Naturally Occurring Canine Glomerulonephritis

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The 8 dogs with glomerulonephritis in this study had progressive renal disease characterized by proteinuria, azotemia and hypoalbuminemia, without peripheral edema and ascites. Observed by light, fluorescence and electron microscopy, the glomeruli were diffusely but irregularly involved. By light microscopy, there was focal mesangial proliferation, and the peripheral portions of many glomerular capillary loops were thickened by eosinophilic material along the endothelial sides of basement membranes. Immunofluorescence studies demonstrated granular deposits of IgG and β1C-globulin outlining glomerular basement membranes and within the mesangium. Ultrastructurally, there were electron-dense deposits in the mesangium and within the endothelial side of glomerular basement membranes. Subepithelial deposits were never seen. The majority of clinical and morphologic features suggested a glomerulonephritis of immune-complex type. However, the unique aspects of the ultrastructural lesion precluded this assumption based solely on morphologic criteria (Am J Pathol 67:471–482, 1972).

ALTHOUGH RENAL DISEASE commonly occurs in dogs, glomerulonephritis is generally considered rare.[1–3] This may explain why the naturally occurring disease has seldom been reported and why existing studies include only a small number of dogs. For example, in his review of 321 cases of canine nephritis, Monlux[4] found glomerulonephritis in only nine instances. Additional reports, based primarily on light microscopic findings, have cited the occurrence of glomerulonephritis in dogs with mastocytomas[5] and in conjunction with canine systemic lupus erythematosus[6] and pyometra.[7] Only one ultrastructural study has been reported, and there are none utilizing immunofluorescence.

Problems inherent to the understanding of an uncommon disease are compounded when there is confusion regarding the morphologic changes which represent its diagnostic lesions. Hottendorf and Nielsen[5] diagnosed glomerulonephritis at necropsy in 10 of 27 old dogs used as controls for another study, whereas Lerner *et al*[8] examined

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15 adult dogs and found no evidence of glomerulonephritis. Guttman and Andersen [9] cautiously used the term "progressive intercapillary glomerulosclerosis" in describing a disorder of aging and irradiated beagles which has many of the light, fluorescence and electron microscopic features of glomerulonephritis. Bloom [1] believes that most reports of glomerulonephritis in dogs actually describe chronic glomerular changes caused by other types of renal disease. This evident confusion in the literature illustrates the inadequate characterization of glomerulonephritic lesions in the dog. To clarify this situation, the present study was undertaken to describe naturally occurring canine glomerulonephritis in terms of light, fluorescence and electron microscopic observations.

Materials and Methods

Twenty dogs were selected, based on the finding of proteinuria, from among animals at the Veterinary Medical Teaching Hospital, University of California at Davis. Eight had similar light, fluorescence and electron microscopic lesions, which we are defining as glomerulonephritis. Complete necropsies were performed on 7 of these 8 dogs and on 4 apparently normal dogs less than a year old which were included in the study as controls. Material from the other affected animal (Dog 1, Table 1) was obtained by open renal biopsy.

Light Microscopy

Renal tissue for light microscopy was fixed in 10% Zenker's formol solution, embedded in paraffin, sectioned at 3–4 μ, and stained routinely with hematoxylin and eosin and the periodic acid–Schiff (PAS) reaction. All other tissue specimens were fixed in 10% neutral-buffered formalin. In selected cases, kidney sections were also examined using Congo red, phosphotungstic-acid-hematoxylin (PTAH), or Mallory's trichrome stains.

Fluorescence Microscopy

Specimens for fluorescence microscopy [10] were frozen in liquid nitrogen and sectioned at 4–6 μ in a cryostat. The tissues were subsequently washed briefly 3 times in phosphate-buffered saline, fixed for 30 minutes in ice-cold acetone, rewashed in buffer, and stained with fluorescein-conjugated rabbit anti-canine gamma globulin (IgG), β1C globulin, or fibrinogen (Cappel Laboratories, Downington, Pa). The specificity of the anti-globulin reagents was established by the presence of characteristic single precipitin lines following immunoelectrophoresis against normal dog serum. Minor contaminants found in the anti-fibrinogen reagent were removed with canine serum; subsequent immunoelectrophoresis and reaction with dog plasma resulted in a single precipitin band. Normal dog kidney did not stain, and the procedure was further controlled by blocking specific staining with appropriate unconjugated rabbit anti-canine antibodies. Specimens were viewed with a Zeiss fluorescence microscope.

Electron Microscopy

Tissue for electron microscopy was fixed overnight at 4 C in 1.5% distilled glutaraldehyde [11] buffered to pH 7.4 with 0.1 M sodium cacodylate, and post-

fixed for 90 minutes in 1% osmium tetroxide.[12] After embedding [13] in either Epon or Araldite, orientation sections approximately 1 μ thick were cut and stained with toluidine blue. Ultra-thin sections of selected glomeruli were then cut and stained with uranyl acetate and lead citrate.[14] Specimens were examined in a Siemens 1A electron microscope at 80 kV with a 35 μ objective aperture.

Results

Clinical Findings

Clinical data are summarized in Table 1. There was no apparent correlation of breed, age or sex with the incidence of glomerulonephritis. Proteinuria ranged from 2 to 4+ by Robert's test. Quantitative protein determinations were performed on the urine from 5 dogs; values ranged from 0.4 to 3.1 g/24 hours. All 6 dogs whose sera were examined by electrophoresis had hypoalbuminemia; values as low as 0.7 g% were recorded. None of the dogs had peripheral edema or ascites, however. Azotemia was a common finding, and the clinical course was progressive, usually terminating in uremia.

Gross Findings

Affected kidneys were tan (in contrast to the red-brown of normal kidneys), with finely pitted subcapsular surfaces. Pinpoint pale tan foci could frequently be distinguished on the granular cut cortical surfaces (Figure 1). The kidneys usually appeared slightly smaller than normal.

Light Microscopy

Light microscopic glomerular lesions (Figures 2, 3) consisted of increased amounts of mesangial cells and matrix, together with irregular eosinophilic thickening of peripheral capillary basement membranes. The glomeruli and their individual capillary loops were unequally affected. Thickening was due to PAS-positive material deposited on the endothelial side of the glomerular basement membrane (GBM). Hyaline nodules, occasionally seen in glomerular tufts, were a characteristic finding. Such nodules were PAS-positive, and did not stain positively with Congo red or PTAH. Neutrophils were not observed with increased frequency within glomerular capillaries. Interstitial changes were generally limited to focal areas of chronic inflammation and fibrosis; arteriolar sclerosis was occasionally present.

Fluorescence Microscopy

The pattern of specific IgG fluorescence (Figure 4) was granular, clearly outlined the peripheral portions of glomerular capillary loops

Table 1—Clinical Summary of Dogs with Glomerulonephritis*

Dog No.	Age (yr)	Sex	Breed	Weight (kg)	PCV	Urinary Protein (24 hr)		Serum Protein (g %)		Serum cholesterol (mg %)	BUN (mg %)	Clinically uremic‡
						(g)	(qual)†	albumin	globulin			
1	8	M	Springer spaniel	20	25	1.5	4+	0.7	3.9	300	183	Yes
2	3	M	Doberman pinscher	25	31	3.1	4+	1.7	3.4	—	165	Yes
3	7	F	Chow	18	24	—	2+	—	—	—	113	Yes
4	7	F	Samoyed	14	36	—	3+	—	—	—	153	Yes
5	12	F	Toy fox terrier	5	34	1.9	4+	0.9	2.8	294	165	Yes
6	6	F	Shetland sheep dog	5	40	0.4	4+	—	—	224	128	Yes
7	12	M	Cocker spaniel	9	42	—	4+	2.8	3.1	231	41	No
8	5	M	Irish setter	26	33	1.8	4+	1.6	3.5	319	40	No

* Laboratory values are representative of the most severe phase of the disease observed
† Qualitative measure, Robert's test
‡ Vomiting, polydipsia, polyuria, nocturia, and diarrhea

and could frequently be localized to the luminal surface of glomerular basement membranes. At times fluorescence appeared linear, but this was an inconstant finding and there were always glomerular loops nearby that exhibited a granular or beaded pattern. Mesangial staining was also noted. Specific fluorescence for β1C globulin was similar in distribution to that observed for IgG.

Fluorescence indicating the presence of fibrin or like material (Figure 5) was distributed in a pattern different from that of IgG and β1C globulin. It was granular but inconstant in distribution, so that capillary loops were not outlined. The bulk of this fluorescence occurred in capillary lumina, mesangium and in association with nodules.

Electron Microscopy

Ultrastructurally, amorphous deposits were observed in mesangial matrix (Figure 6) and within the endothelial side of glomerular basement membranes (Figure 7). The position of the latter corresponded to that of the areas of eosinophilic basement membrane thickening observed by light microscopy. Subepithelial electron-dense deposits were never found. Fusion of foot processes was a constant finding, but varied in extent.

Discussion

There appear to be morphologic similarities between canine glomerulonephritis and other disorders previously reported in the dog. Whether pathogenetic relationships exist remains unclear. Progressive intercapillary glomerulosclerosis,[9] a condition apparently related to the aging of beagles, manifests electron-dense deposits in the luminal side of GBM and a granular pattern of immunofluorescence. The glomerulonephritic lesions described by Obel *et al*.[7] in bitches with pyometra are virtually identical to the glomerular changes found in the 8 dogs of this study. The findings of these investigators included irregular thickening of glomerular capillary walls by PAS-positive material, apparently deposited under endothelial cells, with hyaline nodules in more severe cases. There were varying degrees of cellular proliferation, and the amount of glomerular damage seemed to differ from one glomerulus to another within the same kidney. As seen by electron microscopy, GBM contained dense deposits along endothelial surfaces; dense inclusions were noted within swollen mesangial cells. Fluorescence microscopic observations were not reported.

There also appears to be morphologic similarity between canine glomerulonephritis and passive serum sickness induced in the mouse.

Okumura *et al*[15] injected preformed soluble antigen-antibody complexes and subsequently described GBM deposits like those seen in our dogs with glomerulonephritis. However, as the ultrastructural features of canine serum sickness nephritis have not, to our knowledge, been reported, the comparison of this mouse model to canine glomerulonephritis may not be germane.

The two pathogenetic mechanisms now widely accepted as responsible for at least some types of naturally occurring glomerulonephritis result in morphologically distinct glomerular lesions.[16] Immune-complex glomerulonephritis, caused by antigen-antibody complexes and complement deposition along GBM, is characterized by a discontinuous beaded pattern of immunofluorescence and the corresponding ultrastructural finding of electron-dense deposits along the epithelial surface of the glomerular basement membrane. In contrast, attachment of antibody specifically directed against GBM antigen, followed by fixation of complement, results in anti-GBM type glomerulonephritis. Here GBM immunofluorescence is linear and subepithelial ultrastructural deposits are lacking. Furthermore, specific antibody against GBM can be eluted from affected kidneys.

The lesion described in this paper has elements in common with classical immune-complex type glomerulonephritis. The granular deposition of IgG and β1C globulin along GBM is typical of such immune-complex diseases as membranous glomerulonephritis of man[17] and the domestic cat[18] and serum sickness nephritis.[17] Nevertheless, the canine lesion is morphologically distinct in that the dense subepithelial deposits considered characteristic for this type of glomerulonephritis are missing. It is not clear whether this finding suggests a different pathogenetic mechanism for the canine disease or whether the peculiar ultrastructural orientation of deposits merely indicates species variation. The significance, if any, of the fibrin or like material found in the glomeruli of affected animals is not known. Ultrastructural and immunofluorescence studies of immune-complex type glomerulonephritis experimentally induced in the dog are essential to the resolution of these questions.

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Acknowledgments

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[*Illustrations follow*]

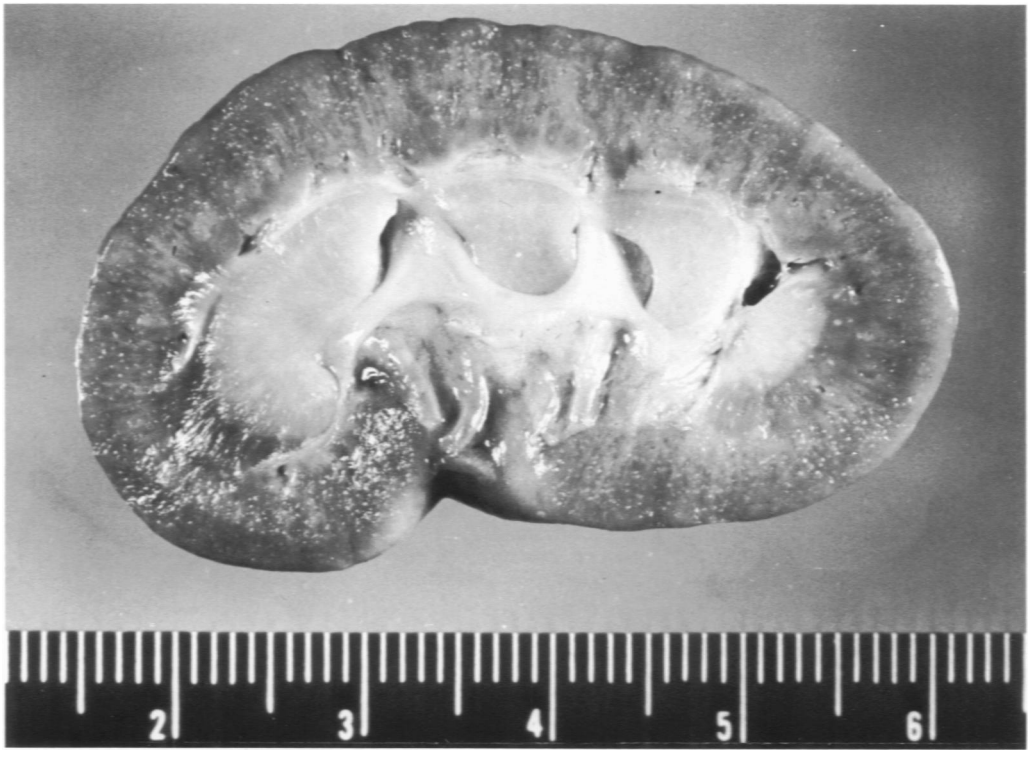


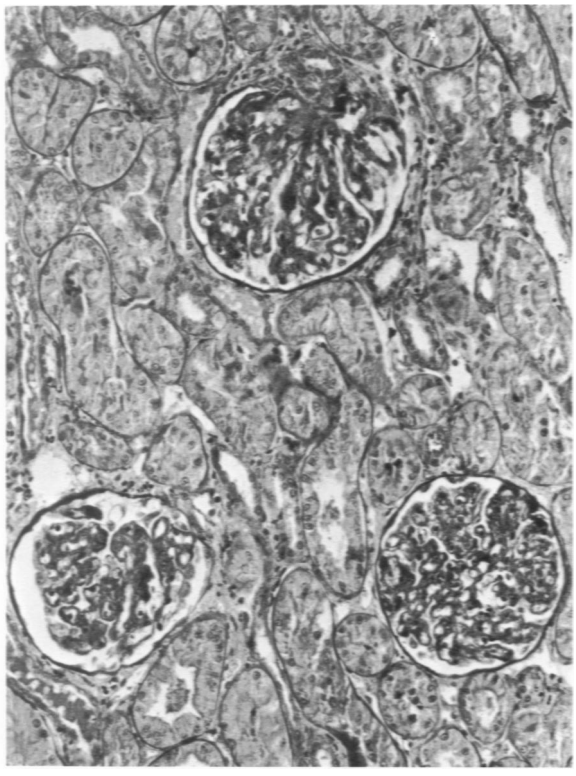
Fig 1—Photograph of a glomerulonephritic canine kidney. The cut cortical surface was tan, granular, and stippled with numerous pinpoint, pale-tan foci (Dog 6; × 2.5).

Fig 2—Photomicrograph of a glomerulonephritic canine kidney illustrating the diffuse involvement of glomeruli. Among glomeruli of the same kidney, the severity of mesangial and capillary changes characteristically lacked uniformity. The appearance of the tubules and interstitium was usually normal, as is depicted here (Dog 2; PAS, × 160).

Fig 3—Photomicrograph of a severely affected glomerulus depicting thickening of capillary loops, hyaline nodules (*arrows*) and irregular mesangial proliferation. It is characteristic that the changes are not uniform throughout the glomerulus (Dog 2; PAS, × 500).

Fig 4—IgG (depicted here) and β1C globulin were similarly deposited in discontinuous fashion along the glomerular capillary basement membranes of dogs with glomerulonephritis. The pattern of immunofluorescence was predominantly granular, and larger deposits were localized to the endothelial side of capillary loops. Mesangial staining was also present (Dog 4; fluorescein-conjugated rabbit anti-canine IgG, × 350).

Fig 5—The distribution of fibrin in the glomeruli of dogs with glomerulonephritis differed from that of IgG and β1C-globulin. The bulk of fluorescence was found in capillary lumina, mesangium, and in association with nodules. Glomerular basement membranes were not outlined by the staining (Dog 8; fluorescein-conjugated rabbit anti-canine fibrinogen, × 350).



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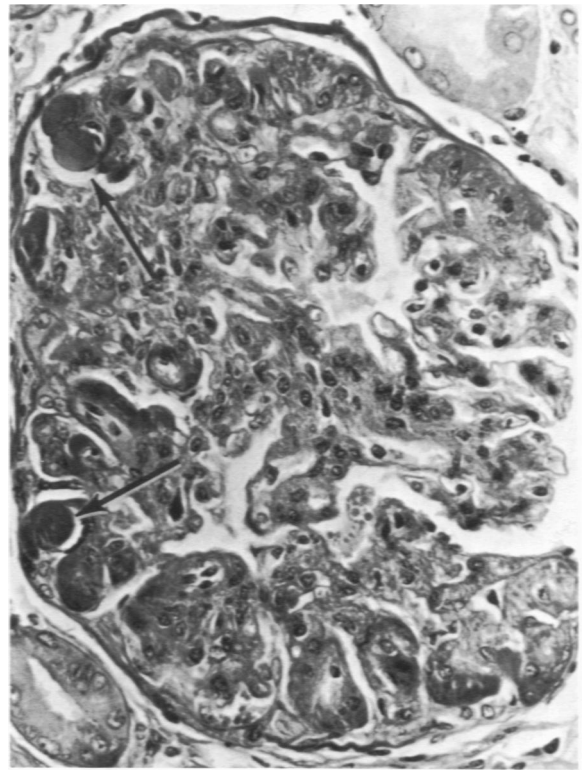

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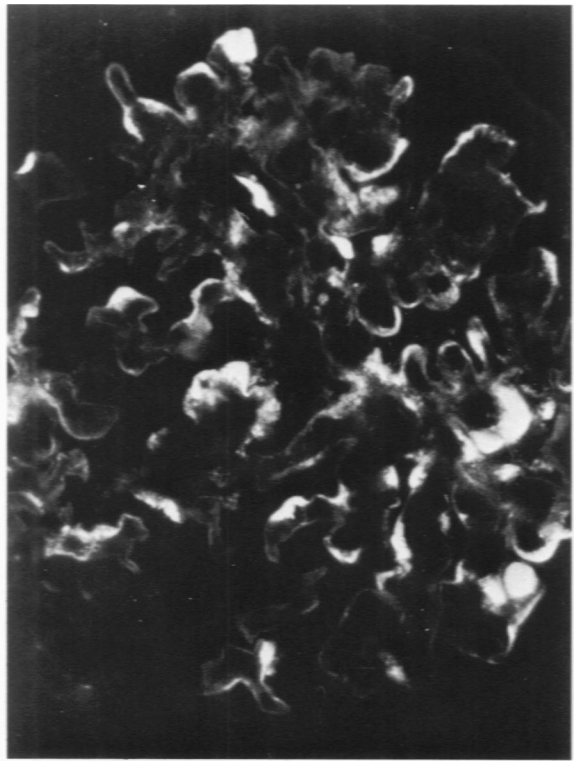

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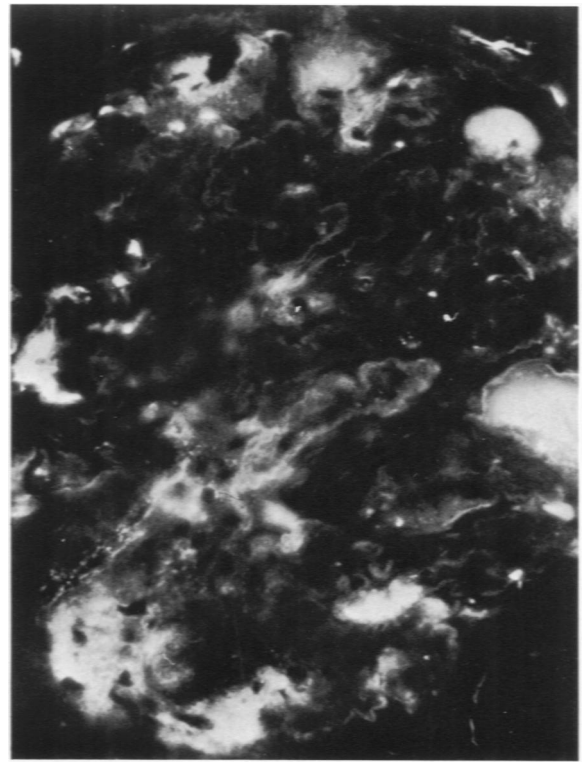

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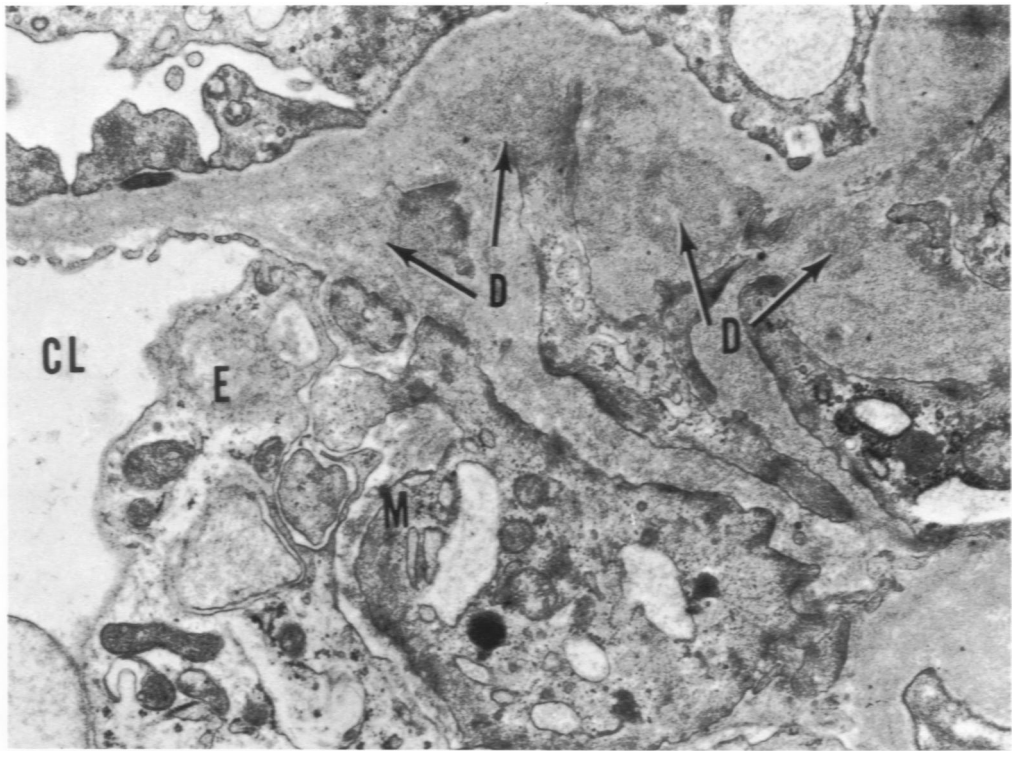

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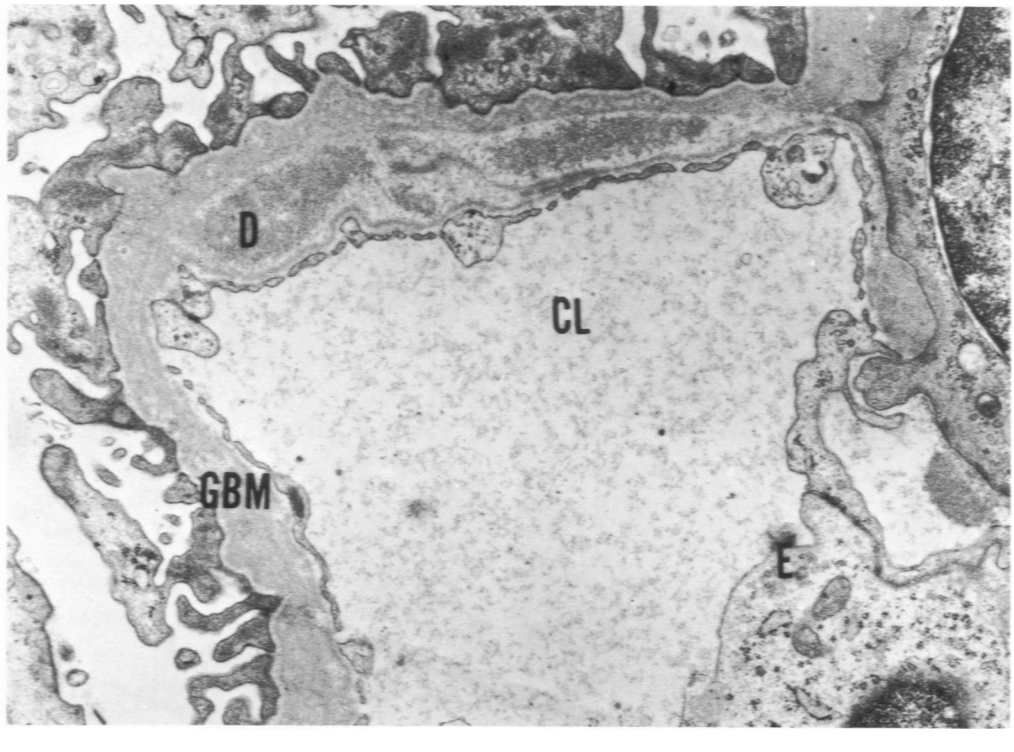

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Fig 6—Electron micrograph of mesangial area from a glomerulonephritic dog, depicting deposits (*D*) within the mesangial matrix. *M*, mesangial cell; *E*, endothelial cell; *CL*, capillary lumen (Dog 8; × 13,800). **Fig 7**—Electron micrograph of a peripheral glomerular capillary loop from a dog with glomerulonephritis. The lamina densa of the glomerular basement membrane (*GBM*) is split and contains large, dense deposits (*D*) within its endothelial side. Subepithelial deposits were never seen. Note the local fusion of epithelial cell foot processes. *E*, endothelial cell; *CL*, capillary lumen (Dog 8; × 15,000).