Virchows Arch. A Path. Anat. and Histol. 384, 29–44 (1979)

*Virchows Archiv A*


# Light and Electron Microscopic Findings in Five Cases of Cryoglobulinemic Glomerulonephritis

T. Faraggiana[1], C. Parolini[1], G. Previato[2], and A. Lupo[2]

[1] Università degli Studi di Roma, I° Istituto di Anatomia Patologica, Roma, Italy
[2] Istituti Ospitalieri di Verona, Divisione di Nefrologia Medica, Verona, Italy

**Summary.** Renal tissue from five patients with cryoglobulinemia was studied by light and electron microscopy and immunofluorescence. None of the histologic features observed at the light microscopic level seems to be specific for cryoglobulinemia. Electron microscopic investigations have shown very large electron dense deposits in almost every examined lobule in all cases. The deposits displayed two main patterns; a homogeneous texture in two cases and tubular or annular structures in three cases. The patients with typically structured deposits had IgG-IgM cryoglobulinemia (2 cases) or monoclonal IgM cryoglobulinemia (1 case). The presence of IgM in cryoglobulinemia may be the cause of the peculiar structure of the deposits.

**Key words:** Glomerulonephritis – Cryoglobulinemia – Electron microscopy.

## Introduction

Renal involvement in patients with cryoglobulinemia is frequent in both primary and secondary forms (Mazzei et al., 1970; Verroust et al., 1971; Brouet et al., 1974). Several morphological patterns of glomerular lesions have been reported, the most frequent being intracapillary proliferation and membranoproli ferative-like changes (Meltzer et al., 1966). Less common are membranous glomerulonephritis (Brouet et al., 1974), hyaline changes without evidence of proliferation (Zinneman et al., 1968), sclerosis and hyaline thrombi within the capillary lumina (Morel-Maroger and Verroust, 1974). Thus, none of these histologic changes seems to be specific for cryoglobulinemia.

Recently, electron microscopic investigation, reviewed by Feiner and Gallo (1977), has shown many electron dense, typically structured deposits throughout the glomeruli in 9 of the 11 reported cases, and it has been suggested that

*Send offprint requests to:* Tullio Faraggiana, I° Istituto di Anatomia e Istologia Patologica, Viale Regina Elena, 324 (Policlinico), I-00161 Roma, Italy

0340-1227/79/0384/0029/$03.20

the only characteristic morphological criterion of the glomerular lesions secondary to cryoglobulinemia is the ultrastructural appearance of the glomerular deposits.

We have had the opportunity to examine the renal biopsy specimens obtained from 5 patients with renal involvement secondary to cryoglobulinemia by light and electron microscopy and immunofluorescence. Three of these cases showed typical structured deposits within the glomeruli. The aim of this paper is to present the morphological aspects of these biopsies, which, despite some individual variations, displayed an impressive common pattern of glomerular lesions.

## Material and Methods

Clinical and biochemical evidence of renal dysfunction were detected in 5 patients with cryoglobulinemia.

For isolation of cryoglobulin, blood was drawn in a warm dry tube and allowed to clot at 37° C for 2 h. The serum, after centrifugation at 37° C, was placed in a refrigerator at 4° C for 48–72 h. The cryoprecipitate was removed by centrifugation at 4° C and washed in the cold three times with phosphate-buffered saline (PBS). The precipitate was then dissolved in PBS at 37° C and the qualitative anlaysis was performed by immuno-electrophoretic study of the purified cryoglobulins against monospecific goat antiserum for the heavy chains of human serum IgG, IgA, IgM and kappa and lambda light chains. The amount of urinary protein was estimated by the sulfo-salicylic acid method. Cellulose acetate electrophoresis, immuno-electrophoresis and Ouchterlony analysis were performed on serum and urine by standard methods.

Rheumatoid factor activity was determined by agglutination of latex particles coated with human IgG and presence of antinuclear antibody with indirect fluorescence technique. Radioimmunoassay for hepatitis B surface antigen (HBs Ag) and its antibody were assayed on serum. The serum levels of total haemolitic complement (CH50) and serum levels of C3, C3PA, and C4 were measured (radial immunodiffusion, Behringwerke) in different instances in four patients while in one patient only CH50 and C3 concentrations were determined. Bone marrow aspirates were done in two patients with abnormal homogeneous increase of serum IgM.

Renal specimens, obtained by needle-biopsies from patients 1–4 were fixed for light microscopy in Bouin's solution and embedded in paraffin. Sections were stained with HE, PAS and Masson's trichrome stain. Small portions of each block were fixed in Karnowsky fixative, postfixed in Osmium and embedded in a mixture of Epon and Araldite.

The biopsy specimen obtained from patient 5 was fixed in 4% paraformaldheyde, postfixed in Osmium and embedded in toto in Epon.

A small portion was also fixed in paraformaldheyde and embedded in glycomethachrylate. Semithin sections were stained with periodic acid-silver methenamine. Ultrathin sections were routinely stained with uranium and lead salts, with periodic acid-silver methenamine (Marinozzi, 1961), and with periodic acid-thiocarbohydrazine-silver proteinate (Thiéry, 1967).

For immunofluorescence microscopy a second specimen from each renal biopsy was immediately frozen on a gelform in isopentane liquid nitrogen. Sections 3 to 4 μ thick were cut, fixed in acetone for 5 min, rinsed in buffered saline solution and covered with monospecific fluorescin labelled antisera directed against human IgG, IgA, IgM, C1q, C3, C4, fibrinogen and albumin (Behringwerke).

## Results

### *1. Clinical Data*

Primary glomerulonephritis, autoimmune disorders and other infectious processes were excluded.

**Table 1.** Summary of clinical features in 5 patients

| | B.L. (1) | J.A. (2) | DF.S. (3) | F.J. (4) | F.G. (5) |
|---|---|---|---|---|---|
| Age/Sex | 39/M | 42/M | 47/M | 50/F | 52/M |
| Presenting symptoms | Weakness, ankle edema, purpura | Purpura, nephrotic Syndrome | Purpura triggered by drug – Nephrotic Syndrome | Purpura, anemia, artralgia temporary blindness | Articular manifestation, skin necrosis, glomerulo nephritis |
| Associated disease | Waldenstrom's macro-globulinemia | None | Waldenstrom's macro-globulinemia | None | None |
| Blood pressure mm Hg | 130/80 | 170/110 | 180/105 | 130/80 | 150/90 |
| Rheumatoid activity | 1/640 | – | 1/640 | 1/160 | 1/640 |
| Cryoglobulin type | IgMkappa (I°) | IgMkappa-IgG (II°) | IgMkappa-IgG (II°) | IgM-IgG (III°) | IgMkappa-IgG (II°) |
| BUN (mg.%) | 14 | 38 | 16 | 24 | 140 |
| Serum creatinine (mg.%) | 1.10 | 2.10 | 0.80 | 1.45 | 2.80 |
| Urine protein (G/die) | 4–6 | 5–20 | 5–8 | < 2 | < 1 |
| Clinical course Stable renal function for | 3 years | 2 years | 3 years | 2 years | Died: autopsy showed systemic vasculitis |

**Table 2.** Results of complement assay

| Patient | CH 50 | $C_3$ PA | $C_3$ | $C_4$ |
|---|---|---|---|---|
| B.L. | 68 | 87 | 30 | 0 |
| J.A. | 50 | not done | 30 | not done |
| DF.S. | 48 | 83 | 7 | 0 |
| F.J. | 51 | 91 | 33 | 17 |
| F.G. | 11 | 90 | 32 | 9 |

Normal values $\pm$ 2SD
CH50 = $97 \pm 27$ as percent of a pool of normal sera
$C_3$PA = $101 \pm 17$ as percent of a pool of normal sera
$C_3$ = $43 \pm 17$ mg/dl serum
$C_4$ = $18 \pm 9$ mg/dl serum

The cryoglobulinemia appeared to be idiopathic in 3 and secondary to Waldenstrom's macroglobulinemia in two patients (Table 1). Of the 3 patients with essential cryoblobulinemia, one had mixed polyclonal IgM-IgG cryoglobulins (Type III) and two patients type II cryoglobulinemia with IgMkappa-IgG cryoglobulin. Of the two patients with Waldenstrom's macroglobulinemia, one had

type I cryoglobulin composed of isolated monoclonal IgMkappa immunoglobulin and one patient type II mixed cryoglobulin (IgM-IgG) with a monoclonal IgMkappa. Renal damage was found in all patients, but renal failure was present only in those with essential cryoglobulin. Patient F.G. died shortly after biopsy and autopsy revealed severe systemic vasculitis. Proteinuria was the major renal abnormality in Waldenstrom's macroglobulinemia. Rheumatoid factor activity was highly positive in all patients. Tests for antinuclear antibody HBs Ag/Ab were negative. The serum levels of total haemolytic complement and specifically of component C4 and C3 were reduced (Table 2). The marrow aspirate from patients with Waldenstrom's macroglobulinemia was moderately hypercellular and showed focal aggregates of lymphocytoid-plasmacells.

## 2. *Morphological Data*

*Light Microscopy*. Diffuse, severe hypercellularity was the most evident feature in all cases of miced IgM-IgG cryoglobulinemia. It showed both intra- and intercapillary distribution and many capillary lumina were completely occluded. Moreover in most glomeruli a marked accentuation of the lobular pattern was observed (Figs. 1 and 2).

Many glomerular cells, among which granulocytes were frequently recognizable, showed plentiful lisosome-like, intracytoplasmic droplets: they were occasionally very large and stained fairly well with the periodic acid silver methenamine method.

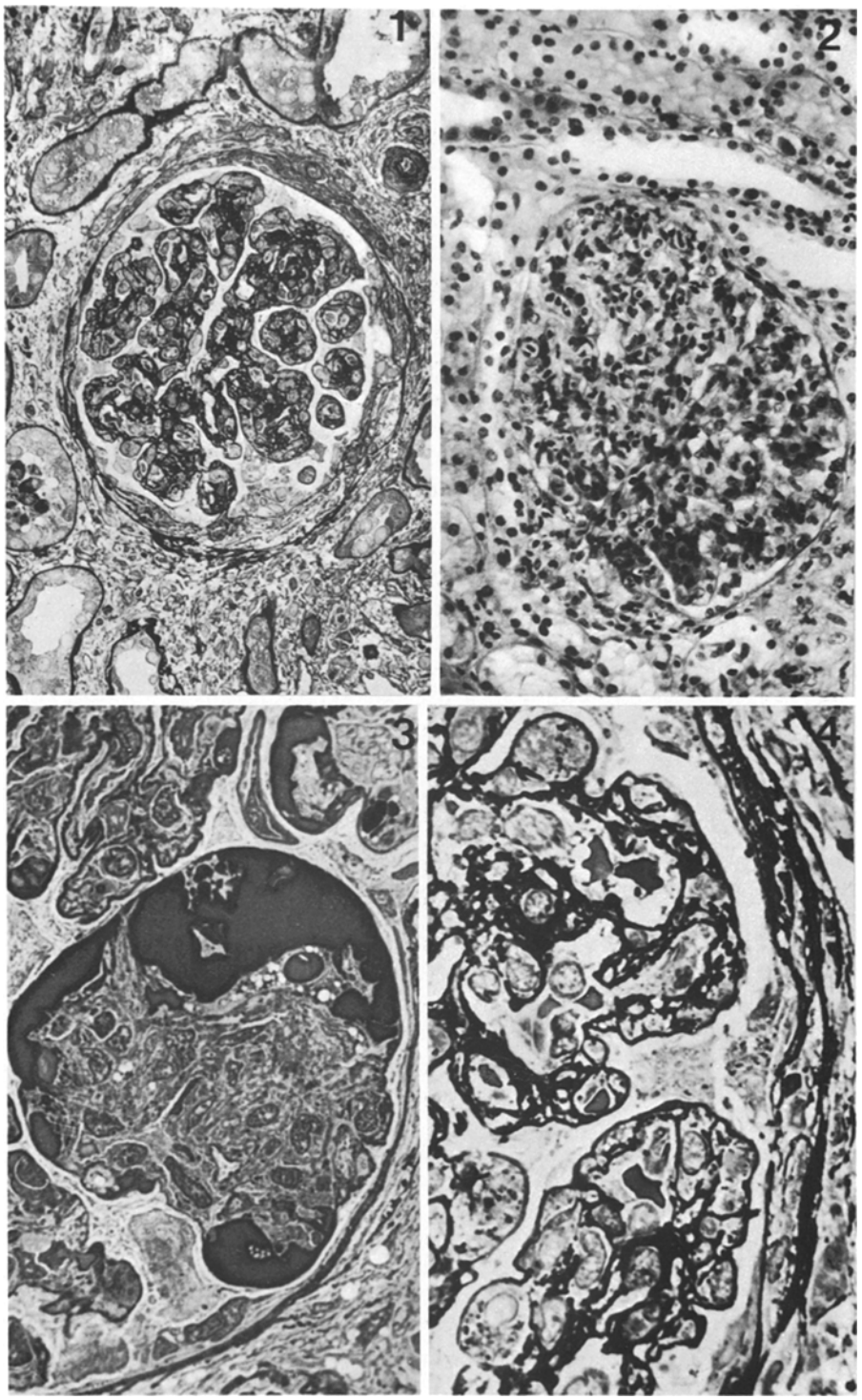
In all cases a focal extracapillary proliferation and accompanying glomerulocapsular synechiae were also seen. In two cases of mixed IgM-IgG cryoglobulinemia (n. 2, 3: Table 1) and the case with monoclonal IgM cryoglobulinemia (n. 1), dense, homogeneous casts (so called hyaline thrombi) were observed in a few capillaries (Fig. 3). More frequently small deposits were visible throughout the glomerular tuft, on the endothelial side of the basal membrane and in the mesangial regions. The basal membrane itself was often split and showed the "double contour" which is described as typical of the membranoproliferative glomerulonephritis. This lesion was also noticeable in the solidified lobules where patent lumina were no longer recognizable (Fig. 4). The tubules contained many hyaline droplets in their cytoplasm; the lumina were often filled with

**Fig. 1.** Case n. 5 – Renal glomerulus showing intracapillary hypercellularity. No patent lumina are still recognizable. The glomerular tuft has a clear lobular pattern. Extracapillary proliferations and glomerulocapsular adhesion can also be seen (PASM × 250)

**Fig. 2.** Case n. 4 – Striking hypercellularity can be seen in the lower half part of the glomerulus. The upper part shows a quite normal appearance (PAS × 250)

**Fig. 3.** Case n. 1 – Portion of a glomerulus showing hyaline "trombi" in some peripheral capillary lumina (PASM × 1,000)

**Fig. 4.** Case n. 2 – Portion of glomerulus showing two lobules with "circumferential mesangial interposition" and double contoured appearance of capillary walls (PASM × 1,000)


1
2
3
4

desquamated cells and compact casts, strongly reactive with the PASM method. Focal areas of tubular atrophy and non specific infiltration were also visible. A typical fibroelastic hyperplasia of the wall of the interlobular arteries was evident in three cases.

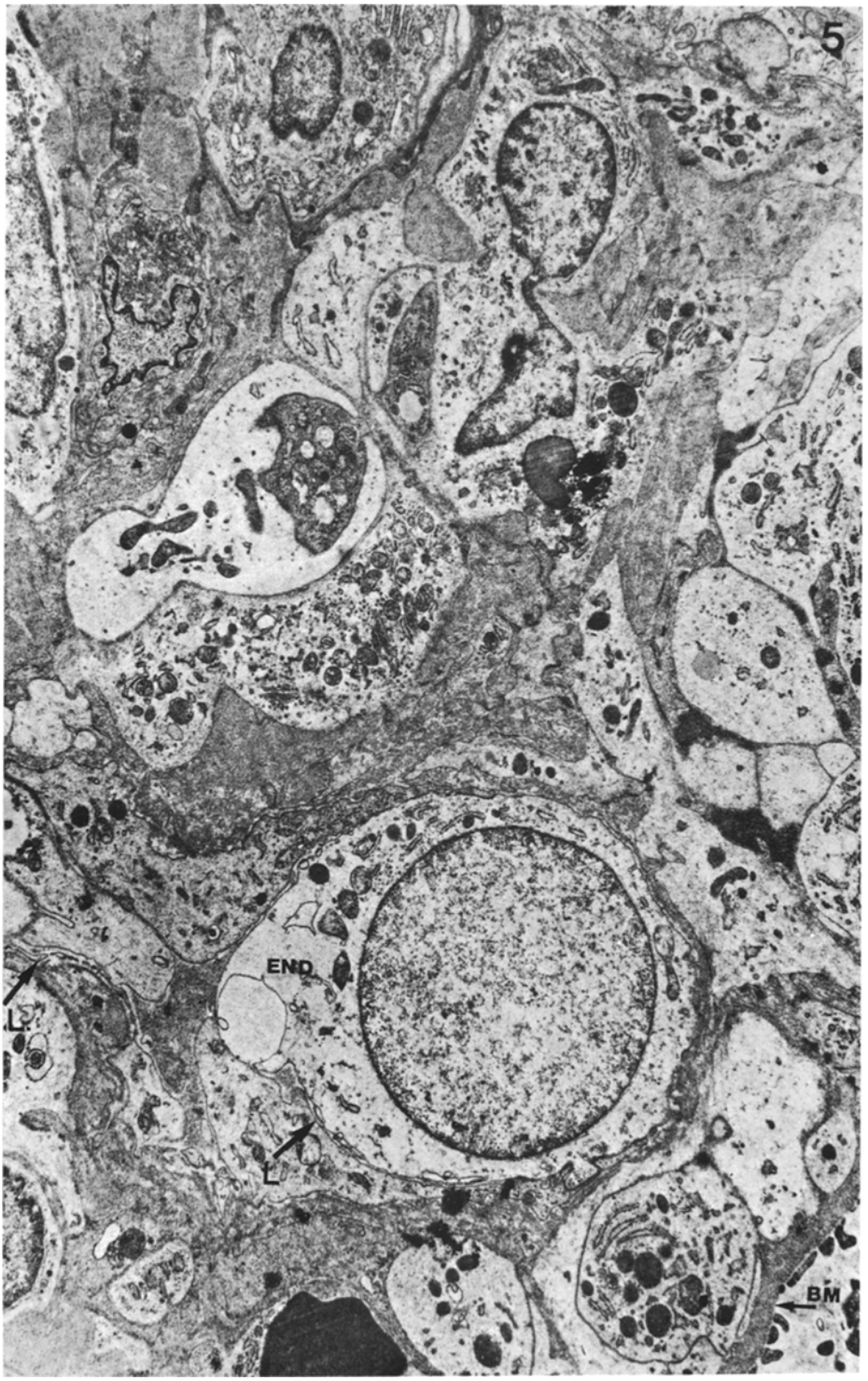
*Ultrastructural Findings.* In cases 2–5 the majority of glomerular capillaries appeared dilated and filled with cells: these included neutrophilic granulocytes, swollen endothelial cells, monocytes and other cells, probably of mesangial origin. Such intracapillary hypercellularity was particularly striking in patients 4 and 5: the capillary lumina were in fact reduced to very small, slitlike spaces (Fig. 5).

In the other two cases (2–3) a few lumina were still apparent, the general picute resembling that of membranoproliferative glomerulonephritis. Cytoplasmic projections were seen to extend from the intercapillary regions towards the periphery of the capillary loop, between an apparently split basement membrane. This was particularly notable in silver-stained sections (Fig. 6). The amount of mesangial matrix was frequently increased, but it showed an uneven distribution throughout the glomerulus. The presence of very dense deposits in almost every lobule examined was the most relevant finding in all cases. Their location varied, but most were placed on the endothelial side of the basement membrane. The endothelium itself was displaced and was frequently absent. Elsewhere the deposits were between the basement membrane and the cytoplasmic extension of the mesangial cells. In the mixed cryoglobulinemia the electron dense deposits showed a peculiar appearance in two out of four cases. In patients 2 and 3 they were formed of tubular structures which, in cross-section, had a circular profile (Fig. 7). The average external diameter of the annulate structures was 330 Å. Translucent subendothelial areas were not infrequently apparent (Fig. 8). Here, too, the typical annular particles were still recognizable. By contrast in cases 4 and 5 the deposits had an apparently homogeneous texture (Fig. 9), Case 1 displayed many features incommon with those reported above (2–3). However the deposits, as a rule, were larger and were frequently recognizable even in an intramembranous and subepithelial position (Fig. 10). Moreover, their fine structure was striking: the annulate particles were much more evident and abundant than in the cases with mixed IgG-IgM cryoglobulinemia, though they were of similar dimensions. It was possible to observe a loss of endothelial cells in the capillaries containing large deposits: in such cases the deposits were in direct contact with the cytoplasm of the neutrophil granulocytes.

In all our cases both the intracapillary and intercapillary cells showed electron dense, membrane-bounded inclusions corresponding to the hyaline droplets described at the histological level.

It was possible to document a phagocytic origin of these inclusions in many sections. Moreover, they retained a structure similar to that of the extracellular

**Fig. 5.** Case n. 5 – Electron micrograph showng a glomerular capillary lumen completely occluded by cells of different kind. END: Endothelial cell. BM: basement membrane. L: lumen (PB × 8,000)


5
END
L
L
BM

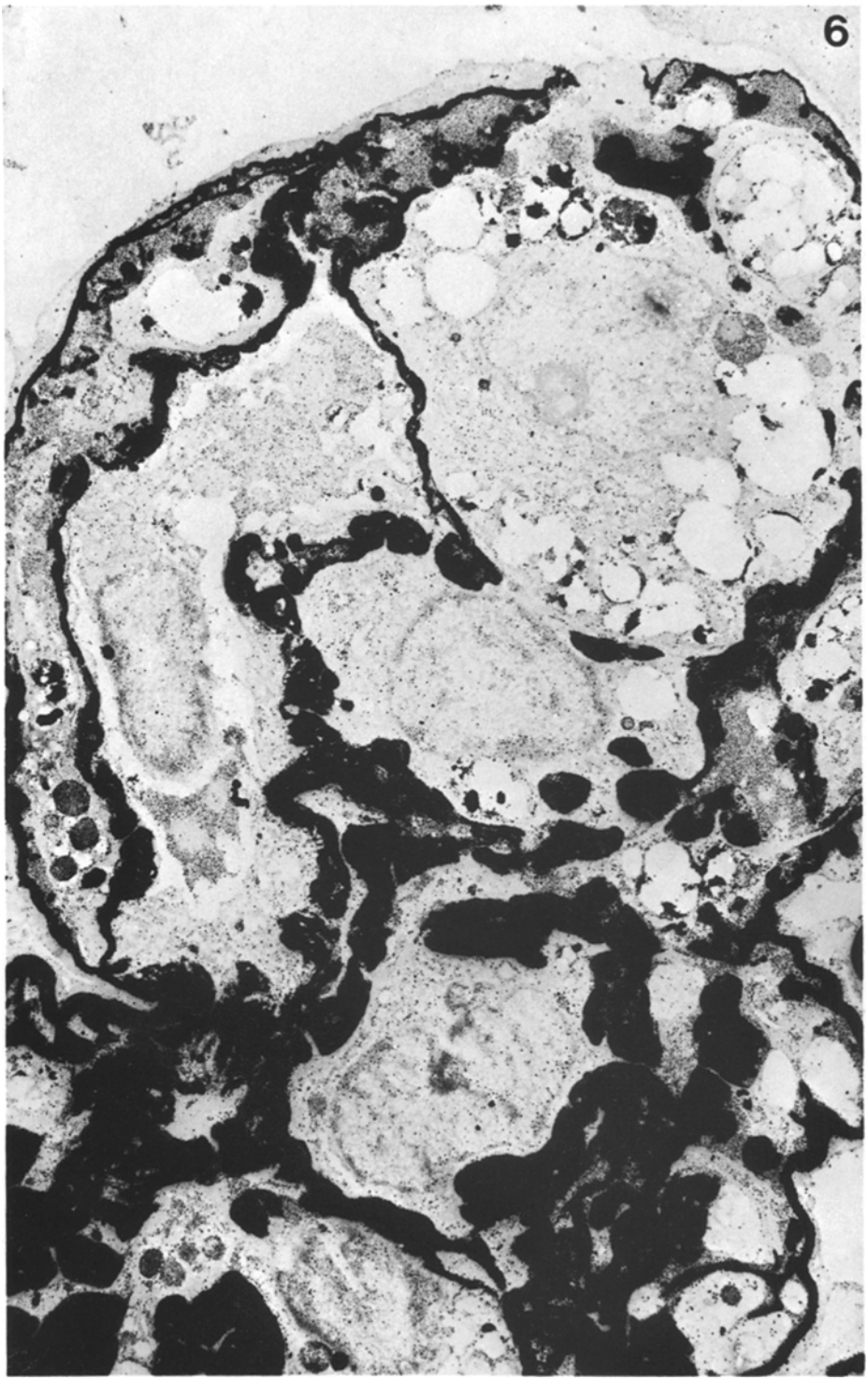



**Fig. 6.** Case n. 2 – Electron micrograph showng a peripheral capillary loop. Two layers of basement membrane (deeply stained with silver methenamine method) enclose a non-homogeneous material, stricly resembling mesangial matrix (PASM × 5,500)

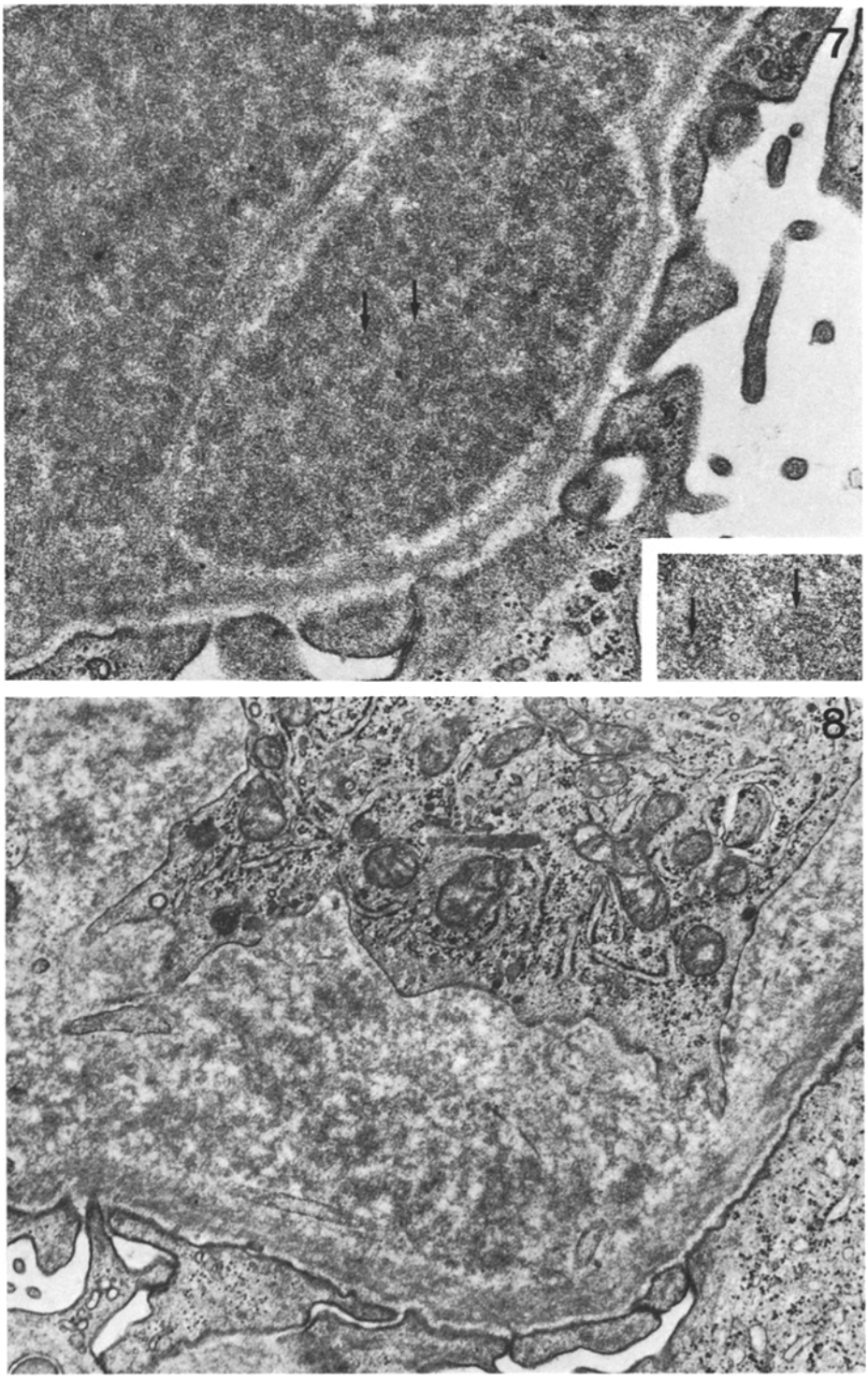
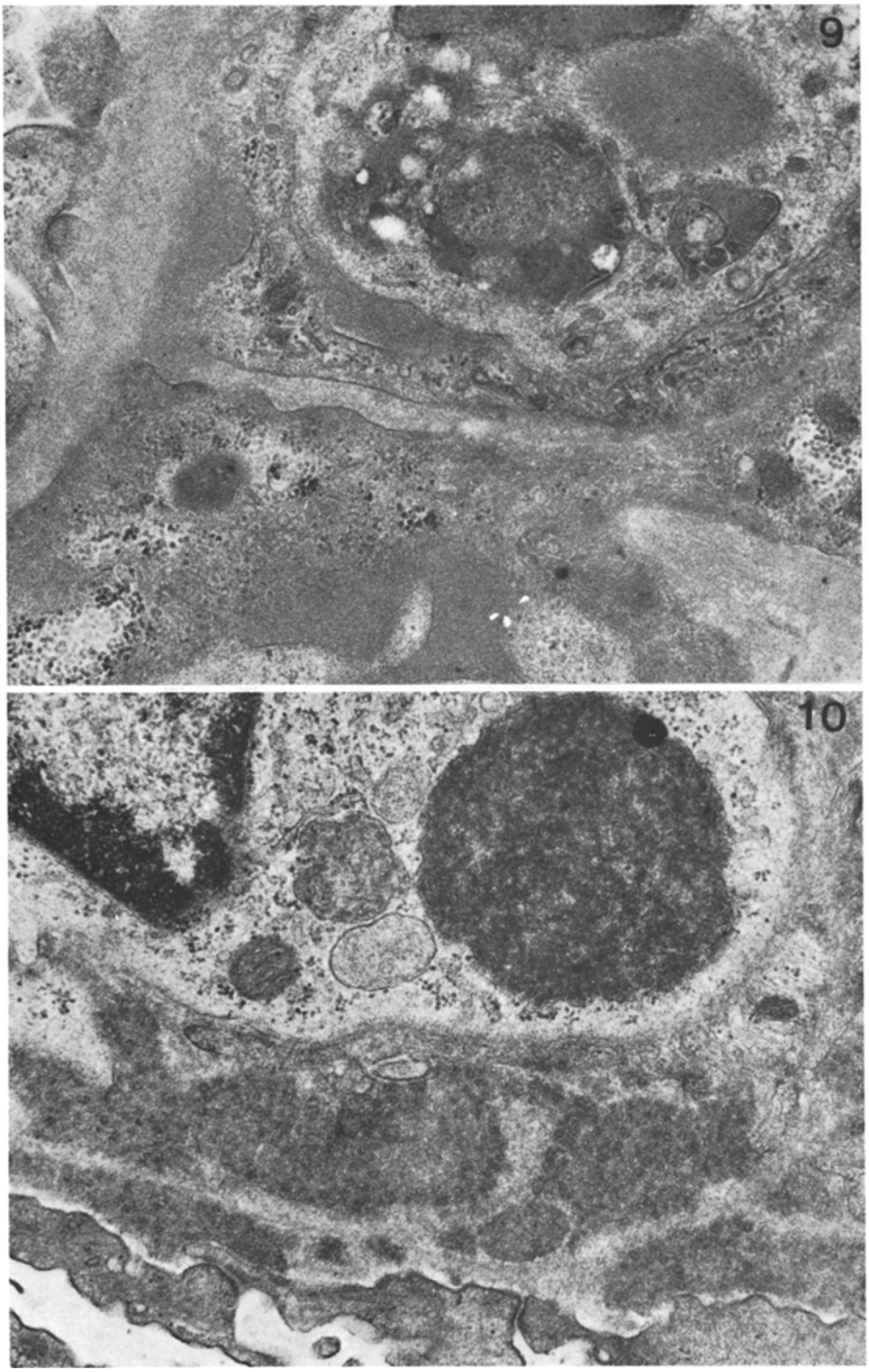



**Fig. 7.** Case n. 2 – Glomerular deposit lying between the lamina densa of basement membrane and endothelial cell. Anular and tubular structures are clearly distinguishable (*arrows*). (UR PB × 37,500). Insert × 75,000

**Fig. 8.** Case n. 2 – Electron micrograph from the same patient as Fig. 7. Tubular structures are scattered in a large translucent subendothelial area. (UR PB × 2,100)




**Figs. 9 and 10.** Cases n. 4 and 1—Intracytoplasmic inclusions showing a very similar appearance to that of the surrounding extracellular deposits: homogeneous in the upper photograph (UR PB × 26,000), granular in the lower (UR PB × 20,000)

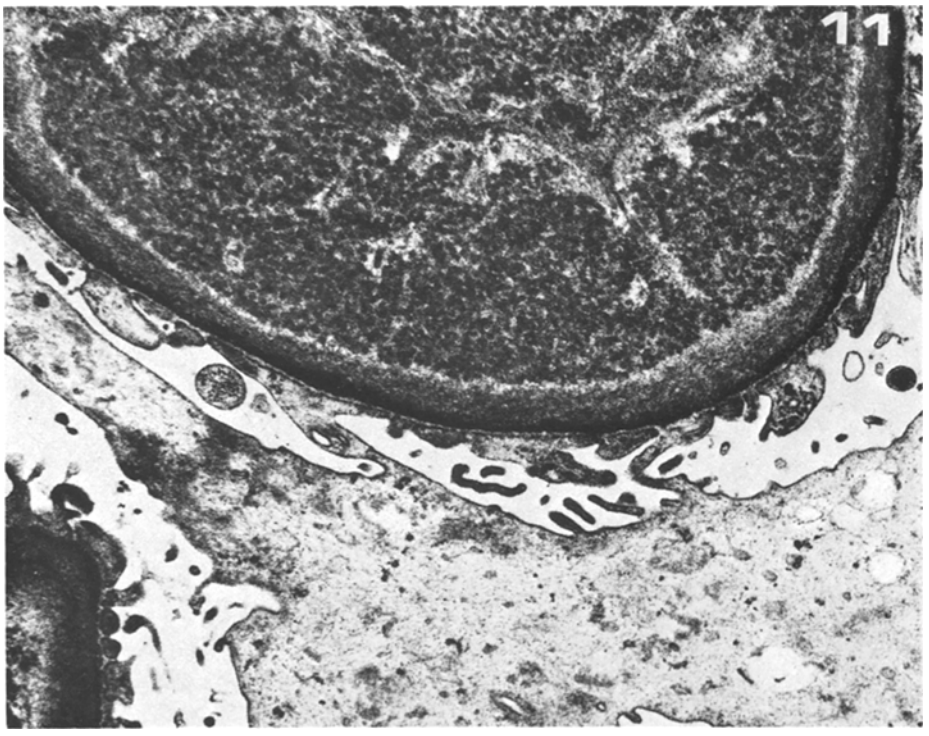



**Fig. 11.** Case n. 2 – Subendothelial deposit in a peripheral capillary loop. The silver stain method enhances the outline of the anulate structures. (PASM × 15,000)

deposits; yet, they were granular in cases 1, 2, 3 (Table 1) (Fig. 10) and more homogeneous in the others (Fig. 9). Silver stains disclosed a non-uniform reactivity of the subendothelial deposits; actually their annular appearance was enhanced by PASM (Fig. 11). On the whole the deposits were always less intensely stained that the adjacent basement membrane and mesangial matrix (Figs. 6 and 11). The periodic acid-thiocarbohydrazide-silver proteinate staining on metachrylate sections preserved the annular structure of the deposits. In this case they were more deeply stained than the dense layer of the basement membrane.

*Immunofluorescence.* IMF results were available in 4 patients. In the two patients with Waldestrom's macroglobulinemia and cryoglobulinemia of type I and II respectively, the only positivity was observed for C3 deposition in segmental granular pattern along the basement membranes (Fig. 12). Patient 2 (J.A.) had high positivity for IgG; IgM and C3 deposition in segmental granular pattern along basement membranes. Patient n. 4 (F.J.) had mild positivity for IgM and C3 deposition in segmental granular and globular pattern along the basement membranes and in solerotic areas (Fig. 13).

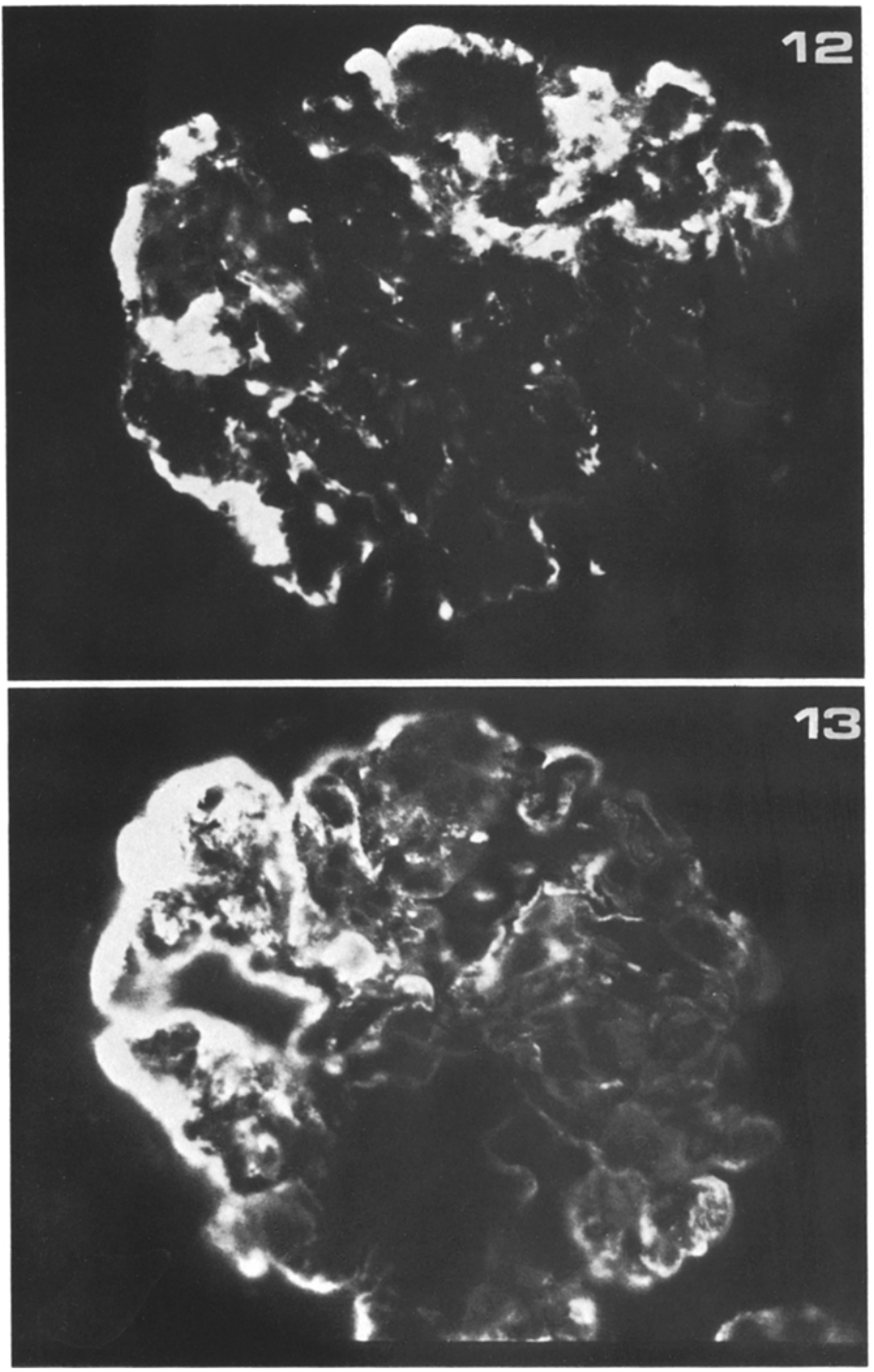



**Fig. 12.** Case n. 1 – $C_3$ deposition in segmental pattern along the basement membranes. (×400)

**Fig. 13.** Case n. 4 – IgM deposition along capillary walls, more evident at the periphery of the glomerulus. (×400)

## Discussion

This study documents the histological and ultrastructural changes in the kidneys of 3 patients with mixed essential IgG-IgM cryoglobulinemia and 2 patients with cryoglobulinemia secondary to Waldenstrom's macroglobulinemia. When evident, the peculiar structure of the deposits seems to be a good criterion to characterize these glomerular lesions as a pathological entity, which has to be distinguished from other glomerulonephritis with accompanying cryoglobulinemia. The histological lesions ranged from a predominantly intra-capillary proliferation similar to that of acute glomerulonephritis, to membranoproliferative changes. The two patterns were frequently associated and the latter differs from the common forms of membranoproliferative glomerulonephritis only in the presence of compact precipitates (thrombi) in many capillaries. While some authors (Meltzer et al., 1966; Verroust et al., 1971) regard these thrombi as a poor prognostic sign, we have observed no clinical or functional signs of progressive renal failure in our patients (Table 1). These findings seems to support the hypothesis (Feiner and Gallo, 1977) that cryoprecipitates occur in vitro rather than in vivo, and that in this latter situation cryoprecipitates are not functionally occlusive. Cellular proliferation seems to be a more accurate prognostic index of renal damage. It is interesting that in patients 1, 2, 3 (Table 1) which show less intense glomerular hypercellularity, there was a peculiar structure of the deposits with a great abundance of tubular and annular structures. Similar structures have already been described by others (Cordonnier et al., 1975; Monga et al., 1976; Feiner and Gallo 1977), always concerning IgG-IgM mixed cryoglobulinemia. Whether this association is of significance and whether it is related in some way to a different composition of IgG-IgM ratio in the precipitates, is not known at present. With regard to our cases, we cannot reach any definite conclusion because of the limited number of patients we have examined and our inability to obtain quantitative data on the composition of the cryoprecipitates. The fact that in case 1 (B.L.), with monoclonal IgM cryoglobulinemia, we observed the greatest abundance of annular structures within the glomerular deposits, seems to suggest that such particles are somehow associated with the presence of IgM in the cryoprecipitates. Therefore, the presence of IgM in mixed cryoglobulinemia may be the cause of the peculiar structure of the deposits. On the other hand, in all cases with monoclonal IgG cryoglobulinemia previously reported, the glomerular deposits displayed a different pattern which was mainly fibrillar and never annular or tubular (Feiner and Gallo, 1977). Moreover, a recent study by Avasthi et al. (1977) has shown the occurrence of subepithelial fibrillar deposits in a patient with benign monoclonal gammaglobulinemia and glomerulonephritis. Nevertheless, a tubular crystal structure for a IgG cryoprecipitable immunoglobulin, obtained from the serum of a patient with multiple myeloma, was described by Bogaars et al. (1973). The tubular units had, in cross section, a uniform circular appearance although their dimensions were considerably smaller than those of the analogous structures we had found in glomerular deposits of patients with IgG-IgM cryoglobulinemia. Unfortunately the lack of renal investigations make it impossible to make any correlation.

Silver stains are very useful in following the morphogenisis of the glomerular changes, both on semithin and ultrathin sections. It is evident from Fig. 6 that the "double contour" of the capillary wall is actually due to new formation of basement membrane; its frequent interruption also explains the accompanying finding of extracepillary proliferation. Moreover silver methods showed that the glomerular deposits are composed of two histochemically different components, since the tubular structures are more reactive than the amorphous matrix in whcih they are dispersed. We were unable, however, to explain why the electon dense deposits are less reactive with the PASM method than the basement membrane, whereas they stain darker with the PATC-SP method: both procedures are known to stain selectively the periodic acid generated aldehyde sites. It is not clear why all the patients of our study as well as the many others previously reported show a great abundance of subendothelial deposits and membranoproliferative-like features in glomeruli. Cryoglobulins, whose direct influence on the genesis of glomerular electron dense deposits has been shown by immunological (Golde and Epstein, 1968; Baldwin and McCluskey, 1968) and morphological investigation (Tornroth and Skrifvars, 1973; Bartlow et al., 1975) are known to be immunocomplexes of relatively large size (Lo Spalluto et al., 1962; Meltzer et al., 1966).

It is believed that these complexes are trapped by the renal glomerulus, mainly in the mesangial area and if present in large excess, in the subendothelial zone. Here they can induce hypercellularity (mainly intracapillary) or in the more chronic forms, a membranoproliferative glomerulonephritis [7–9] (Cochrane and Koffler, 1973; Germuth and Rodriguez, 1973). The two forms we describe may be a quantitative variation of the same process; an inflammatory response to large complexes (cryoglobulins) trapped in the glomerulus.

Many of the intracapillary cells and those cells projecting along the periphery of the capillary loops are of obvious mesangial origin: they contain many cytoplasmic filaments and are sometimes in close contact with basal membrane-like material. Mesangial cells are known to be able to take an intracapillary position as a consequence of immunological injury (Vernier et al., 1971), other cells, however, which have a pale cytoplasm and a wide Golgi complex, could be blood-borne monocytes. Mazzuco et al. (1977) were able to demonstrate esterase activity in glomeruli from many cases of cryoglobulinemia; this enzyme is very active in monocytes but is not usually present in glomerular cells. Whether monocytic or mesangial in origin, these cells display intense phagocytic activity. It is possible, in fact, to see that they capture a small fraction of the electron dense deposits by infolding their cytoplasm and that they contain many lysosome-like bodies. Sometimes it is also possible to observe a striking analogy between the deposits and the cellular inclusions. Thus there is some support for the hypothesis that the electron dense deposits are cleared by the phagocytic activity of the glomerular cells. Elsewhere, however, the finding of electron lucent areas, such as those seen in Fig. 8, are consistent with a different mechanism of reabsorption of the deposits: it is known, that Ag-Ab complexes can be directly solubilized by complement "in vitro" (Miller et al., 1975).

The results of complement assay in our patients are in keeping with those reported by other authors (Brouet et al., 1974) and support the hypothesis

that cryoglobulins may activate the complement system through the classic pathway (Riethmuller et al., 1966). It must be remembered that Muller et al. (1976) have documented the possibility that cryoglobulins may also activate the complement system through the alternate pathway. That complement activation is important in the pathogenesis of renal damage is suggested by C3 deposition in a granular pattern in all of our patients. The lack of C4 deposition is not easly explained and might support the hypothesis that in the kidney the complement system is activated via the alternate pathway. Moreover, the evidence for $C_3$ glomerular receptors, obtained by Gelfand et al., (1976) points to this complement fraction as that which has an important role in glomerular immuno-complexe deposition.

Other immunofluorescence studies in cryoglobulinemic glomerulonephritis have demonstrated granular deposition along the capillary walls (sometimes associated with intraluminal thrombi) of immunoglobulins belonging to the same class of those forming the circulating cryoglobulins (Golde and Epstein, 1968; Verroust et al., 1971; Brouet et al., 1974; Morel-Maroger and Verroust, 1974; Feiner and Gallo, 1977; Mazzucco et al., 1978). In our patients, however, this correlating between circulating and deposited immunoglobulins was not always found, as in some other reports (Mathison et al., 1971; Cordonnier et al., 1975). This difference was also evident in a patient from Morel-Maroger and Verroust's series and in another patient from Feiner and Gallo's series, findings which might be explained on the basis of a "blocking effect" by IgM on IgG at glomerular level (Golde and Epstein, 1968).

With regard to the immunofluorescence pattern in cryoglobulinemia secondary to Waldenstrom macroglobulinemia, IgM deposition was observed in 2 out of 4 patients by Verroust et al. (1971) and in no patient was there evidence of IgG or $C_3$ deposition. In the authors' opinion the lack of $C_3$ might lead to a passive deposition of IgM in capillary walls. However, $C_3$ deposition in patients 1 and 3 seems to suggest that complement activation has a pathogenetic role in the renal damage seen in cryoglobulinemia secondary to Waldenstrom disease.

*Acknowledgments.* The authors wish to thank Mrs. Fausta Fanella, Mr. Lucio Virgili and Mr. Giancarlo Bolognesi for their skillful assistance and generous support.

## References

Baldwin, D.S., McCluskey, R.T.: Renal involvement in systemic lupus erythematosus, periarteritis nodosa, scleroderma, and cryoglobulinemia. In: Structural basis of renal desease, Becker, E.L. (ed.). New York: Hoeber Medical Division, Harper and Row 1968

Bogaars, H.A., Kalderon, A.E., Cummings, F.J., Kaplan, S., Melnicoff, I., Park, C., Diamond, I., Calabresi, P.: Human IgG cryoglobulin with tubular crystal structure. Nature [New Biology] **245**, 117–118 (1973)

Brouet, J.C., Clauvel, J.P., Danon, F., Klein, M., Seligmann, M.: Biologic and clinical significance of cryoglobulins. A report of 86 cases. Am. J. Med. **57**, 775–787 (1974)

Cochrane, C.G., Koffler, D.: Immune complex disease in experimental animals and man. Adv. Immunol. **16**, 185–264 (1973)

Cordonnier, D., Martin, H., Groslambert, P., Miconin, C., Chenais, F., Stoebner, P.: Mixed IgG-IgM cryoglobulinemia with glomerulonephritis. Am. J. Med. **59**, 867–872 (1975)

Feiner, H., Gallo, G.: Ultrastructure in glomerulonephritis associated with cryoglobulinemia. A report of six cases and review of the literature. Am. J. Path. **88**, 145–155 (1977)

Gelfand, M.C., Shin, M.L., Nagle, R.B., Green, I., Frank, M.: The glomerular complement receptor in immunologically mediated renal glomerular injury. N. Engl. J. Med. **295**, 10–14 (1976)

Germuth, F.G., Rodriguez, E.: Immunopathology of the renal glomerulus. Boston: Little Brown and Co. 1973

Golde, D., Epstein, W.: Mixed cryoglobulins and glomerulonephritis. Ann. Intern. Med. **69**, 1221–1227 (1968)

Lo Spalluto, J., Danward, B., Miller, W.Jr., Ziff, M.: Cryoglobulinemia based on interaction between a gamma macroglobulin and 7 S gamma globulin. Am. J. Med. **32**, 142–147 (1962)

Marinozzi, V.: Silver impregnations of ultrathin sections for electron microscopy. J. Bioph. Biochem. Cytol. **9**, 121–125 (1961)

Mathison, D. A., Condemi, J.J., Leddy, J.P., Callerame, M.L., Panner, B.J., Vaughan, J.H.: Purpura, arthralgia and IgM-IgG oryoglobulinemia with rheumatoid factor activity: response to cyclophosphamide and splenectomy. Ann. Intern. Med. **74**, 383–390 (1971)

Mazzei, D., Quarto di Palo, F., Cattaneo, R.: Cryoglobulinemia and nephritis. Lancet **1**, 369–370 (1970)

Mazzucco, G., Barbiano di Belgioioso, G., Galato, R., Monga, G.: Presenza di monociti nelle lesioni glomerulari della crioglobulinemia mista essenziale IgG-IgM: studio istochimico e correlazioni con gli aspetti in microscopia ottica e in immunofluorescenza. Minerva Nefrol. **25**, 45–50 (1978)

Meltzer, M., Franklin, C., Elias, H., McCluskey, R.T., Cooper, N.: Cryoglobulinemia. A clinical and laboratory study. Cryoglobulins with rheumatoid factor activity. Am. J. Med. **40**, 837–856 (1966)

Miller, G.W., Nussenzweig, V.: A new complement function: solubilisation of antigen antibody aggregates. Proc. Natl. Acad. Sci. USA **72**, 418–422 (1975)

Monga, G., Mazzucco, G., Coppo, R., Piccoli, G., Coda, R.: Glomerular findings in mixed IgG-IgM cryoglobulinemia. Light, electron microscopic, immunofluorescence and histochemical correlations. Virchows Arch. B Cell Path. **20**, 185–196 (1976)

Morel-Maroger, L., Verroust, P.: Glomerular lesions in dysprotidemia. Kidney Int. **5**, 249–252 (1974)

Muller, S., Rother, U., Westerhausen, M.: Complement activation by cryoglobulin. Evidence for a pathogenic role of an IgG (kappa) cryoglobulin in cutaneous vasculitis. Clin. Exp. Immunol. **23**, 233–235 (1976)

Riethmuller, G., Meltzer, M., Franklin, E., Miescer, P.A.: Serum complement levels in patients with mixed (IgG-IgM) cryoglobulinemia. Clin. Exp. Immunol. **1**, 337–339 (1966)

Vernier , R.L., Mauer, S.M., Fish, A.J., Michael, A.F.: The mesangial cell in glomerulonephritis. Adv. Nephrol. **1**, 31–46 (1971)

Verroust, P., Mery, J.P., Morel-Maroger, L., Clauvel, J.P., Richet, G.: Les lésions glomérulaires des gammopathies monoclonales et des cryoglobulinémies idiopathiques IgG-IgM. Actualités Nephrologiques de l'Hôpital Necker, pp. 167–202. Paris: Ed. Med. Flammarion 1971

Zinnemann, H.H., Lewis, D., Seal, U.S.: On the nature of cryoglobulins. J. Immunol. **100**, 594–603 (1968)

Received April 8, 1979