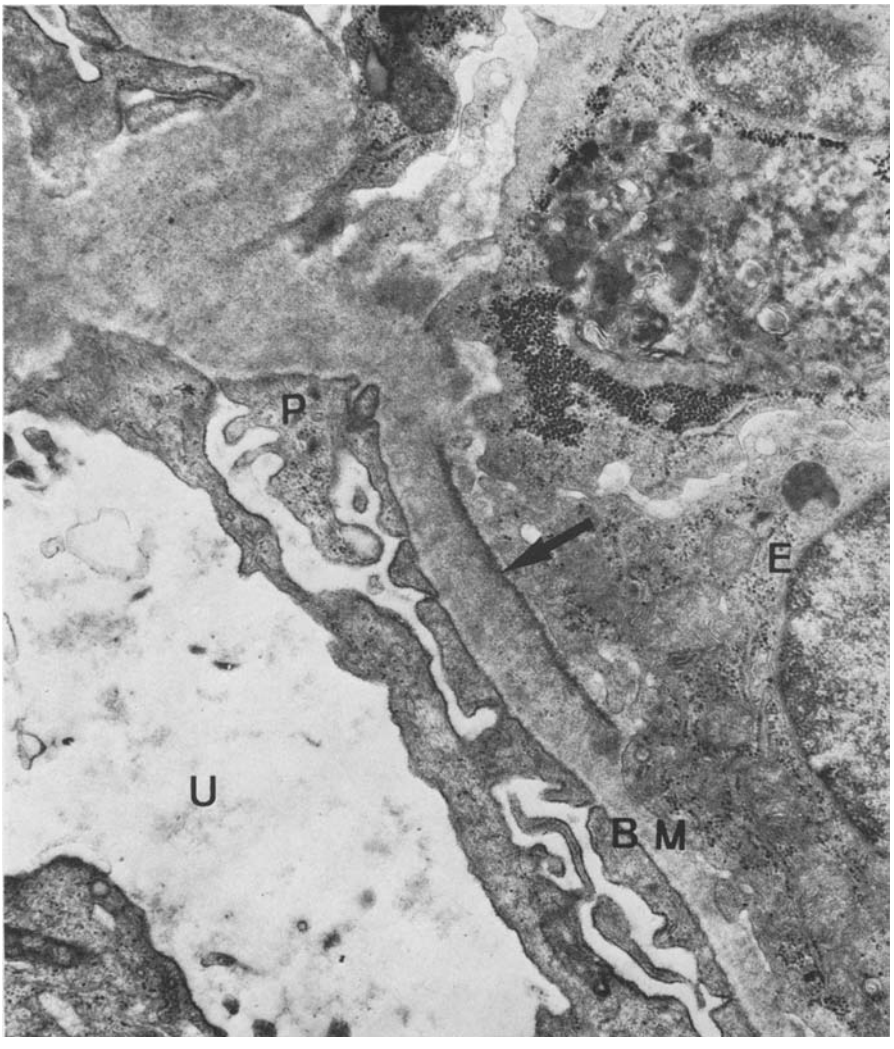

Fig. 2. Linear deposit of ERGM localized in the luminar side of a segment of the basement membrane (arrow). *E* endothelial cell, *BM* basement membrane, *P* pedicels, *U* urinary space. ×21900

The ERGM appeared under three aspects: (1) powdery trails, (2) isolated heterogeneous masses, (3) heterogeneous masses coupled with powdery trails.

1. *The powdery trails* were made of fine particles. The dispersion of these particles was variable.

a) They were sometimes scattered in an irregular manner in the mesangial membranoid material that they could entirely impregnate (Fig. 1).

b) They could be distributed in the capillary loops always in the membranoid material and with a gradient: highest concentration of ERGM being on

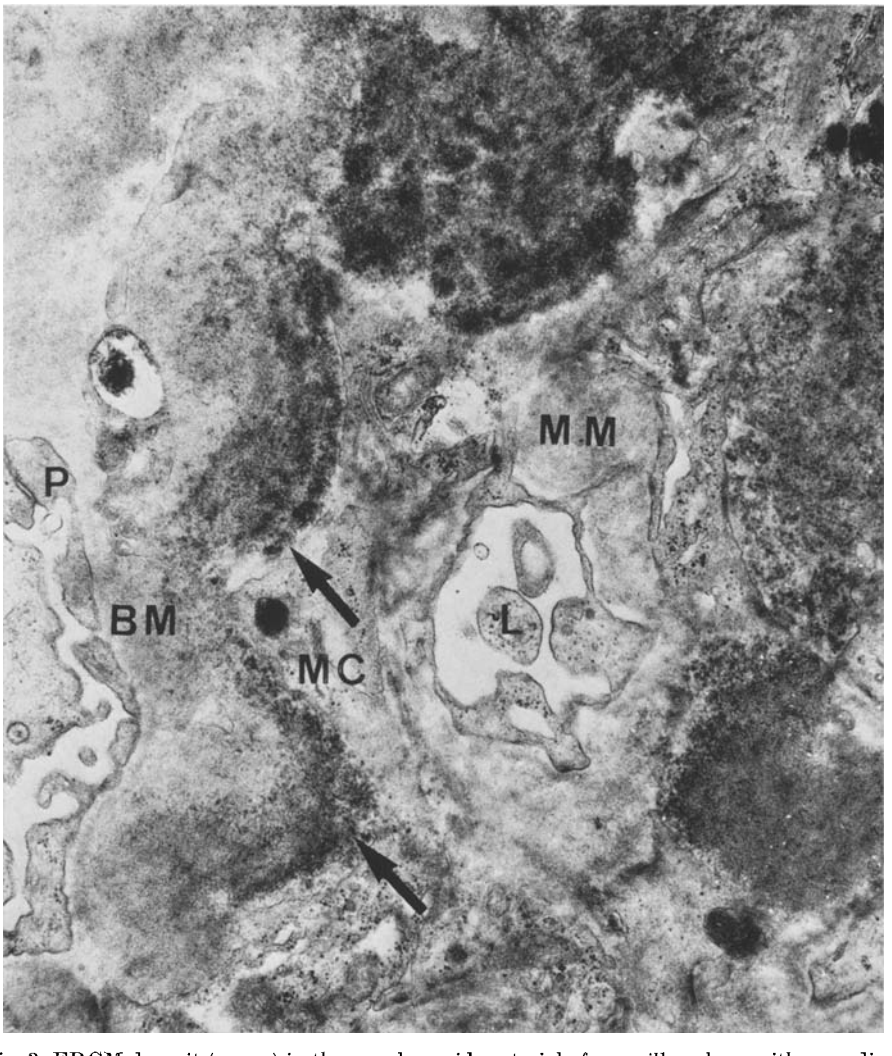

Fig. 3. ERGM deposit (arrow) in the membranoid material of a capillary loop with a gradient (arrow), the highest concentration being on the luminar side of the capillary loop, the lowest towards the urinary space. *BM* basement membrane, *MC* mesangial cell interposed in the capillary loop, *MM* mesangial matrix, *L* lumen, *P* pedicels. ×19500

the luminar side of the capillary loop and lowest concentration, towards the urinary space (Figs. 2, 3, 6, 7, 8).

2. *The isolated heterogeneous masses* were between 3000 and 33000 Å in diameter. They were constituted of a surface which appeared granulated (granules from 200 to 300 Å) with on it one or more very electron dense areas (Figs. 4, 5). The masses were more or less round or oblong in shape. They were hemmed by an electron dense hatching of more or less regular thickness

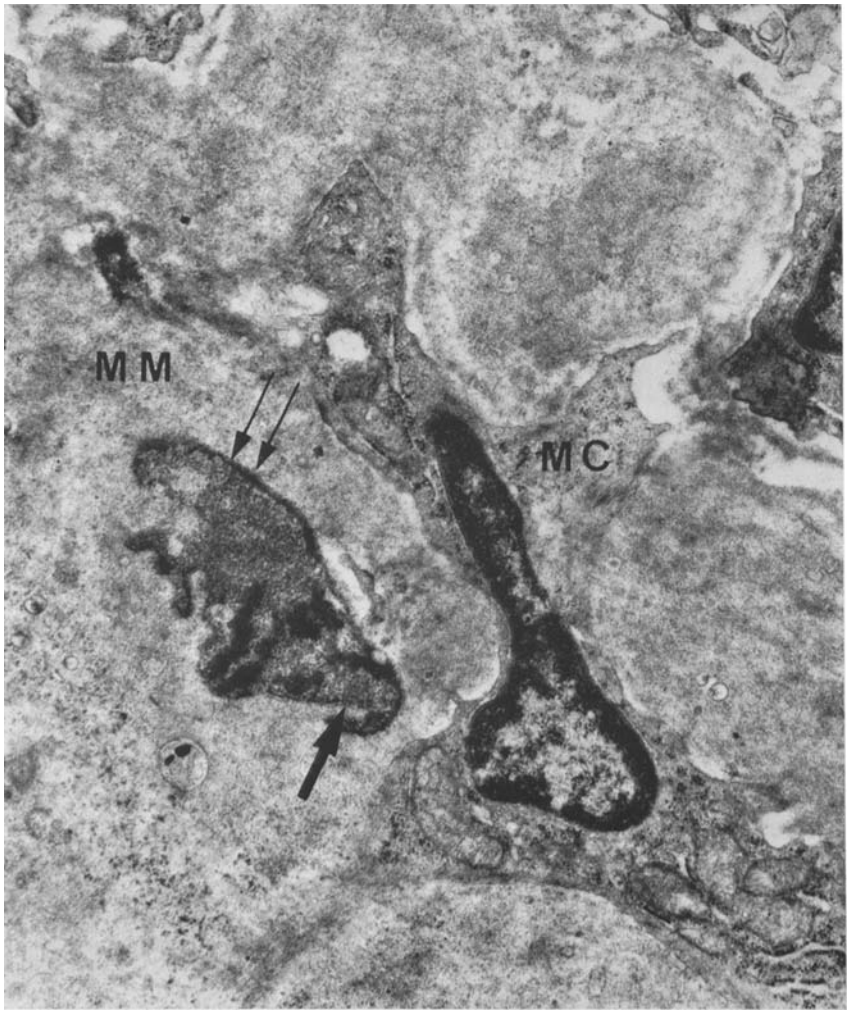

Fig. 4. Isolated heterogeneous mass of ERGM in the mesangial membranoid material (double arrows). It isconstituted of several electron dense areas. Note the thin membrane contiguous to the mass (arrow). *MC* mesangial cell, *MM* mesangial matrix. ×21900

(Fig. 5). This hatching gave a neat limit to these masses. In addition, sometimes a thin membrane different from the plasma membrane of the neighbouring cells, has been seen near to the hatching (Figs. 4, 7). The heterogeneous masses were only seen in the mesangial area. They were often found grouped (Fig. 5).

3. *The heterogeneous masses* in continuity (Figs. 6, 7) or contiguous (Fig. 8) to the powdery trails showed exactly the same characteristics as their constituents above described.

Light Microscopy. When ERGM was particularly abundant, it was possible to see it by light microscopy. With Massons's method, it appeared deep ruby,

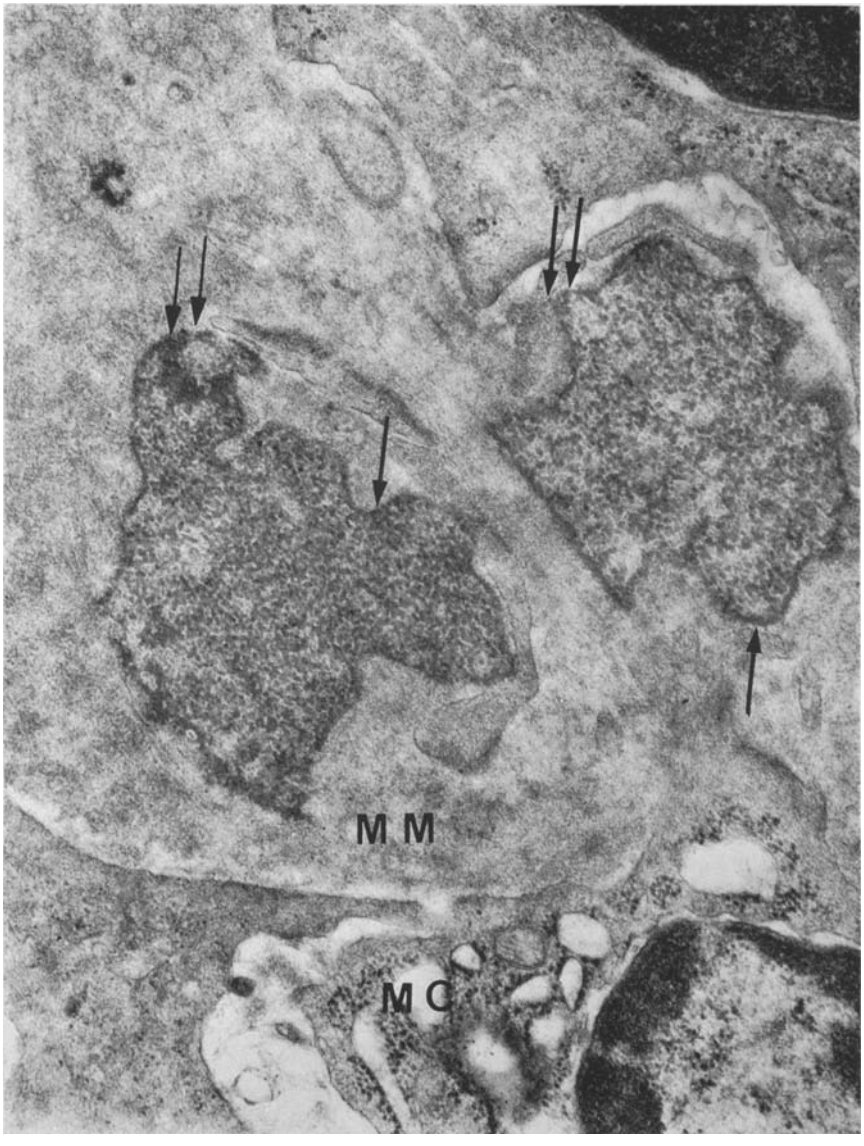

Fig. 5. Grouped heterogeneous masses of ERGM in the mesangial matrix (double arrows). Note the hatching of constant thickness and constant electronic density (arrow). *MM* mesangial matrix, *MC* mesangial cell. × 36000

purplish-blue or mauve. It appeared red with hematoxilin-phloxin-saffron. The fact that this material was not distinguishable from membranoid material, neither with periodic acid Shiff-colloidal iron stain nor by the Marinozzi stain, may be due to its place on the membranoid material.

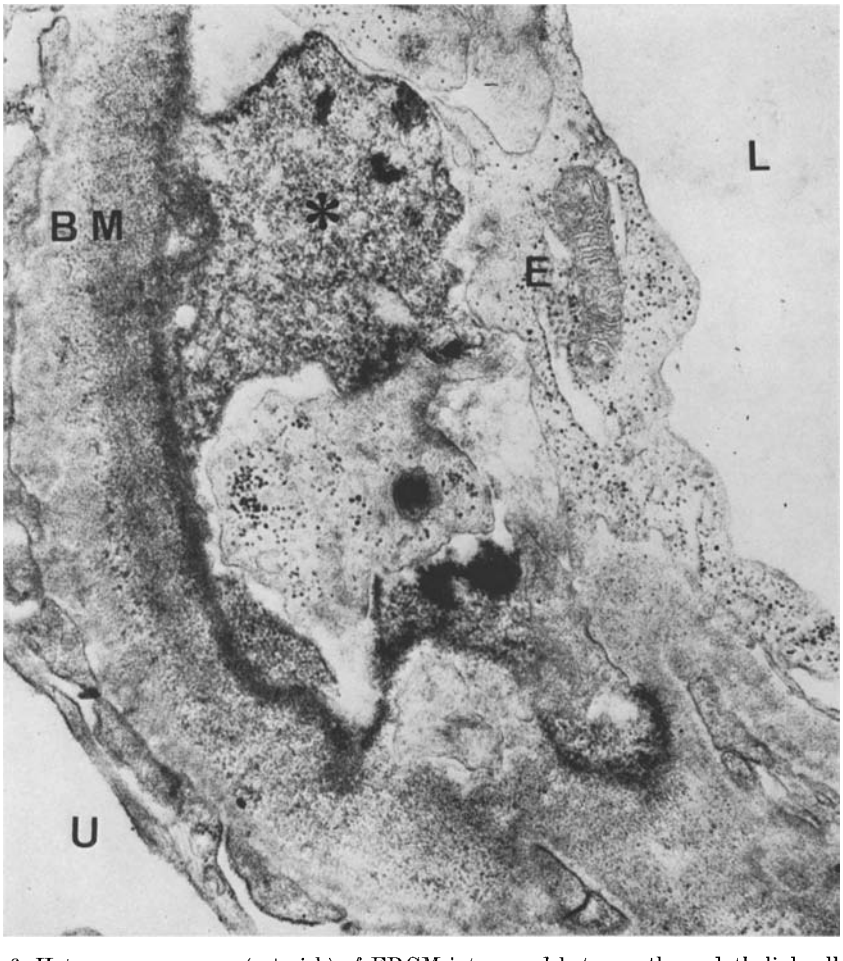

Fig. 6. Heterogeneous mass (asterisk) of ERGM interposed between the endothelial cell and the basement membrane and in continuity with the powdery trails. Note again the gradient in the basement membrane, with the highest concentration on the luminar side of the capillary loop, the lowest towards the urinary space. *BM* basement membrane, *E* endothelial cell, *L* lumen, *U* urinary space. ×24 000

Distribution According the Different Diseases. In our series, ERGM was only found in cases of endocapillary proliferative glomerulonephritis, idiopathic membranoproliferative glomerulonephritis, acute glomerulonephritis and systemic lupus erythematosus glomerulonephritis (Table 1).

Immunochemistry. The results are indicated in the Table 2.

Discussion

The ERGM possesses ultrastructural characteristics which allow to distinguish it easily from membranoid material, collagen, amyloid and ordinary immune

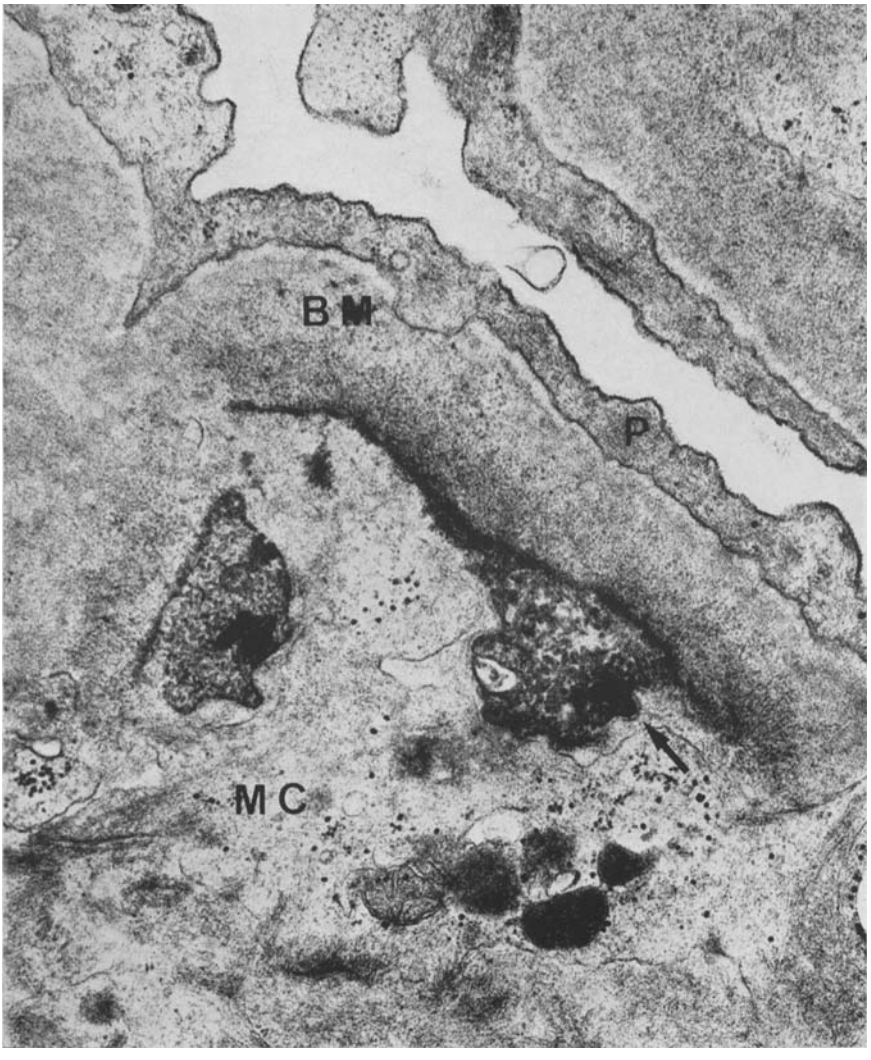

Fig. 7. Heterogeneous mass of ERGM in continuity with a powdery trail. Note the thin membrane, different from the plasma membrane of the neighbouring cell (arrow). *BM* basement membrane, *MC* interposed mesangial cell, *P* fused pedicels. ×28000

deposits (Churg *et al.*, 1972). The powdery trails and the isolated heterogeneous masses can be considered as being of the same nature since they can be seen in close continuity.

This study does not allow us to establish the nature of the ERGM. However they are several arguments in favour of a nuclear origin:

1. The shape and dimensions of the heterogeneous masses are compatible with a nuclear origin, especially their inner arrangement which appeared sometimes to be very similar to that of a nucleus.

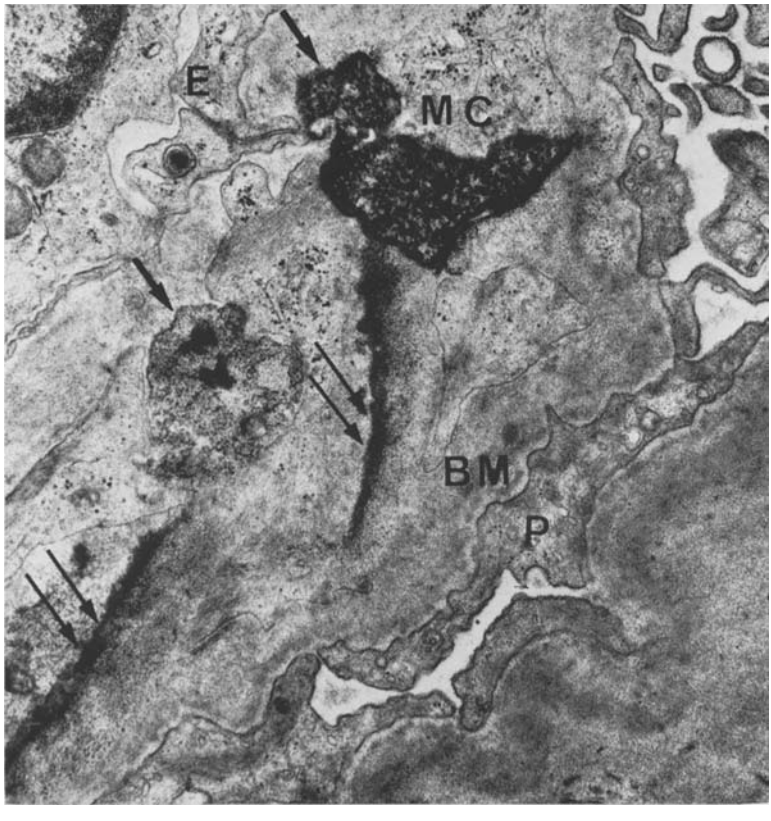

Fig. 8. Heterogeneous masses of ERGM interposed in the capillary loop (arrow). They are contiguous to the powdery trails (double arrows). Note the gradient (double arrows). *BM* basement membrane, *MC* mesangial cell, *P* fused pedicels, *E* endothelial cell. ×18000

In addition, on some sections, the masses are surrounded by a membrane distinct from the plasma membrane of the neighbouring cells, and recalling the remnants of perinuclear space.

2. ERGM was generally found in proliferative glomerulonephritis characterized by mesangial cell proliferation and leukocytic accumulation. Accordingly, the ERGM was found in glomerulonephritis with a very high content of nuclear material.

3. ERGM was seen most frequently in proliferative glomerulonephritis with high content of immune deposits. The anti-B_1C globulin sera were often fixed in large amounts in the glomeruli. This fact suggests that the complexes with very high complement content are endowed with a high cytolytic activity.

However, it is not possible to identify the ERGM with B_1C globulin, since the anti-B_1C globulin sera are fixed on the glomerulus, diffusely and in large masses, whereas ERGM localization is much more focal. In addition, there was no anti-B_1C globulin serum fixation in two cases where ERGM was present.

Table 1. Occurence of ERGM in different instances

Diagnoses	Cases studied	Biopsies studied	Biopsies with ERGM observed
Minimal glomerular changes	29	29	0
Membranous nephropathy	13	14	0
Membranoproliferative Glomerulonephritis	29	33	13[a]
Acute glomerulonephritis	8	9	3[b]
Glomerulonephritis with IgA mesangial deposits	19	19	1
Focal hyalinosis	3	4	0
Systemic lupus erythematosus	24	24	1
Rapidly progressive glomerulonephritis	6	6	1
Immediate renal graft biopsies	28	28	0
Kidney transplants more than one year after transplantation	20	25	1
Amyloidosis	5	5	0
Diabetic nephropathy	7	7	0
Rheumatoid arthritis	5	5	0
Toxemia of pregnancy	3	3	0
Primary hypertension	2	2	0
Miscellaneous	24	24	1

[a] ERGM was detected in the renal samples of three patients biopsied twice.
[b] ERGM was detected in the renal samples of a patient biopsied twice.

Table 2. Immunohistochemical results in the biopsies where ERGM was detected by electron microscopy

Cases	IgA	IgG	IgM	Fibrin	β_1C
1	0	0	0	0	++
2	+	−	−	+	+
3	−	−	0	±	+
4	−	+	+	+	+
5	+	+	+	+	++
6	−	−	−	−	−
7	0	+	0	0	+
8	−	−	−	−	±
9	−	−	−	±	+
10	−	+	+	±	+
11	±	−	±	+	++
12	−	−	+	+	+
13	−	+	+	−	+
14	−	+	−	+	+
15[a]	−	+	+	−	+
15[b]	+	+	+	−	−
16	0	0	0	0	0
17[a]	±	+	±	±	++
17[b]	+	+	+	−	+
18	+	+	+	+	++
19	−	+	−	±	++

0 = not done; − = no fixation; ± = discrete fixation. + = clear fixation; ++ = very important and diffuse fixation.
[a] First biopsy.
[b] Second biopsy.

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References

Arakawa, M., Kimmelstiel, P.: Circumferential mesangial interposition. Lab. Invest. **21**, 276–284 (1969).

Bariéty, J., Druet, P., Lagrue, G., Samarcq, P., Milliez, P.: Les glomérulopathies "extramembraneuses" (GEM). Etude morphologique en microscopie optique, électronique et en immunofluorescence. Path. et Biol. **18**, 5–32 (1970).

Bariéty, J., Druet, P., Loirat, P., Lagrue, G.: Les glomérulonéphrites pariétoprolifératives (GNPP). Etude histopathologique en microscopie optique, électronique et en immunohistochimie de 49 cas. Corrélations anatomocliniques. Path. et Biol. **19**, 259–283 (1971).

Berger, J.: IgA glomerular deposits in renal disease. Transplant. Proc. **1**, 939–944 (1969).

Berger, J., Yaneva, A., Hinglais, N.: Immunofluorescence des glomérulonéphrites. Actual. néphrol. Hôp. Necker, p. 161–177. Paris: Flammarion 1971.

Burkholder, P. M., Marchand, A., Krüger, R. P.: Mixed membranous and proliferative glomerulonephritis: a correlative light, immunofluorescence and electron microscopic study. Lab. Invest. **23**, 459–479 (1970).

Churg, J., Grishman, E.: Ultrastructure of immune deposits in renal glomeruli. Ann. intern. Med. **76**, 479–486 (1972).

Druet, P., Bariéty, J., Bernard, D., Lagrue, G.: Les glomérulonéphrites primitives à dépôts mésangiaux d'IgA et d'IgG. Etude clinique et morphologique de 52 cas. Presse méd. **78**, 583–587 (1970a).

Druet, P., Leloup, B., Bariéty, J., Lagrue, G.: Application à la pathologie rénale de la technique des anticorps couplés aux enzymes. Europ. J. clin. biol. Res. **15**, 119–122 (1970b).

Ehrenreich, T., Churg, J.: Pathology of membranous nephropathy. In: Pathology Annual (Sommers, S.C. ed.), p. 145–186. New York: Appleton 1968.

Habib, R., Kleinknecht, C.: The primary nephrotic syndrome in childhood. Classification and clinicopathologic study of 406 cases. In: Pathology Annual (Sommers S.C., ed.), p. 417–474. New York: Appleton 1971.

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