Spontaneous Glomerulonephritis in the Prosimian Primate Galago

A Correlative Light, Immunofluorescence and Electron Microscopic Analysis

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EXAMPLES OF SPONTANEOUS or experimentally induced proliferative glomerulonephritides in animals other than man are now rather plentiful. Glomerulonephritis apparently originating in hypersensitivity related to a variety of microorganisms has been observed in mice with lymphocytic choriomeningitis,[1,2] Coxsackie B,[3] or murine leukemia viruses [4]; in hogs with hog cholera virus [5]; in the nephritis of Aleutian mink disease [6,7] and the lupus-like nephritis of NZB mice in association with viral infections [8,9]; in dogs infected with Brucella organisms [10]; and in rabbits infected with nephritogenic, β-hemolytic, Group A streptococci.[11] Experimental hypersensitivity glomerulonephritis has also been induced in rabbits, rats and sheep by sensitization with a variety of foreign antigens,[12] nephrotoxic antiserums[13,14] or homologous or heterologous kidney antigens.[15,16] In addition, spontaneous glomerulonephritis in sheep [17] and lupus nephritis in dogs [18] have been described. Spontaneous glomerulonephritis in primates other than man has been described,[19,20] and attempts to induce poststreptococcal and other forms of hypersensitivity glomerulonephritis resembling that in man, in a variety of simian primates have met with variable success.[21–28] To our knowledge, nothing has been reported with respect to spontaneous or experimentally induced glomerulonephritis in the prosimian primate *Galago*.

In the course of routine pathologic examination of four species of galagos with acute or chronic illnesses, 26 of 33 necropsied animals housed at the Primate Facility, Duke University were observed to have some form of proliferative glomerulonephritis.[29] Three additional animals had a membranous glomerular lesion.

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The galago, though frequently seen in zoos and utilized in some primate centers for behavioral and other biomedical studies, has not been examined very extensively from the pathologic point of view. Two species of galagos have been studied extensively with respect to their being carriers of yellow fever.[30] In those studies only clinical and epidemiologic studies, with special reference to behavior, social structure of colonies and feeding habits, were made. It is very difficult to come by any information regarding the clinical or pathologic aspects of diseases in general, and in particular of renal disease, in these small primates, in either wild or captive state. To our knowledge, nothing has been reported on the incidence or nature of glomerulonephritis in galagos. The present report, therefore, concerns a histopathologic analysis of the renal alterations observed in several species of captive galagos displaying a variety of glomerular lesions. Evidence is presented that indicates possible hypersensitivity etiology of at least one form of nephritis in these animals and that contrasts the morphology of the renal lesions with those of proliferative glomerulonephritides reported in man and other experimental animals.

Materials and Methods

The galagos examined in this study were housed at the Primate Facility, Duke University and were utilized principally for genetic and behavioral studies. Animals were routinely necropsied when found dead or after euthanasia when they became incapacitated or moribund from illness.[29] Blocks of tissue from all major organs including kidney were fixed in 10% formalin or Zenker in preparation for processing for routine light microscopy. In addition, in 5 animals, a portion of kidney was frozen in a matrix of gelatin at −70 C[31] for later immunohistochemical study and another portion of kidney was fixed in cold 4% glutaraldehyde for later ultrastructural examination.[32]

Blocks of tissue fixed for routine histologic preparation were run-up through alcohols and xylene and impregnated with paraffin in an Autotechnicon according to standard procedures. Paraffin blocks of kidney were sectioned at 2–3 μ and stained with H&E, periodic acid-Schiff reaction (PAS), periodic acid-methenamine silver-Masson stain (PAMM),[32] or Van Gieson connective tissue stain (VG).

Frozen blocks of tissue were sectioned at 4 μ with a cryostat microtome. The frozen sections were fixed on glass slides in acetone for 5 minutes, air-dried, washed in phosphate-buffered (pH 7.3, 0.01 M phosphate) physiologic saline (PBS), and then treated with the appropriate fluorescent antibody reagent. After incubation with a drop of fluoresceinated antigalago γ_2-immunoglobulin or antigalago C3 globulin, the slides were rinsed in two changes of PBS and mounted under coverslips with 50% glycerine in PBS. The antiserum reactive with galago γ_2-globulin was prepared by immunization of rabbits with γ_2-galago globulin isolated from pooled galago serum by DEAE-cellulose chromatography. Rabbits immunized with this preparation of γ-globulin produced an antiserum that precipitated in microimmunoelectrophoresis in a single band with galago γ_2-globulin. Galago C3 (third component of hemolytic complement) was isolated from pooled galago serum by DEAE-cellulose chromatography, partially purified

by Pevicon block preparatory electrophoresis, and repurified by CM-cellulose chromatography. Rabbits immunized with this repurified galago C3 produced an antiserum that precipitated in microimmunoelectrophoretic analysis a B1-globulin analogous to the BIC (C3) globulin of man and guinea pig.[33,34] The sodium sulfate-precipitated γ-globulin fraction of these antiserums was conjugated with fluorescein isothiocyanate by slight modification[32] of a previously published technic.[35] Controls for the immunohistochemical staining reactions were those generally used in this type of study.[32] Fluoresceinated antigalago γ_2-globulin and antigalago C3 were absorbed with respective specific antigen prior to application to the tissues to demonstrate absorptive removal of the conjugated antibodies reactive with tissue-localized host γ_2 or C3 globulins. Other sections were rinsed in acid buffer (pH 3.4) prior to reaction with conjugated antigalago γ_2-globulin to dissociate host γ_2-globulin, presumably localized *in vivo* in the kidneys in immune aggregates. An additional control consisted of treatment of tissue sections with conjugated immune globulins possessing immunologic reactivity with serum proteins heterologous to galago globulins (antihuman γ_2-globulin, antihuman C3, and anti-guinea pig complement).

Blocks of tissue, fixed in glutaraldehyde for ultrastructural study, were processed by procedures outlined in detail elsewhere.[32] Briefly, the fragments of kidney were postfixed in osmium tetroxide, stained *en bloc* with uranyl acetate, embedded in Epon, sectioned at 50 mμ on a Porter-Blum ultramicrotome, and finally stained on copper grids with lead citrate and uranyl-magnesium acetate.

Sections stained by routine histologic technics or with fluorescent antibody reagents were examined and photographed with a Leitz Ortholux-Orthomat photomicroscope equipped with HBO 200W mercury vapor UV light source, incandescent light source, specially designed substage condenser for combined phase-fluorescence observation of specimens, and conventional bright-field and dry dark-field condensers. Specimens prepared for ultrastructural study were examined and photographed with an Hitachi HS-7 or HS-8 electron microscope.

Results

A complete histopathologic survey of the variety of lesions observed in various organs of these galagos has been reported recently.[29] Of particular concern in the present report is a more thorough analysis of the renal lesions observed in 26 of 33 necropsied animals of the four species, *Galago crassicaudatus argentatus, G c crassicaudatus, G demidovii* and *G senegalensis*. In general, five forms of glomerular lesions were observed. Four of these are proliferative in nature and may represent different renal diseases, a spectrum of lesions in the natural history of a single renal disease, or morphologic variations of a single disease process. The other form of glomerular alteration consists of a membranous thickening of glomerular capillary walls. The variety of renal glomerular lesions will be described by categories based on the major glomerular changes and are summarized by species distribution in Table 1. A variety of alterations were observed in renal convoluted tubules and interstitium, and these will be described in association with the respective glomerular alterations.

Diffuse Proliferative Glomerulonephritis

There were 4 animals with a diffuse proliferative glomerulonephritis: 2 *G c argentatus,* and 2 *G seneglensis.* In these 4 cases there was a moderate diffuse increase in the number of glomerular endocapillary cells (mesangial and endothelial cells) with concomitant moderate-to-marked narrowing of the capillary lumens. An occasional neutrophilic leukocyte was present in some tufts. With PAMM and PAS stain, the mesangial membrane matrix increased considerably, but with PAMM stain no "proteinaceous" deposits, such as those seen in human acute proliferative glomerulonephritis,[32,36] could be identified along the subepithelial surface of glomerular capillary walls. Unfortunately, no renal tissue from any of these 4 animals was available for immunohistochemical or ultrastructural examination. In one *G c argentatus* (No. 705), there were many sites of tubular and interstitial calcification and focal hydropic swelling of epithelial cells in proximal

Table 1. Incidence of the Various Glomerular Lesions in Four Species of Galagos

	Distribution of lesions by species and animal No.*				
Glomerular lesion	G c crassicaudatus	G c argentatus	G senegalensis	G demidovii	Total No. of animals with lesions
Diffuse proliferative glomerulonephritis	0	705 110	312 301	0	4
Stalk glomerulonephropathy	007 004 404 417	107 713 712 101 710 105	69-7‡	0	11
Stalk-lobular glomerulonephritis	0	701 704†	0	0	2
Progressive-sclerotic glomerulonephritis	012 011†	706 108† 707† 709†	0	0	6
"Membranous" glomerulonephropathy	008	0	310	1003	3
Total No. of animals with lesions/ animals necropsied	7/9	14/14	4/6	1/4	26/33

* Animals are listed by colony identification No.

† Renal tissue was available in this animal for immunohistochemical and ultrastructural study (See Table 2).

‡ Necropsy case No.

convoluted tubules. In one *G senegalensis* (No. 312) there were a few interstitial foci of infiltration by large and small lymphoid cells.

Stalk Glomerulonephropathy

There were 11 animals with thickening of glomerular mesangium (stalk) by slight or moderate increase in mesangial cells and/or membrane matrix, usually without any significant compromise of capillary lumens. Four of these animals were *G c crassicaudatus*, 5 were *G c argentatus*, and 1 was a *G senegalensis*. The pattern of stalk thickening closely resembled that observed in a similar glomerular lesion of man.[36] Again, unfortunately, no renal material was available from these 5 animals for immunohistochemical or ultrastructural examination. In 1 *G c crassicaudatus* (No. 417) there was local thickening of occasional peripheral portions of glomerular capillary walls in a fashion resembling the major alterations seen in a more progressive form of glomerular disease in some animals, which is described below. In a *G c argentatus* (No. 101) there was focal cytoplasmic hydropic alteration of proximal tubular epithelial cells.

Stalk-Lobular Proliferative Glomerulonephritis

Two animals, both *G c argentatus* (No. 704 and 701), had a form of glomerular mesangial proliferative disease more severe than that described above. The increase in mesangial cells and membrane matrix was so extensive as to result in enlarged glomeruli with lobular accentuation of capillary tufts (Fig 2). In association with this rather marked increase in size of mesangial areas, the glomerular capillary lumens were moderately compromised. In addition, there was local thickening of peripheral portions of glomerular capillary walls but formation of collagen in glomerular capillary walls had not occurred. By immunofluorescence microscopy, there were no detectable sites of host immunoglobulins or third component of complement (C3) in the glomeruli of the one animal (No. 704) studied by this technic (see Table 2). Examination by electron microscopy of five glomeruli from Animal No. 704 revealed several findings that correlated with the light mircroscopic observations. Mesangial regions were greatly enlarged by excessive amounts of basement membrane matrix and increased number of mesangial cells (Fig 3). The cross-sectioned profile of the markedly thickened capillary basement membranes had many swiss-cheese holes or vacuoles, particularly along the subendothelial portion of the basement membrane (Fig 3). Endothelial cells generally were not enlarged. There was local obliteration of filtration slit pores be-

Table 2. Summary of Results of Immunohistochemical and Ultrastructural Studies

Diagnostic category of renal disease	Animal No.	Immunofluorescence				Electron microscopy				
		Galago γ-globulin	Galago C3	Anti-membrane activity in serum	Galago γ-globulin eluted at pH 3.4	Subendothelial deposits	Collagen fibers in capillary walls	Thickened basement membrane	Subepithelial deposits	Cystic epithelial cells
Galago crassicaudatus argentatus										
Stalk-lobular glomerulonephritis	704	0	0	0	ND	0	0	+	0	+
Progressive	108	+	+	0	Yes	+	+	0	+	+
sclerotic	707	+	+	0	Yes	+	+	±	0	0
glomerulonephritis	709	+	+	ND	ND	+	+	0	0	0
Galago crassicaudatus crassicaudatus										
Progressive sclerotic glomerulonephritis	011	+	+	0	Yes	+	+	0	0	0

ND indicates test not done.

cause of "fusion" of epithelial foot processes. Many epithelial cells contained large cytoplasmic vacuoles filled with nondescript, finely granular material. There were no electron-dense deposits or collagen fibers in or along the glomerular capillary basement membranes.

Other histologic findings in the kidneys of these 2 animals were frequent cystic dilation of glomerular spaces and tubules (some of the cysts were large enough to be seen in the gross specimen) (Fig 1), diffuse interstitial fibrosis, and focal interstitial inflammatory infiltrates consisting of small and large lymphocytes and plasma cells. In No. 701 there were many focal deposits of calcium salts in the interstitium and wall of convoluted tubules. There were no alterations in renal arterioles and arteries.

Progressive-Sclerotic Glomerulonephritis

Six animals had a progressive or "chronic" form of glomerulonephritis; 2 of these animals were *G c crassicaudatus* and 4 were *G c argentatus*. By light microscopy, the following glomerular alterations were shared in variable degrees by these 6 animals. There was moderate-to-marked, local or diffuse thickening of peripheral portions of glomerular capillary walls by solid or fibrillar, and eosinophilic, "Masson" red or green, Van-Gieson positive material (Fig 4 and 8). This, along with cellular and argyrophilic membrane matrix thickening of mesangial regions, greatly compromised glomerular capillary lumens. No glomerular capsular crescents or completely sclerosed glomeruli were seen. Cystic dilation of a few or many glomerular spaces and convoluted tubules was noted in 2 cases (No. 706 and 108).

Immunohistochemical examination of kidney in 4 of these animals (see Table 2) revealed limited or extensive local distribution of host γ-globulin and C3 in thickened portions of glomerular capillary walls (Fig 5 and 9). By combined phase-fluorescence microscopy, the deposits of γ-globulin and complement were observed to lie beneath the glomerular capillary basement membranes on the endothelial side of the capillary wall (Fig 5). Much of the localized γ-globulin could be eluted from the glomerular capillaries in the three kidneys tested (No. 108, 707 and 011) by washing of sections in acid buffer of pH 3.4. Such acid-washed sections had only a few isolated remaining sites of glomerular fluorescence when treated with fluoresceinated anti-galago γ_2-globulin. Indirect immunofluorescence tests for membrane-reactive antibodies in the serum of these animals were essentially negative. Sections from a *G c argentatus* with stalk-lobular glomerulonephritis (No. 704) free of localized γ-globulin were incubated in a

first step with nephritic host galago serum and then in a second step with fluorescein-conjugated antigalago γ_2-globulin. No diffuse linear deposition of host γ-globulin was observed; however a few isolated segments of glomerular capillary walls displayed slight-to-moderate immunofluorescence reactions in this indirect test. This finding is contrary to the presence of membrane-reactive antibodies in the nephritic serums and is instead compatible with the presence of immunoglobulins reactive with some unidentified material distributed locally in glomerular tufts of Animal No. 704. Treatment of sections of nephritic galago kidneys with fluoresceinated antihuman γ-globulin or antihuman albumin as controls for nonspecific fluorescence gave negative results. Absorption of fluoresceinated antigalago γ_2 or antigalago C3 with γ_2 or C3, respectively, abolished the ability of these antiserums to react with glomerular sites of the respective localized host globulins. Finally, treatment of sections with unconjugated antigalago γ_2 or antigalago C3 prior to treatment in a second step with the respective conjugated "fluor" reagent only slightly or moderately blocked the immunofluorescence reaction.

Ultrastructurally, glomeruli in all of the kidneys from this group of animals displayed rather similar alterations except for minor quantitative variations among the 4 animals examined and one major exception to be described below in animal No. 108. The thickened capillary walls observed by light microscopy and the corresponding deposits containing host immunoglobulin and complement observed by immunofluorescence microscopy had their counterpart in small, medium-sized or massive subendothelial deposits observed by electron microscopy (Fig 6 and 10). These deposits comprised mixtures of homogeneous, fairly solid or loose, coarsely granular electron-dense material (Fig 6 and 10); remnants of endothelial cell cytoplasmic structures; and intermixed large collagen fibers with an average band periodicity of 695 Å (Fig 6, 10, 12 and 13). In several capillary loops of one out of four glomeruli examined from animal No. 108, unique subepithelial, moderately electron-dense nodules or "humps" were identified (Fig 13). At high magnification, these nodules are seen to possess a substructural "crystalline" lattice with a unit spacing of about 170 Å (Fig 13). Other ultrastructural alterations noted were widening of mesangial regions with increased amounts of basement membrane matrix intermixed with a deposit material described above and mesangial cells; local intramembranous, "zebra stripe" alterations in Animal No. 108 (Fig 14); rare subepithelial, local, lumpy distortion of the capillary basement membrane; and prominent vacuolization of epithelial

cells in Animal No. 108 (Fig 11). Curiously, the animal with glomerular epithelial vacuolization also had cystic dilation of glomerular spaces and convoluted tubules by light microscopy.

Other general histologic alterations noted in these animals with the progressive sclerotic form of glomerulonephritis were focal interstitial inflammatory infiltrates of lymphoid and plasmacytic cells, focal or diffuse interstitial fibrosis, focal interstitial collections of "foam cells" similar to those observed in some human nephrotic and chronic renal diseases, and in 1 animal (No. 012) focal, heterotopic, interstitial bone formation.

"Membranous" Glomerulonephropathy

Three animals had diffuse thickening of the basement membranes of glomerular capillary tufts (Table 1). This PAS-positive, eosinophilic and argyrophilic membranous thickening was different in pattern from that observed in human membranous glomerulonephropathy [32,37] in that no "proteinaceous" intramembranous deposits or membrane spikes could be demonstrated with PAMM stain of the galago kidneys. In addition there was a slight-to-moderate increase in argyrophilic basement membrane matrix in thickened glomerular mesangial regions, but no increase in the cellularity of the tufts. Unfortunately, no immunohistochemical or ultrastructural studies were done on the kidneys from these 3 animals. No significant interstitial, tubular or vascular alterations were seen in kidneys from these three animals.

Discussion

In a captive population of galagos, utilized mainly for genetic and behavorial studies, routine necropsy on 33 animals over a 4-year period revealed, in addition to a fair variety of disease processes,[29] five histopathologic types of renal glomerular disease. For purposes of presentation and discussion of these glomerular diseases, they have been divided into descriptive diagnostic categories as follows: (1) diffuse proliferative glomerulonephritis, (2) stalk proliferative glomerulonephritis, (3) stalk-lobular proliferative glomerulonephritis, (4) progressive-sclerotic glomerulonephritis and (5) "membranous" glomerulonephropathy. In this histopathologic survey of these renal diseases, it has not been possible to ascertain the etiology or pathogenesis of the various morphologic forms of proliferative glomerular diseases. Furthermore, any possible pathogenetic interrelationship of these nephritides has not been worked out because of the few animals available and the predominantly retrospective nature of this study. The

localization of host γ_2-globulin and complement in diseased glomeruli is suggestive of some "hypersensitivity" mechanism for development of the progressive-sclerotic form of glomerulonephritis. The presence of recurrent and chronic cutaneous infections (demonstrated to be infected, in some instances, with candida species) is also compatible with a microbial hypersensitivity etiology. The patterns of glomerular alteration at the light and electron microscopic levels in animals with the latter disease resemble those glomerular lesions seen in the nephritis of Aleutian mink disease[6,7] and NZB hybrid mice with lupus-like syndrome.[8,9] The inflammatory glomerular lesions in the galagos differ somewhat from those seen in the variety of apparently hypersensitivity-induced, acute proliferative glomerulonephritides of man; the lesions in galagos have prominent amounts of giant collagen fibers, a cross-hatched crystalline substructure in subepithelial "humps" (observed in one animal) and cystic dilation of glomerular spaces and cystic alteration of glomerular epithelial cells. The frequency of occurrence of proliferative glomerular disease in the two galago subspecies *G c crassicaudatus* and *G c argentatus* suggests not only a possible pathogenetic sequential relationship of diffuse proliferative, stalk-proliferative and progressive-sclerotic glomerular lesions, but also a hereditary etiologic factor. The correlative clinical data is unfortunately too scant and poorly documented to establish any meaningful proof of either of these two possibilities; as in man, the pathogenetic interrelationships of the morphologic varieties of proliferative glomerular diseases is yet to be clearly established.

The results of these preliminary morphologic studies on renal glomerular diseases in galagos are presented here for several reasons. It is hoped that other investigators working with these prosimian primates will be stimulated to attempt characterization of the nature and incidence of glomerulonephritides in these animals in both the captive and wild state. Thorough prospective clinicopathologic studies may provide meaningful information on the etiology and natural history of the nephritides in these animals. Finally, these proliferative inflammatory renal diseases in galagos may provide a convenient model for study of the nature of hypersensitivity glomerulonephritis in man.

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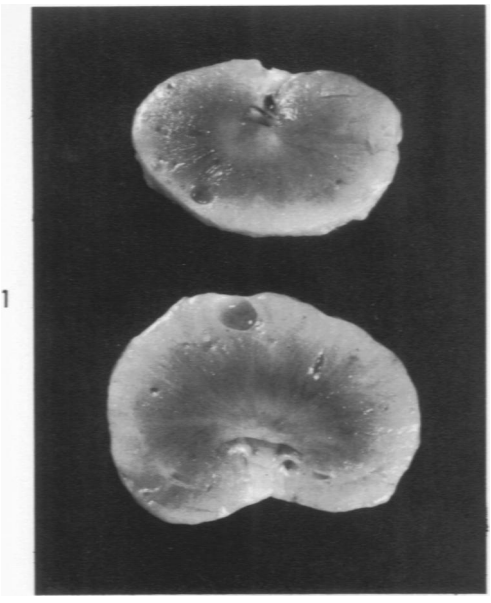
[*Illustrations follow*]



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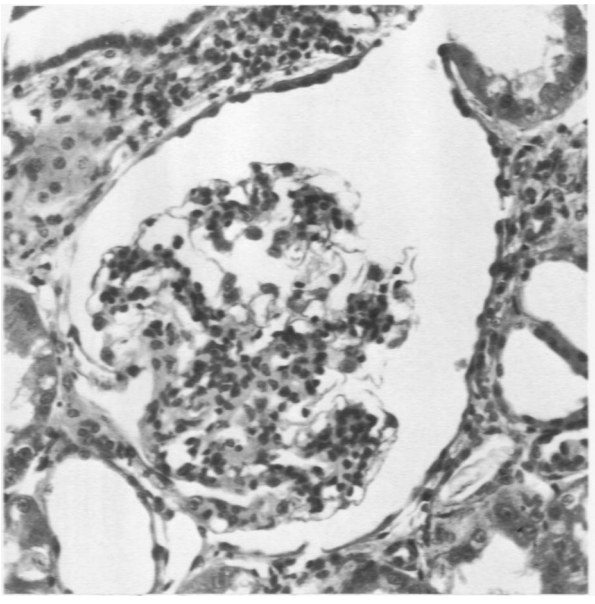

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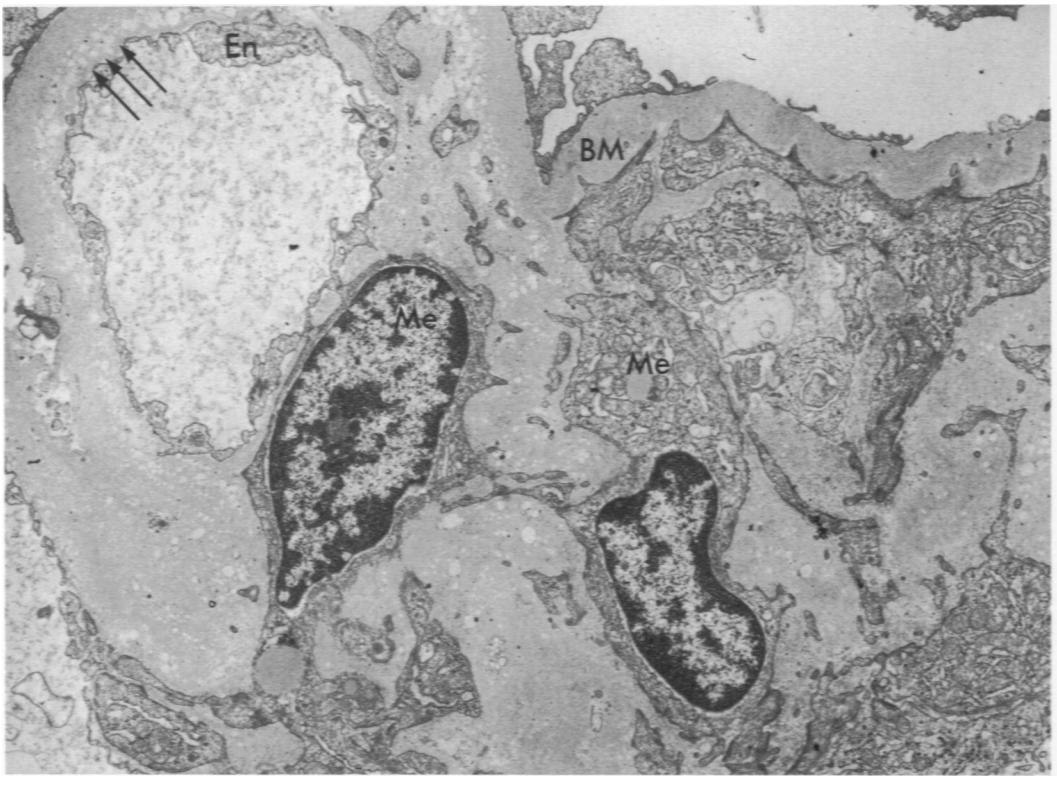

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Fig 1.—Cut surface of the kidneys from Galago No. 704 showing occasional large and many small cortical cysts. Gross photograph, approximately life size.

Fig 2.—Glomerulus from Galago No. 704 showing mesangial hypercellularity and "cystic" dilation of Bowman's space. Adjacent to glomerular capsule is focus of chronic inflammatory cell infiltrate in interstitium. H&E. × 217.

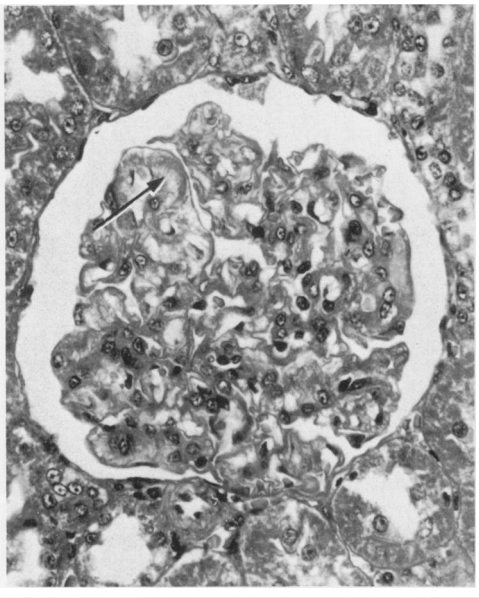
Fig 3.—Portion of glomerular tuft from kidney of Galago No. 704 with diffuse thickening of capillary basement membrane *(BM)*, subendothelial vacuolization of basement membrane *(three arrows)*, and increase in size and number of mesangial cells *(Me)*. Electron micrograph. × 6300.

Fig 4.—Glomerulus in kidney from Galago No. 011 showing thickening of capillary walls by subendothelial deposits, some of which have lacy pattern *(arrow)*, slight increase in cellularity, thickening of mesangial regions and marked narrowing of capillary lumens. H&E. × 200.

Fig 5.—Another glomerulus in frozen section of kidney from Galago No. 011, observed by phase-fluorescence microscopy showing sites of immunofluorescence *(white)* for localized host immunoglobulins beneath capillary basement membranes *(thin dark lines outlining many of deposits)*. Fluorescent antigalago γ-globulin. × 415.

Fig 6.—Portion of another glomerular tuft from kidney of Galago No. 011 with huge subendothelial deposit *(d)* containing bundles of collagen fibers *(c)*, remnants of cytoplasmic organelles and vacuoles. Capillary basement membrane is slightly thickened *(bm)* and small capillary lumen is lined by thin endothelial cell *(En)*. Foot processes of epithelial cells *(Ep)* are locally "fused" with obliteration of slit pores. Electron micrograph. × 12,000.

Fig 7.—Microtubular "honeycomb" structure, presumably of endoplasmic reticulum, in epithelial cell *(Ep)* of another glomerulus from Galago No. 011. Basement membrane *(bm)* with overlying epithelial foot processes. Electron micrograph. × 15,000.



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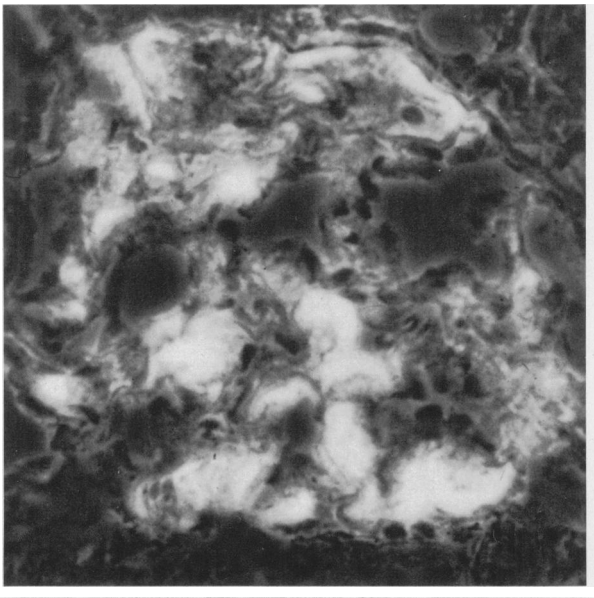

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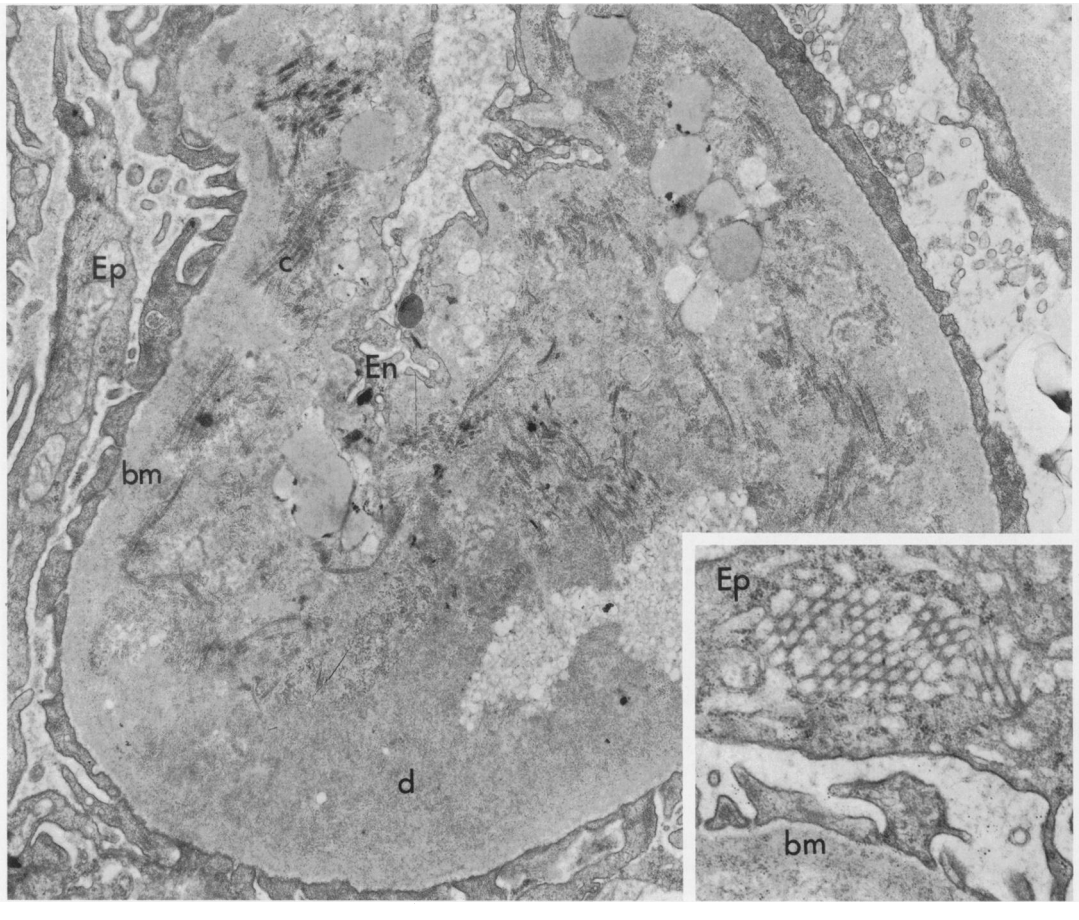

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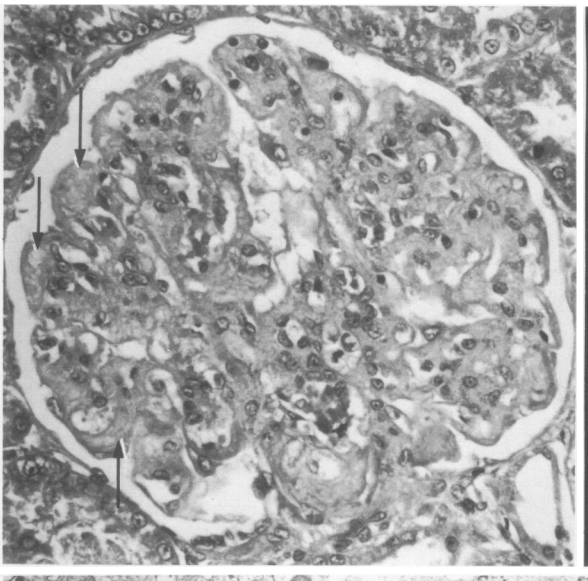

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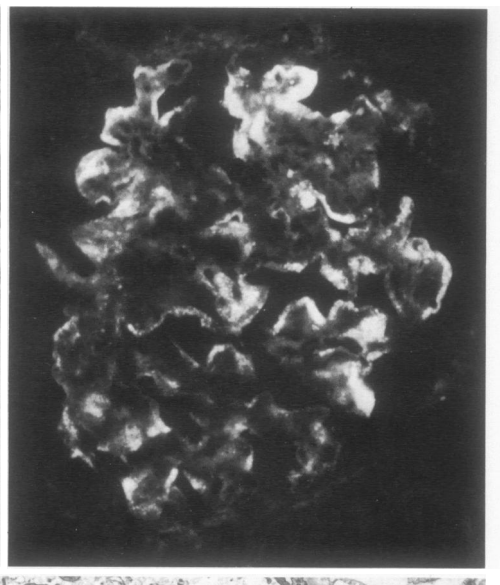

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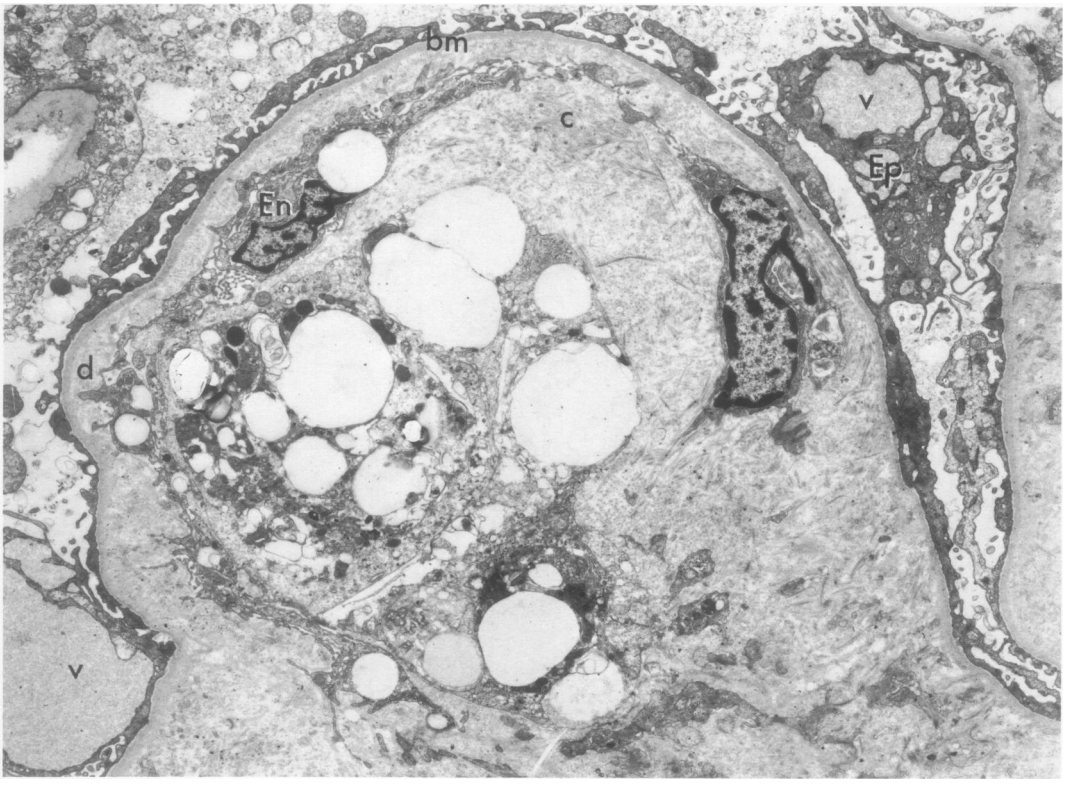

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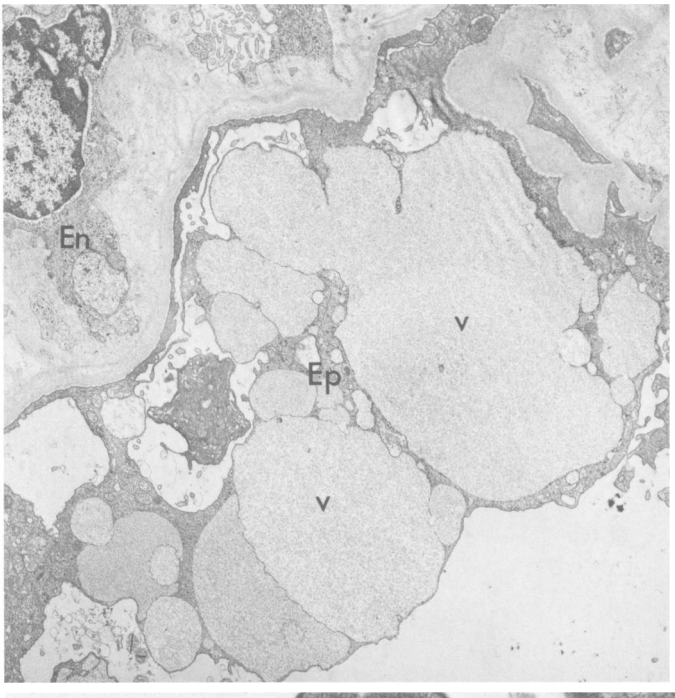
Fig 8.—Glomerulus in kidney from Galago No. 108 shows increased cellularity, thickening of mesangial regions and capillary walls, and subendothelial deposits often with lacy pattern *(arrows)*. H&E. × 260.

Fig 9.—Another glomerulus in frozen section from kidney of Galago No. 108 with local thick linear, globular or granular deposits of host antibody globulin along capillary walls. Fluorescent antigalago γ-globulin. × 225.

Fig 10.—Portion of capillary tuft from another glomerulus from Galago No. 108 with subendothelial, loose granular deposits *(d)* containing bundles of collagen fibers *(c)*, marked narrowing of capillary lumen nearly filled with endothelial cells *(En)*, thin capillary basement membrane and vacuolization *(v)* of epithelial cells *(Ep)*. Electron micrograph. × 4200.



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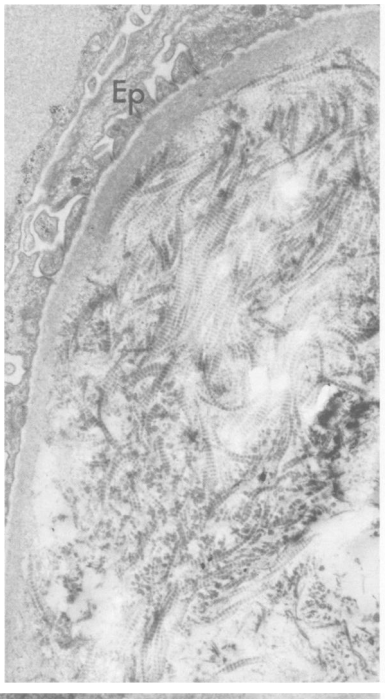

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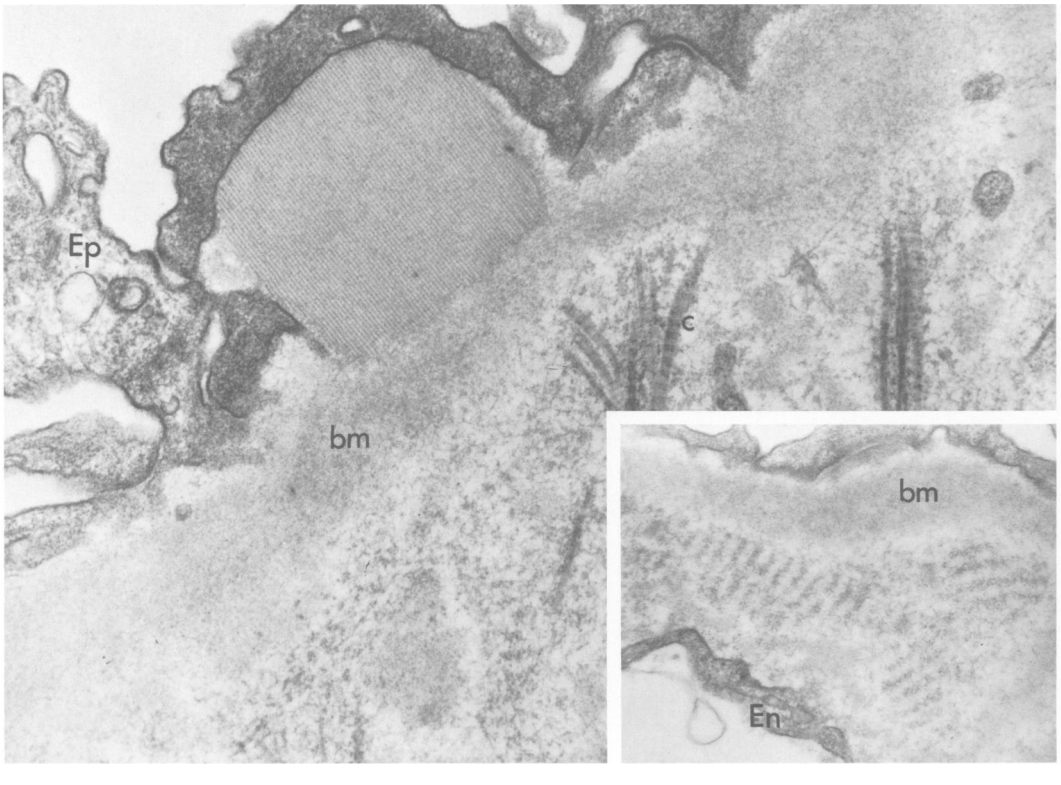

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Fig 11.—Portion of capillary tuft in another glomerulus from Galago No. 108 showing marked vacuolization *(v)* of epithelial cells *(Ep)*. Endothelial cell *(En)*. Electron micrograph. × 4500. **Fig 12.**—Portion of capillary wall in another glomerulus from Galago No. 108 showing large number of collagen fibers beneath capillary basement membrane. Epithelial cell *(Ep)*. Electron micrograph. × 8000. **Fig 13.**—Portion of capillary wall in same glomerulus as in Fig 10 from Galago No. 108 showing a subepithelial deposit with "crystalline" substructural lattice. Basement membrane *(bm)*, epithelial cell *(Ep)*, collagen fibers *(c)*. Electron micrograph. × 26,000. **Fig 14.**—Portion of capillary wall in same glomerulus as in Fig 10 and 13 from Galago No. 108 showing zebra-stripe pattern in thickened glomerular capillary basement membrane *(bm)*. Endothelial cell *(En)*. Electron micrograph. × 28,600.

[*End of Article*]