Virchows Arch. Abt. A Path. Anat. 357, 125—135 (1972)


# Round "Virus-Like" Extracellular Particles in Glomerular Tufts

## An Electron Microscopic Study of 190 Human Renal Biopsies

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Received May 27, 1972

*Summary.* The round "virus-like" extracellular particles (REP), observed in 86 of 190 renal biopsies are round or oval entities with an outer diameter ranging from 400 to 1200 Å. Sometimes they are electron-dense, homogeneous particles; sometimes they assume the shape of small vesicles with or without an electron-dense core. The REP are localized between the podocytes or the mesangial cells on one side and membranous material (glomerular basement membrane or mesangial matrix) on the other side. Their number ranges from a few units to several hundreds. They occur in widely differing instances. No evidence supports the hypothesis of their viral identity; a localized degenerative process is more likely.

Several authors have mentioned the presence of small round or oval particles in the glomeruli: in subepithelial spaces of the capillary wall (Rowlands *et al.*, 1970; Bariéty *et al.*, 1971; Churg and Grishman, 1972), in basement membrane (Busch *et al.*, 1971) or in extramembranous deposits (Bariéty *et al.*, 1970). Those particles were suggested to be herpes-like virus (Busch *et al.*, 1971) or particular forms of protein deposits (Churg and Grishman, 1972) or they were simply called "virus-like" (Rowlands *et al.*, 1970).

In the present paper we will study the shape and the localization of such particles as well as their prevalence and occurence according to the types of nephropathies which necessitated the biopsies.

## Material

190 renal biopsies were studied by light and electron microscopy and 138 of these were also studied by immunohistochemistry. The 190 renal biopsies showed a broad spectrum of diagnoses (Table 1): amyloidosis, diabetes mellitus, systemic lupus erythematosus with or without nephropathy, rheumatoid arthritis without nephropathy, toxemia of pregnancy, essential hypertension, chronic pyelonephritis, kidney transplants more than one year after transplantation, immediate renal graft biopsies, acute glomerulonephritis, minimal glomerular changes with or without nephrotic syndrome, focal hyalinosis (Habib and Kleinknecht, 1971), glomerulonephritis with diffuse subepithelial deposits (Bariéty *et al.*, 1970; Rosen, 1971), membranous and proliferative glomerulonephritis (Burkholder *et al.*, 1970; Bariéty *et al.*, 1971; Seymour *et al.*, 1971), glomerulonephritis with extracapillary proliferation (Habib and Kleinknecht, 1971; Seymour *et al.*, 1971), glomerulonephritis with IgA mesangial deposits (Berger, 1969; Druet *et al.*, 1970), miscellaneous and unclassified renal lesions.

Table 1. Occurrence of REP in different instances

| Diagnosis | Cases studied | REP observed |
|---|---|---|
| Amyloidosis | 6 | 2 |
| Rheumatoid arthritis | 2 | 1 |
| Diabetes | 8 | 7 |
| SLE | 18 | 9 |
| Toxemia | 3 | 0 |
| Essential hypertension | 5 | 2 |
| Pyelonephritis | 3 | 1 |
| KT | 11 | 6 |
| RGB | 30 | 14 |
| Minimal glomerular changes | 19 | 7 |
| Focal hyalinosis | 6 | 2 |
| GN with diffuse extramembranous deposits | 14 | 9 |
| Membranoproliferative GN | 13 | 6 |
| GN with IgA mesangial deposits | 17 | 6 |
| Acute GN | 11 | 5 |
| GN with extracapillary proliferation | 4 | 1 |
| Miscellaneous and unclassified | 20 | 8 |

SLE = systemic lupus erythematosus; KT = kidney transplants more than one year after transplantation; RGB = immediate renal graft biopsies.

## Methods

The techniques for light microscopy and for immunohistochemistry have been previously described (Bariéty *et al.*, 1970; Druet *et al.*, 1970). 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.

## Results

REP were observed in 86 out of 190 renal biopsies. They were seen close to the podocytes and to the mesangial cells but never close to the endothelial cells or to the cells of the Bowman's capsule.

REP were round or oval juxtaposed structures of different sizes along the outer side of the plasma membrane (Figs. 1–8). Most often they were spherical homogeneous electron dense structures. Sometimes, they were granules appearing as clear areas limited by a thin membrane. Other particles assumed the shape of "cockades": an electron dense center was surrounded by a lucent halo, limited by a single membrane (Fig. 2). The total diameter of the particles ranged from 400 to 1200 Å with an average diameter of 500 Å. Their number varied, ranging from a few granules in a single linear pattern, to hundreds of granules occuring in clusters (Fig. 1). However, rarely more than one cluster was observed in one section of a glomerular tuft.

Most often, REP were observed near the podocytes, between the basement membrane and the plasma membrane of the podocyte. Usually, they were present:

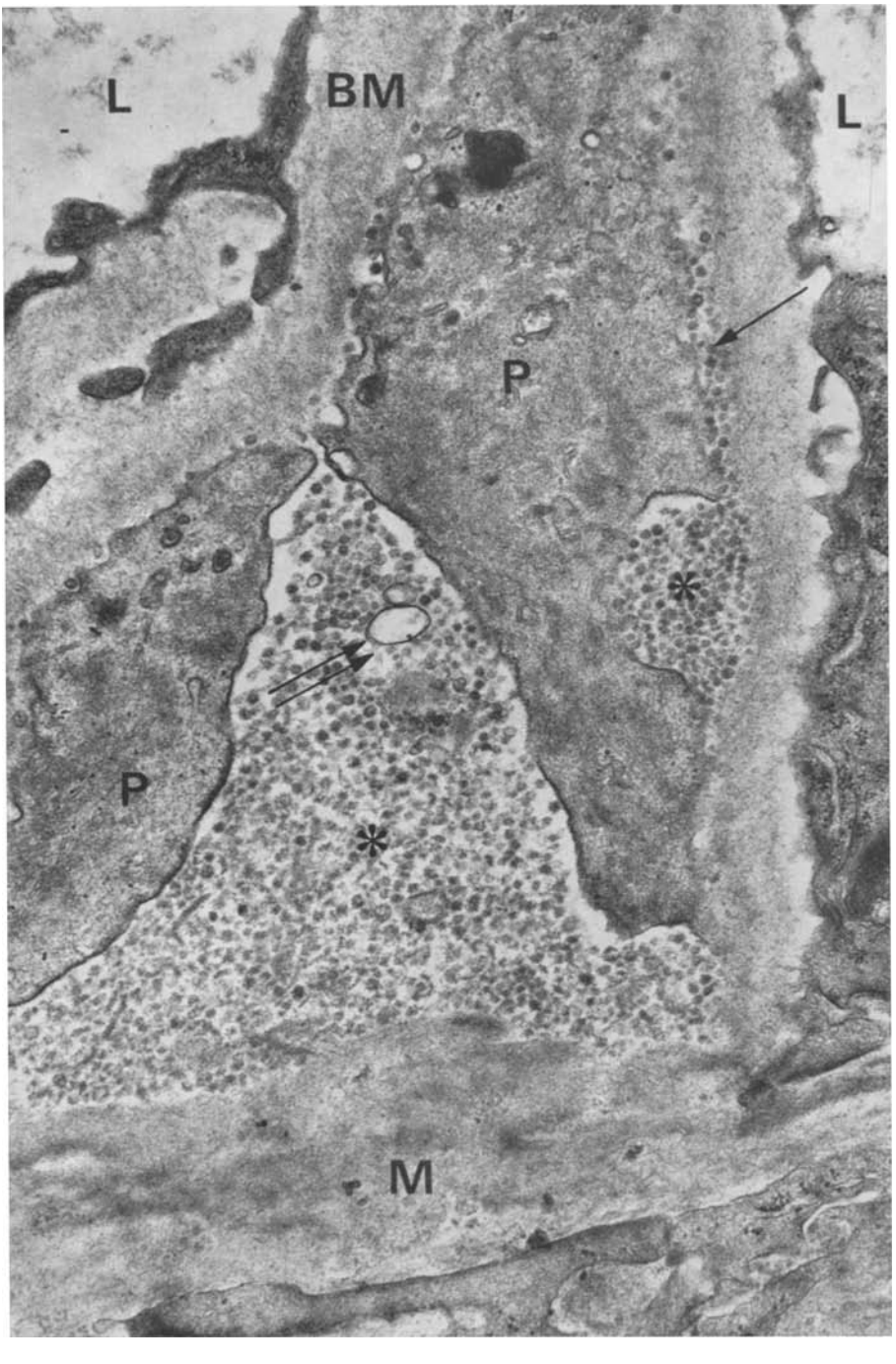


Fig. 1. SLE. Clusters of small cytoplasmic particles (*). Small cell particles set in a linear pattern (arrow). Remaining cytoplasmic vesicules (arrows). Podocyte (*P*), lumen (*L*), mesangial matrix (*M*), basement membrane (*BM*) (×30500)

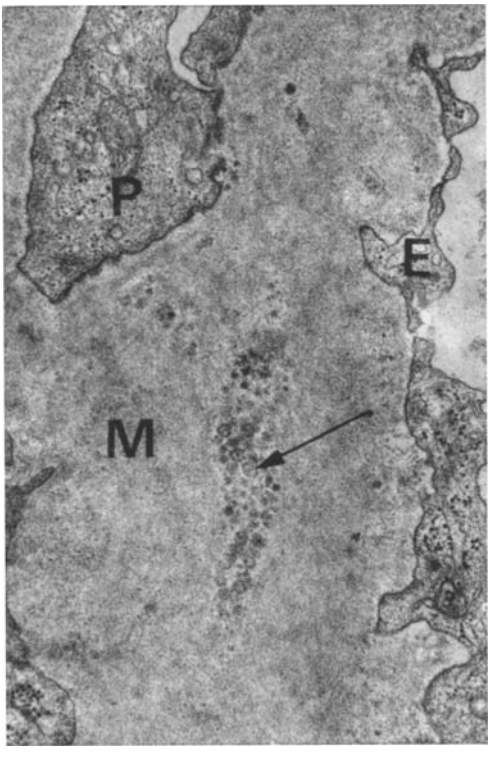


Fig. 2. Nephroangiosclerosis. REP enclosed within the mesangial matrix. Some of them assume the shape of cockades (arrow). Mesangial matrix (*M*), podocyte (*P*), endothelial cell (*E*) (×27800)

1. beneath fused foot processes (Figs. 1, 3, 4); 2. adjacent to the cytoplasmic strips which tightly joined together two contiguous capillary loops (Figs. 3, 4); 3. at the end of the cytoplasmic expansions which were inserted into the infoldings formed by the basement membrane lying over the mesangial area. Sometimes REP clusters seemed intracytoplasmic, bound within a vacuole (Fig. 4). This pattern was probably due to tangential section. REP were also observed near the mesangial cells, and generally occured: 1. at the end of the cytoplasmic ramifications of the mesangial cell in a centrolobular position (Fig. 5); 2. along the cytoplasmic membrane of mesangial cells interposed in capillary loops (Arakawa and Kimmelstiel, 1969).

Occasionally, REP were observed within some subepithelial deposits (Fig. 6), within the basement membrane or within the mesangial matrix (Fig. 2), far from the cells. In these cases, the granules were often associated with membranous structures formed by 2 parallel densities from 200 Å to 300 Å apart. Along their length, a transverse banding periodicity was seen (Fig. 8). The presence of these membranous structures was seen in 65 out of the 86 biopsies with observed REP (76%), and in 47 of the 104 specimens without REP (45%). No REP were observed

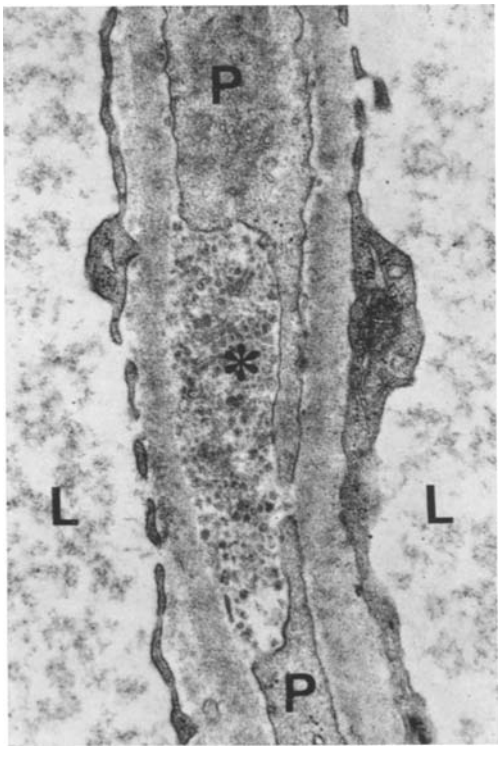


Fig. 3. SLE. Podocytes (*P*) joining two capillary loops. Lumen (*L*). Cluster of REP (*) in a site normally filled by a strip of podocyte cytoplasm (×20000)

either in the cytoplasm or in the nuclei of glomerular cells. Nevertheless some foot processes seemed to be completely transformed into REP (Fig. 7).

Moreover, intraendothelial microtubular virus-like structures identical to those reported previously (Györkey *et al.*, 1969; Sinkovics *et al.*, 1969) were frequently observed in patients with or without proved systemic lupus erythematosus: in 22 cases out of the 104 without REP and in 27 cases out of the 86 cases with REP. Typical herpes-like particles (Epstein, 1962; Swanson *et al.*, 1966) were very rare.

REP occured in a great variety of instances (Table 1). No correlation has been found between the occurence of REP and pathological or immunohistochemical features. No relationship could be established between their presence and clinical or biological findings (sex, age, proteinuria, nephrotic syndrome, renal function, LE cells, antinuclear antibodies). Several patients were biopsied before any treatment, others had been treated by immunosupressive agents, steroid or antiinflammatory drugs.

## Discussion

There exists a high prevalence of REP in glomerular tufts since they were detected in 86 out of the 190 specimens reviewed. Their prevalence is certainly

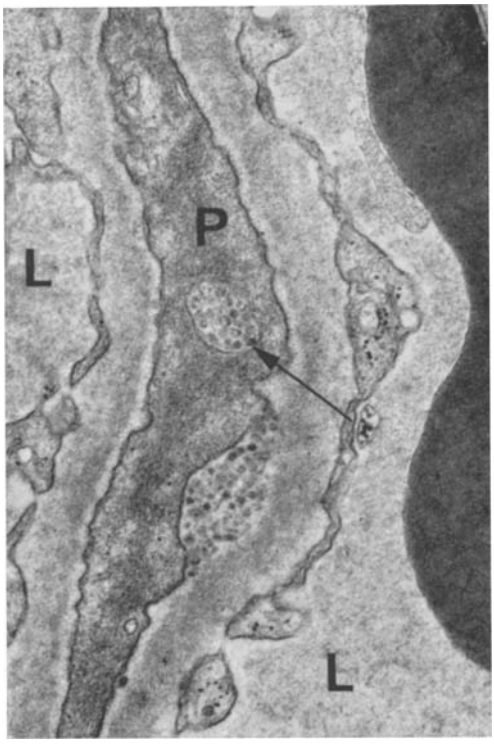


Fig. 4. Acute glomerulonephritis. Podocyte (*P*) joining two contiguous loops. Lumen (*L*). One of the two clusters is apparently enclosed within a cytoplasmic vacuole (arrow) (×25000)

under-rated, as in most biopsies no more than four glomeruli were examined. They were found in very different instances, but no significant correlation could be surmised from their varying concentration and distribution. They even occured in morphologically normal kidneys, for instance in patients with postural proteinuria or in immediate renal-graft biopsies. Moreover, in the absence of a control group it cannot be established that the presence of REP is pathological in nature. It may be the physiological situation.

The nature of the REP is unknown. Three hypotheses may be discussed: 1. their viral identity; 2. their non specific cytoplasmic origin; 3. a special form of protein deposit.

The term "small extracellular particles" was used by Moses *et al.* (1968) to designate formations observed in cultured lymph cells from patients with infectious mononucleosis. Large aggregates of REP, identical to those detected in our specimens, were viewed only in cell cultures which concomitantly happened to contain typical herpes-like particles. In those cell cultures arrays of tubules 22 mμ in diameter, similar to the intraendothelial virus-like structures (Györkey *et al.*, 1969; Sinkovics *et al.*, 1969) were also present. Particles similar in shape to REP were detected in the glomeruli of transplanted kidneys. These formations, called

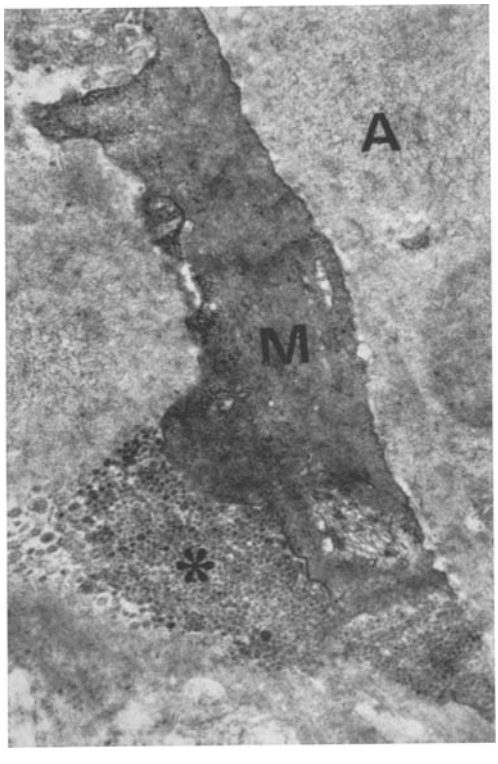


Fig. 5. Amyloidosis. Amyloid fibrils (*A*). Cluster of REP (*) in the vicinity of a mesangial cell (*M*). Notice the differing sizes of some particles (×13000)

virus-like by Rowlands *et al.* (1970), suggested to be herpes virus capside by Busch *et al.* (1971) were localized in subepithelial space (Rowlands *et al.*, 1970) or in the subepithelial portion of the glomerular basement membrane (Busch *et al.*, 1971).

Though the viral identity of the REP cannot be absolutely dismissed, in our series morphological grounds for this hypothesis were absent: 1. absence of intranuclear particles; 2. absence of the prominent nuclear changes which are currently observed in the course of herpes virus infections; 3. absence of budding processes along the cytoplasmic membrane; 4. lastly, the heterogeneous sizes of the REP.

No evidence supports the hypothesis that the REP are virus-associated particles: 1. entities which might have been human herpes virus (Epstein, 1962; Swanson *et al.*, 1966) were scarcely detected. 2. Intraendothelial virus-like structures (Györkey *et al.*, 1969; Sinkovics *et al.*, 1969) were often observed in the endothelial cells of our specimens. But a non-significant positive relationship existed between the presence of intraendothelial microtubular virus-like structures and that of REP.

On the other hand several arguments support the hypothesis of a degenerative cytoplasmic origin: 1. the utter change of a whole foot process into a cluster of

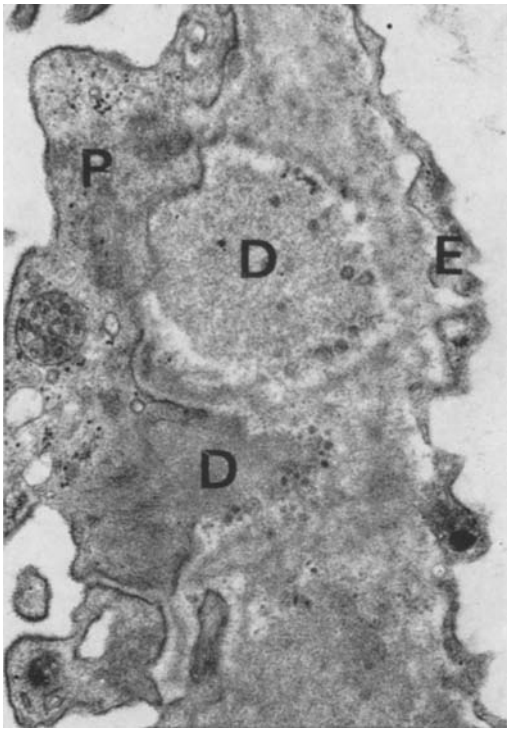


Fig. 6

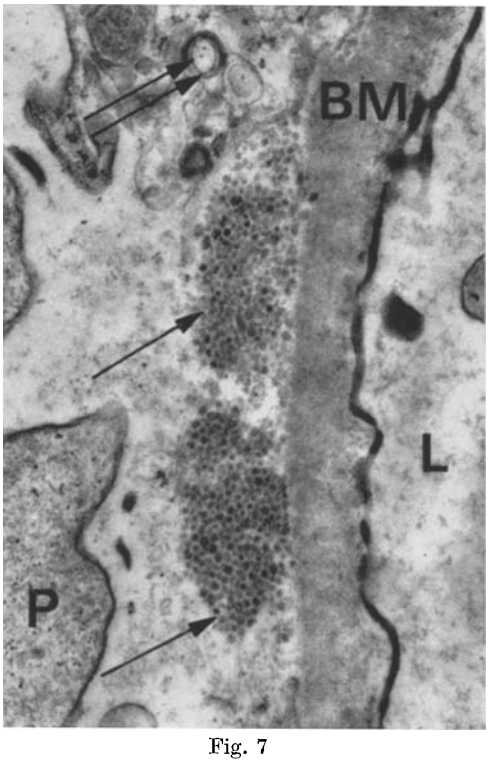


Fig. 7

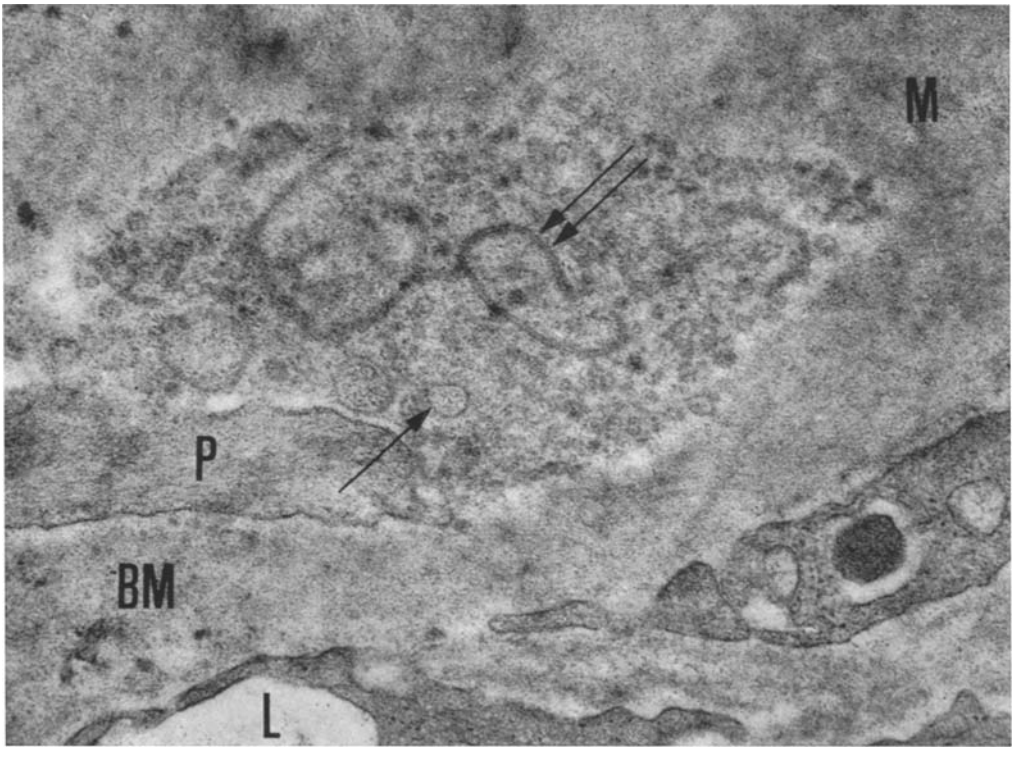


Fig. 8. Transplant kidney. Cluster of differing formations: REP, vesicles (arrow), membranous structures (arrows). Podocyte (*P*), basement membrane (*BM*), mesangial matrix (*M*), lumen (*L*) (×36500)

granules may be accounted for only by a cell lysis. 2. As a rule the granules were localized within an area that should be filled with cytoplasm. Some clusters of REP took the place of a strip of cytoplasm joining two contiguous loops. Other clusters, incorporated within membranous material, seemed to be the remains of the cytoplasmic process which they continued. The high prevalence of REP in a subepithelial or mesangial localization may be accounted for if it is born in mind that these entities, trapped between the cytoplasmic membrane on one side and membranous material on the other side cannot, be eliminated into the urinary space or into the capillary lumen. 3. Their frequent association with large vesicular structures or membranous profiles which probably arise from cells (Nagle *et al.*, 1969) also support the hypothesis of a degenerative origin of REP.

Fig. 6. Glomerulonephritis with extramembranous deposits. Podocyte (*P*), endothelial cell (*E*), sparse REP in the subepithelial deposits (*D*) (×23500)

Fig. 7. Diabetes mellitus. Two clusters of REP (arrow) have taken the places of foot processes. Podocyte (*P*), basement membrane (*BM*), lumen (*L*). Notice the degeneration lesions in the cytoplasm of the adjacent podocyte (arrows) (×15500)

The granules may be a special form of protein deposit; however, their occurence in cell cultures (Moses *et al.*, 1968) and their presence in some glomeruli where no homogeneous or finely granular deposit was detected make this hypothesis improbable. Also the clusters cannot be considered as immune deposits for they are very often seen in glomeruli where no immunoglobulin or $\beta_1$ C- $\beta_1$ A-globulin can be detected by immunohistochemistry.

The causes, mechanism and consequences of REP are unknown. However they do no seem to hinder the elaboration of the basement membrane or the mesangial matrix. The occurence of REP in these components and even in the subepithelial deposits may represent the different steps of the particles' incorporation into the newly formed membranous material or into developing subepithelial deposits.

We gratefully acknowledge the expert technical cooperation of Miss J. Grossetete and the fine secretarial assistance of Miss G. Bachet. We are indebted to Mrs. D. Cernigoj for the translation.

## 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).

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

Busch, G. J., Galvanek, E. G., Reynolds, E. S., Jr.: Human renal allografts. Analysis of lesions in long-term survivors. Hum. Pathol. **2**, 253–298 (1971).

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 (1970).

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 (1970).

Epstein, M. A.: Observations on the fine structure of mature herpes simplex virus and on the composition of its nucleoid. J. exp. Med. **115**, 1–11 (1962).

Györkey, F., Sinkovics, J. G., Min, K. W., Györkey, P.: Systemic lupus erythematosus and myxovirus. New Engl. J. Med. **280**, 333 (1969).

Habib, R., Kleinknecht, C.: The primary nephrotic syndrome of childhood. Classification and clinicopathologic study of 406 cases. Pathology Annual, 1971, p. 417–474. Edited by SC Sommers. New York: Appleton-Century-Crofts 1971.

Moses, H. L., Glade, P. H., Kasel, J. A., Rosenthal, A. S., Hirshaut, Y., Chessin, L. N.: Infectious mononucleosis: detection of herpes-like virus and reticular aggregates of small cytoplasmic particles in continuous lymphoid cell lines derived from peripheral blood. Proc. nat. Acad. Sci. (Wash.) **60**, 489–496 (1968).

Nagle, R. B., Kohnen, P. W., Bulger, R. E., Striker, G. E., Benditt, E. P.: Ultrastructure of human renal obsolescent glomeruli. Lab. Invest. **21**, 519–526 (1969).

Rosen, S.: Membranous glomerulonephritis: current status. Hum. Pathol. **2**, 209–231 (1971).

Rowlands, D.T., Jr., Burkholder, P. M., Bossen, E. H., Lin, H.: Renal allografts in HL-A matched recipients: light, immunofluorescence and electron microscopic studies. Amer. J. Path. **61**, 177–210 (1970).

Seymour, A. E., Spargo, B. H., Penksa, R.: Contributions of renal biopsy studies to the understanding of disease. Amer. J. Path. **65**, 550–598 (1971).

Sinkovics, J. G., Györkey, F., Thoma, G.W.: A rapidly fatal case of systemic lupus erythematosus: structures resembling viral nucleoprotein strands in the kidney and activities of lymphocytes in culture. Tex. Rep. Biol. Med. **27**, 887–908 (1969).

Swanson, J. L., Craighead, J. E., Reynolds, E. S.: Electron microscopic observations on herpes virus hominis (herpes simplex virus) encephalitis in man. Lab. Invest. **15**, 1966–1981 (1966).

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