A MORPHOLOGICAL STUDY OF THE GLOMERULAR LESIONS IN CHRONIC NEPHRITIS IN THE DOG

By

ANDREW JOHN SPENCER B.V.M.S. M.R.C.V.S.

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Department of Veterinary Pathology,

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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DECLARATION

Cases 1, 4-8, 26, 27, 31-33, 35-39 of this study formed a preliminary study of chronic interstitial and chronic glomerulonephritis in dog (Wright et al. 1976). I am indebted to Professor Wright and his co-authors for allowing me to undertake further investigations on these cases so that they could be included in this study, and for the use of Fig. 6 which also appeared in the aforementioned article.

SUMMARY

Renal disease is of major importance in the dog, in terms of both morbidity and mortality. However, while the last two decades have seen great advances in the knowledge of Human and certain experimental animal nephropathies, very few in depth investigations of canine renal diseases have been published. In particular, despite the prominence of glomerular injury in many canine nephropathies, this process has largely been ignored. In Part 1 of this thesis, the literature on the most important canine nephropathies, viz: interstitial nephritis, glomerulonephritis, amyloidosis and suppurative nephropathies, was reviewed. An attempt was made to highlight the most important gaps in our knowledge with particular reference to pathogenesis of the lesions and morphological changes in the glomeruli.

The most common cause of renal failure in the dog is chronic interstitial nephritis. In Part 2 of this thesis the first detailed combined light, electron and immunofluorescence microscopic study was carried out on this nephropathy as it is described in Britain. In addition, elution studies were performed to investigate further the immunopathology of the kidney. The diagnosis of chronic interstitial nephritis was found to cover a heterogeneous group of dogs. Of the 30 cases studied, 24 were probably the result of previous L.canicola infection. The remaining 6 cases appeared less likely to have resulted from L. canicola infection, but no positive evidence emerged to implicate another cause. However all 30 cases

were alike in respect of immunopathology; neither autoantibodies nor immune complex deposition played a role in the severe renal scarring that characterized these cases.

Recently a morphologically similar nephropathy, chronic glomerulonephritis, has been described in the dog. 10 cases were studied with the same techniques as listed above, and included in Part 2 of this thesis, for a comparative evaluation. In contrast to chronic interstitial nephritis, chronic glomerulonephritis is an immunologically mediated nephropathy. In all cases widespread deposits of immunoglobulin and bound complement were present in the glomeruli. In 9 cases granular deposits were found, a pattern highly suggestive of the involvement of immune complexes. In the remaining case linear deposits were present along the capillary walls, a pattern suggestive of the presence of anti-glomerular basement membrane antibodies, but this was not confirmed by the elution studies.

Despite this difference in aetiology, both nephropathies were characterized by the same progressive scarring and obliteration of the glomeruli. This process was described in detail in Part 2 of this thesis for the first time in the dog. The major components of this process were found to be a) thickening, wrinkling and duplication of both glomerular and capsular basement membranes, b) expansion of the mesangial cells and matrix, c) the deposition of fibrin obliterating capillaries and leading to the formation of capsular adhesions, d) the formation of collagen particularly in the urinary space.

Fibrin deposition appeared to play a major role in the progression of glomerular scarring. This concept has been supported by studies on several experimental animal models, but none of this work had ever been carried out on the dog or the results applied to canine nephropathies. Therefore, Part 3 of this thesis was devoted to a combined light, electron and immunofluorescence microscopic study of two experimental canine nephropathies characterized by fibrin deposition in the glomeruli. In the first, a transient period of glomerular thrombosis, resulting from disseminated intravascular coagulation induced by Liquoid (sodium polyanetholsulphonate) injection, was followed by mild focal glomerular scarring. In the second, severe diffuse glomerular scarring followed a prolonged period of extensive fibrin deposition induced by nephrotoxic serum containing anti-glomerular basement membrane antibodies. Thus in both experimental immunological and non-immunological nephropathies glomerular scarring, similar to that encountered in both chronic interstitial nephritis and chronic glomerulonephritis, followed the deposition of fibrin in the glomeruli. This gave credibility to the view that fibrin deposition plays an important role in the progression of glomerular injury and obsolescence in chronic nephropathies of the dog.

GENERAL INTRODUCTION: RENAL DISEASE IN THE DOG

Renal disease is of major importance in both veterinary and Human medicine. In Man, although the mortality rate is not striking when compared with heart disease, cancer and cerebrovascular disease, morbidity is high (Robbins 1967). This, plus the fact that present day management of cases relying heavily on dialysis and transplantation is expensive, means that renal disease is a heavy burden on the world's medical resources (Wing 1977). In the last twenty years a vast amount of research has been published on Human and experimental animal renal disease, and the use of modern techniques such as electron microscopy and immunofluorescence in this work has led to great advances in the understanding of the pathogenetic mechanisms operating in the various nephropathies. This is particularly true with regard to the role of immunological mechanisms and the reactions of the glomerulus in glomerulonephritis, the commonest human nephropathy (Kerr 1975).

In veterinary medicine, renal disease in the dog is one of the major problems that a small-animal practitioner meets. Bloom's survey of 1939 (Table 1) and the records of Glasgow University Veterinary School (Table 2) show that, not only is clinical renal disease common, it is also a major cause of death. In addition, Bloom's 1954 figures (Table 1) show that when sub-clinical lesions are taken into account a much higher overall incidence is found. The figures summarized in tables 1 and 2 show that in contrast to Man, chronic interstitial nephritis is the most important canine nephropathy in terms of both a sub-clinical lesion and as a cause of renal failure. However these figures

were from histological studies and no recent surveys using the more modern investigative techniques of electron and immunofluorescence microscopy have been published. The application of such techniques in recent years has led to an increasing number of reports of glomerulonephritis (Kurtz et al. 1971, Murray and Wright 1974; Rouse and Lewis 1975; Müller-Peddinghaus and Trautwein 1977a,b). Whether these reports reflect a genuine increase or just a greater awareness that glomerulonephritis can occur in dogs, coupled with the increasing use of immunofluorescence and electron microscopy in diagnostic work remains,to be determined. However, although glomerulonephritis is probably more common than was once thought, interstitial nephritis is still regarded as the commonest cause of renal failure in the dog (Morrison and Wright 1976a).

The number of recent studies on the whole range of canine nephropathies is still small, and although much experimental work has been done in laboratory animals elucidating mechanisms of renal,and in particular glomerular injury, little of this has been done in, or applied to,the dog. Thus at present, knowledge of many aspects of canine renal disease, particularly concerning the pathogenesis and details of glomerular injury, is incomplete and considerably behind that of Man. This thesis is an attempt to remedy this situation and the first part is a resume of the salient features of the major canine nephropathies with particular emphasis on the glomerular lesions. Because of this remit, nephropathies where the glomerulus is not involved i.e. primary tubular

diseases, inherited congenital abnormalities, and renal tumours, are excluded.

TABLE 1

Incidence of Renal Disease in Dogs

Published Figures

BLOOM (1939)

i) Incidence of clinical Renal disease

Number of dogs examined clinically	4,123	
Number of dogs with clinical renal disease	274	(6.6%)
Mortality rate in affected dogs	25.5% (70/274)	

ii) Diagnosis in 70 uraemic dogs

Interstitial nephritis	62	(88.7%)
Suppurative nephritis	5	(7.1%)
Nephrosis	2	(2.8%)
Amyloidosis	1	(1.4%)

iii) Incidence of non-clinical interstitial nephritis

Number of dogs necropsied	200	
Number of dogs with focal interstitial nephritis	108	(54%)

BLOOM (1954)

i) Incidence of various renal lesions

Interstitial nephritis -	in all necropsies	55%
	in animals > 8 years	80%
	in uraemic animals	89%
Pyelonephritis -	in all necropsies	5%
Focal embolic nephritis		"not uncommon"
Glomerulonephritis		"rare"
Amyloidosis		"rare"

MONLUX (1953)

i) Incidence of various renal lesions

Number of cases with distinct renal lesions	395	
Number of cases with "nephritis"	321	(81.3%)
Number of cases with metabolic, neoplastic, degenerative or congenital lesions	74	(18.7%)

TABLE 1 (Contd)

ii) Diagnosis in 321 nephritic cases

Interstitial nephritis (including suppurative nephritis and specific infections)	283	(88.2%)
Inflammatory vascular disease (glomerulonephritis, infarction and "sclerosing nephropathy")	38	(11.8%)

WETTIMUNY (1963)

i) Diagnosis in 178 dogs with a histological lesion of nephritis

Chronic interstitial nephritis	97	(54.5%)
Acute interstitial nephritis	36	(20.2%)
Primary glomerular disease (glomerulonephritis, amyloidosis, lipidosis)	20	(11.2%)
Pyelonephritis (including 1 case of tuberculosis)	16	(9.0%)
Embolic nephritis	9	(5.1%)

TABLE 2

Incidence of Renal Disease in Dogs

Glasgow University Veterinary School

Pathology Department Records 1971-76

Total number of dogs necropsied	1633
Total number dying or destroyed with renal failure	110
% of dogs dying or destroyed with renal failure	6.7%

Number of dogs with acute interstitial nephritis	7 (6.4%)
" " " " sub-acute interstitial nephritis	3 (2.7%)
" " " " chronic interstitial nephritis	53 (48.2%)
" " " " chronic pyelonephritis	7 (6.4%)
" " " " proliferative glomerulonephritis	6 (5.4%)
" " " " chronic glomerulonephritis	9 (8.2%)
" " " " membranous nephropathy	4[a] (3.6%)
" " " " amyloidosis	7 (6.4%)
" " " " nephrosis	9 (8.2%)
" " " " miscellaneous lesions	5[b] (4.5%)
TOTAL	110 (100%)

a 2 cases presented with the nephrotic syndrome.

b 2 cases of nephrosis complicating proliferative glomerulonephritis,

1 case of nephrosis complicating chronic interstitial nephritis,

1 case of combined pyelonephritis and amyloidosis,

1 case of renal lymphosarcoma.

PART 1

A REVIEW OF THE MAJOR NEPHROPATHIES OF THE DOG

PRIMARY INTERSTITIAL DISEASE

Interstitial nephritis can be broadly classified as being either acute or chronic. Acute cases are characterized by the presence of mononuclear cell infiltrates confined to the interstitial tissue, while chronic cases are characterized by progressive renal scarring (McIntyre and Montgomery, 1952; McIntyre 1954).

Acute Interstitial Nephritis (AIN)

The incidence of AIN is at present low in Great Britain, probably as a result of widespread vaccination against Leptospira canicola, the acknowledged causal agent (Morrison and Wright 1976a). Typically the disease is seen in young ($<$ 2 years old), male dogs from urban areas, and any breed may be affected (McIntyre 1954).

Clinical signs vary from thirst and polyuria in an otherwise normal dog to an acutely ill uraemic animal (Bloom 1937, McIntyre and Montgomery 1952, McIntyre 1954, Wettimuny 1963). The latter dogs are depressed, anorexic, thirsty and oliguric. They often vomit repeatedly and have a brown discolouration of the mouth. In severe cases ulceration of the mouth and tongue may be extensive and lumbar pain present; mortality in such animals is high.

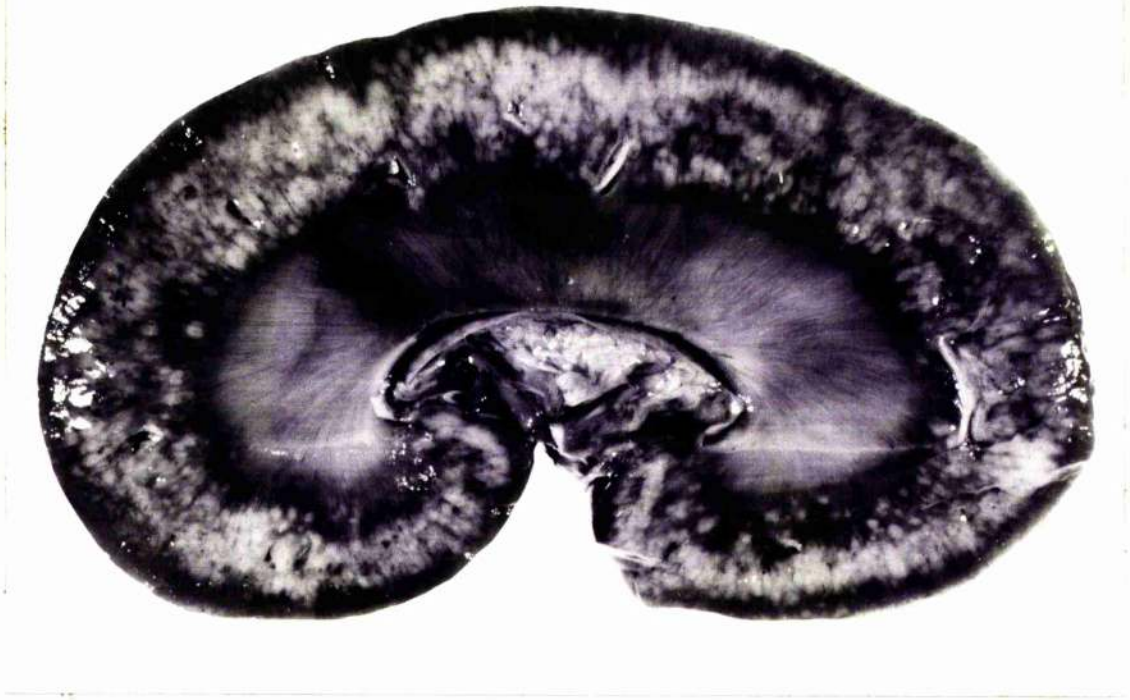
The pathological features are well known (Bloom 1937, 1939, 1954, Platt 1951a, McIntyre and Montgomery 1952, Monlux 1953, McIntyre 1954, Wettimuny 1963). Macroscopically the kidneys are swollen and mottled with distinctive pale areas present throughout the inner, and to a lesser degree the outer, cortex (Fig. 1). In less severely affected animals

Fig. 1 Acute Interstitial Nephritis

The kidney is swollen due to extensive mononuclear cell infiltration in the cortex and outer medulla.

Fig. 2 Chronic Interstitial Nephritis (CIN), case 16.

As a result of interstitial scarring the kidney is pale particularly in the area of the cortico-medullary junction, and the cortex reduced in width. Small cyst are scattered throughout the kidney mostly in the outer medulla.



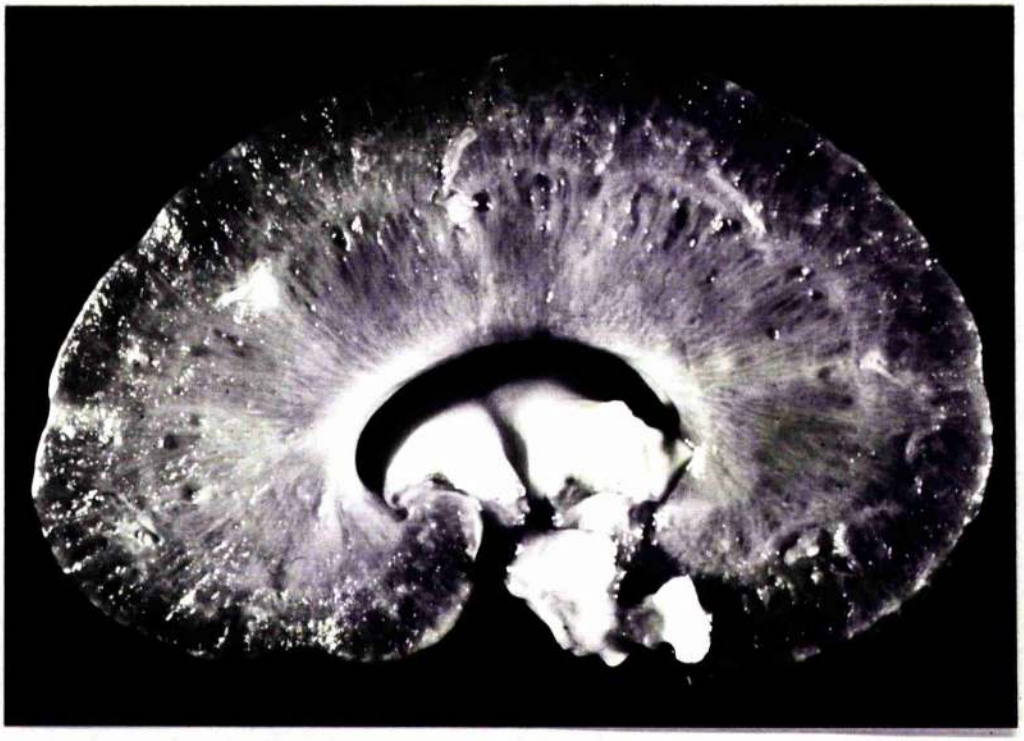
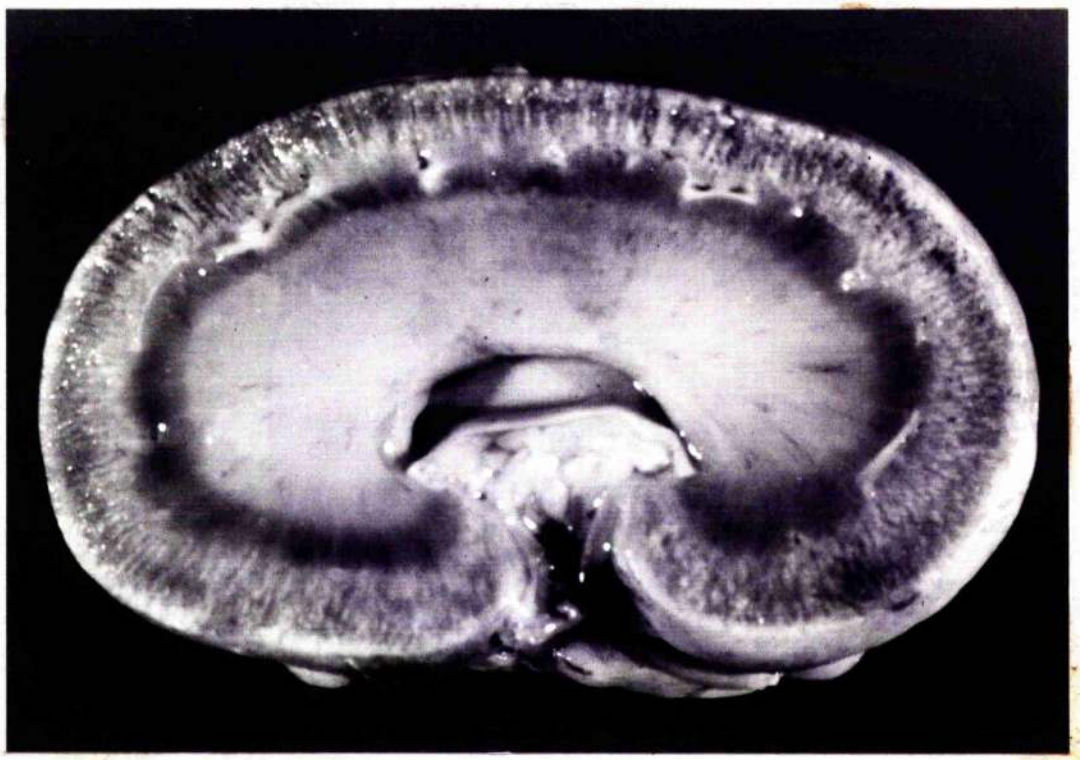


Fig. 3 CIN, Case 15

The most severe case of CIN seen in this study. The cortex is reduced to a thin band, but the kidney is normal in size due to the extreme cystic dilation of the medulla.

Fig. 4 "Inherited" Renal Disease (in a cocker spaniel)

The kidneys resemble other cases of CIN except for the extreme pallor reflecting extensive renal calcification.



only a few scattered pale foci may be seen. These areas are composed of massive cellular infiltrates composed of plasma cells, lymphocytes and macrophages with only occasional polymorphonuclear leucocytes. There is widespread degeneration and necrosis of the tubules in these areas but the glomeruli are unaffected.

Leptospirae can be identified in the urine (by dark ground microscopy) and in the kidneys themselves (by silver staining methods) from cases of AIN (McIntyre and Montgomery 1952, McIntyre 1954) and good evidence has now been produced to implicate L. canicola as the causal organism. This organism has been cultured from blood and urine of clinical cases (McIntyre 1954) and such cases are characterized by a rising or high level (> 1:10,000) of serum antibody to it (McIntyre 1954). Protein eluted from affected kidneys also contains high levels of such antibody, while L. canicola antigen can be identified in the kidney with immunofluorescence as intact and effete organisms, and apparently phagocytosed by macrophages (Morrison and Wright 1976b). Despite this volume of evidence, conclusive proof involving the consistent reproduction of the clinical disease by experimental infection with the organism is lacking. Many attempts, using various methods of culturing the organism and various routes of inoculation, have been made (McIntyre 1954, Jull and Heath 1959, Wettimuny 1963, Anderson 1967, Low et al. 1967, Taylor et al. 1970) and although some of these authors occasionally produced quite extensive renal lesions (Anderson 1967, Taylor et al. 1970) the clinical syndrome of renal failure due to AIN has never been reproduced experimentally.

Further unanswered questions arise over the nature and function of the cellular infiltrate. A recent immunopathological study suggests it has two functions: local production of anti-leptospiral antibody by the plasma cells; and phagocytosis of cell debris and leptospiral antigen complexed with antibody by the macrophages (Morrison and Wright 1976b). Whether or not some of the lymphoid cells and macrophages are involved in cell mediated immunity has never been investigated.

In addition, the possible role of autoimmunity is not known. Torten et al. (1967) found the presence of serum antibody directed against the renal interstitial tissue in 1 of 4 dogs which had been experimentally infected with L.canicola and they suggested that in the acute phase of the disease kidney antigens could be released into the circulation so stimulating the production of autoantibodies which could cause further renal damage. However, no further evidence has been published to support this possibility of an autoimmune reaction, while Morrison and Wright (1976b) on the other hand, failed to find any anti-kidney antibody in renal eluates from 14 cases of AIN.

Chronic Interstitial Nephritis (CIN)

CIN is regarded as the commonest cause of renal failure in the dog (see above). It rarely occurs in dogs less than a year old and its incidence increases with age (McIntyre 1954). As with AIN, males are more commonly affected than females and there is no apparent breed susceptibility (McIntyre 1954).

The clinical picture is one of chronic renal failure (Bloom 1937, 1954, McIntyre and Montgomery 1952, McIntyre 1954, Wettimuny 1963). Cases are characterized by thirst, polyuria, gradual weight loss, pallor of the mucous membranes, proteinuria and hypertension. If the animal becomes uraemic (blood urea > 7.9 m.mol l^{-1}), anorexia, frequent vomiting (often containing blood), halitosis and oral ulceration are then seen. In addition, osteodystrophia fibrosa is often present, and when severe this leads to "rubber jaw" where the canine teeth can be moved in their sockets (Platt 1951b, Brodey et al. 1961).

Several detailed accounts of the pathology of CIN exist (Bloom, 1937, 1939, 1954, Platt 1951a, McIntyre and Montgomery 1952, Monlux 1953, McIntyre 1954, Wettimuny 1963, Mackey 1965). The dominant feature is the replacement of nephrons by extensive irregular areas of fibrosis concentrated particularly around the cortico-medullary junction. This produces a firm, pale, contracted kidney with a shrunken granular cortex (Figs. 2, 3). Microscopically there is extensive derangement of nephrons in these areas of scarring while elsewhere the renal architecture is relatively well preserved. Unlike AIN cellular infiltration is minor but small foci composed of lymphocytes, plasma cells and occasional macrophages are always present in the cortex particularly in the areas of fibrosis.

Glomerular lesions are a prominent feature of CIN but they have only been briefly described in the literature (Platt 1951a, McIntyre and Montgomery 1952, Wettimuny 1963, Mackey 1965, Anderson 1968b). The typical lesion described is one of scarring leading eventually to complete glomerular

obsolescence. The major factor in this process is the progressive accumulation in the glomerulus of amorphous, eosinophilic material; this has a staining reaction for fibrin when first formed but with time this alters and it stains for collagen (Platt 1951a, Mackey 1965, Anderson 1968b). This material builds up both in the tuft obliterating the capillaries, and in the urinary space where it forms capsular adhesions. Eventually this results in a non-functional glomerulus composed of a shrunken mass of collagen staining material with few cells or capillaries. As a result of this progressive destruction of glomeruli, those remaining show compensatory hypertrophy with enlargement of the tuft and dilation of the capillaries (Bloom 1954). A less common glomerular lesion is collapse and atrophy of the tuft accompanied by gross distension of Bowman's capsule; occasionally however, this may be the dominant glomerular lesion (Mackey 1965, Anderson 1968b).

A variety of changes are seen in the tubules and collecting ducts (Bloom 1937, 1939, 1954, Platt 1951a, McIntyre and Montgomery 1952, Monlux 1953, Wettimuny 1963). In the cortex some tubules are compressed and atrophied, others are hypertrophied and others still are dilated and lined by atrophic epithelium so appearing cystic. Many of the tubular basement membranes are also thickened (Platt 1951a). In the medulla there is often marked dilation of the collecting ducts which may be lined by a single layer of flattened atrophic epithelium or multiple layers of hyperplastic epithelium. Hyaline casts are commonly found at all levels of the nephron, particularly in "cystic" tubules and

collecting ducts. Granular casts are less common while leucocytes and erythrocytes are only very occasionally seen (Wettimuny 1963).

Abnormalities occur commonly in the arteries and these are associated with the presence of hypertension (Mackey 1965, Anderson 1968a). Two main lesions have been described. Firstly, there is often hypertrophy of the tunica media and hyperplasia of the adventitial connective tissue of the interlobular arteries; thickening and splitting of the internal elastic laminae may accompany this change although this is not a common feature (McIntyre and Montgomery 1952, Mackey 1965, Anderson 1968a). The other main lesion, plasmatic vasculosis, can be present in the arcuate and interlobular arteries and the afferent arterioles (Platt 1952, Monlux 1953, Bloom 1954, Mackey 1965, Anderson 1968a). This lesion is caused by the extrusion of eosinophilic hyaline material into the walls of these vessels; this material stains for fibrin when first formed but with time it undergoes a change in staining reaction to that of collagen, and occasionally deposits have a mixed staining reaction. (Mackey 1965, Anderson 1968a). The severity of the lesion varies from a small subepithelial deposit lying on an intact internal elastic lamina, through a larger mass causing focal necrosis of the tunica media, to rupture of the external elastic lamina with liberation of the material into the surrounding connective tissue (Mackey 1965, Anderson 1968a).

Finally, calcium deposits are often found in the kidneys, particularly in the basement membranes of the proximal convoluted tubules, and Bowman's capsules, but all parts can

be affected (Platt 1951a, Bloom 1954, Ichijo 1966). Such deposits probably result from the disturbance in the renal excretion of calcium and phosphate ions (Ichijo 1966).

Despite the high incidence of CIN there has been only one ultrastructural study (Krohn et al. 1973). However, doubts have been cast as to whether these were cases of CIN (see below) so the ultrastructural features must be regarded at present as unknown.

The aetiology of CIN remains an enigma. Most evidence suggests that CIN follows a non-fatal episode of AIN associated with L.canicola infection (McIntyre and Montgomery 1952, McIntyre 1954). To support this concept these authors described 3 cases of non-fatal CIN which died of renal failure at varying times (4 months to 2 years) after the acute illness. At necropsy these dogs were all found to have CIN. In addition, a poorly documented sub-acute interstitial nephritis has been described which is considered to be an intermediate stage between AIN and CIN (Bloom 1954, Mackey 1965). Such cases have pathological features in common with both AIN and CIN, with both an extensive mononuclear cell infiltrate and widespread interstitial fibrosis present.

However, doubt has been shed on the relationship between L. canicola and CIN, as many cases of CIN have no definite history of a prior acute phase (McIntyre 1954). Moreover, direct evidence implicating L. canicola infection in chronic cases is poor. Leptospirae are only rarely seen in the kidney using silver staining methods, and when present are only found in small numbers (McIntyre and

Montgomery 1952, Monlux 1953, McIntyre 1954, Bloom 1954). Reported titres of serum anti-L. canicola antibody are also equivocal. McIntyre (1954) always found a positive, albeit low titre (< 1:1,000) while Bloom (1954) stated that titres were often negative. In addition, CIN is common in Scandinavia where the incidence of L. canicola infection is reputed to be very low (Persson et al. 1961a, Krohn et al. 1971).

Because of this lack of firm evidence to link cases with a previous phase of AIN caused by L. canicola, coupled with the inability to reproduce the clinical syndrome with experimental infections (see above), several workers have investigated the possible role of other microbial agents.

Leptospira icterohaemorrhagiae has been implicated as a cause of CIN by a serological survey (Timoney et al. 1974). These authors found a significant increase in the number of animals with serum agglutinins to L. icterohaemorrhagiae in a group of dogs with diffuse sub-acute or chronic interstitial nephritis, compared with dogs with only small focal lesions of interstitial nephritis or normal kidneys (23.4% compared with 8.8%). However, Bush and Evans (1972) found no increase in levels of antibodies to L. icterohaemorrhagiae in dogs with clinical signs of CIN compared to those without such signs. In addition, experimental infection of dogs produces only mild focal infiltrates of mononuclear cells (Gleiser 1957).

Canine adenovirus (CAV) has also been implicated on the basis of serology. Both Bush and Evans (1972) and Timoney et al. (1974) showed that dogs with evidence of

interstitial nephritis had a serum titre to CAV indicative of recent infection more often than those without. Bush and Evans found such titres in 75% of dogs with clinical signs of CIN compared with 40% of dogs with no signs of nephritis. Timoney et al. (1974) found that 30% of dogs with diffuse sub-acute or chronic interstitial nephritis at post mortem examination had such titres combined with negative titres to L. canicola or L. icterohaemorrhagiae, compared with 9.3% of dogs with only mild focal interstitial nephritis or normal kidneys. It is also known that the virus persists in the kidney in about 70% of cases of both natural and experimental infections leading to a focal interstitial nephritis (Wright 1976). However, against this evidence is the fact that repeated attempts to produce severe interstitial lesions leading to renal failure have always failed (Wright 1976). In addition, Wright et al. (1976) in a series of 8 cases of CIN found no CAV antigen in the kidneys using immunofluorescence techniques, and antibody eluted from these kidneys showed no activity against CAV. Moreover, Persson et al. (1961a) found no relationship between CAV infection and nephritis in dogs in Sweden.

Further controversy exists over the mechanisms acting to produce the excessive scarring seen in the kidney in cases of CIN. To date two such processes have been proposed; hypertension and immune-mechanisms.

In Man, it has been recognized for many years that hypertension is a frequent secondary complication of renal disease, and that it is instrumental in causing further vascular damage and nephron injury in the kidney (Heptinstall

1974). A similar relationship probably exists in interstitial nephritis in the dog (Mackey 1965, Anderson and Fisher 1968). These workers found that hypertension could be present in all stages of interstitial nephritis and that the incidence increased as the dogs passed from the acute to the chronic state. In addition, this increase paralleled the increase in incidence of plasmatic vasculosis in the arterial system. This lesion was seen in all hypertensive chronic cases that were necropsied but only in a proportion of the hypertensive acute or sub-acute cases, suggesting that hypertension, once initiated, produces further damage to the kidney.

The role of immunological mechanisms in CIN is not clear at present. Krohn et al. (1971, 1973) from Finland, proposed that immune complex deposition occurred in the glomeruli in CIN and this led to progressive glomerular scarring. To support this concept they described 8 cases in 1971 and 24 cases in 1973 where immunofluorescence showed there to be granular and globular deposits of IgG and complement (C_3) in the capillary walls and mesangium; such a pattern is widely acknowledged to reflect immune complex deposition (see below: glomerulonephritis). In contrast, in an immunofluorescence study of 8 cases of CIN from Britain, Wright et al. (1976) found only very occasional deposits of IgG in a few glomeruli and no C_3; a picture that probably reflected non-specific trapping of plasma proteins in areas of structural damage. Moreover, in the same paper Wright et al. described 8 cases of chronic glomerulonephritis (see below) which were distinguished from

CIN by the presence of extensive deposition of IgG and C_3 in the glomeruli. Krohn et al.'s cases are very similar to these and would appear to be more accurately classified as glomerulonephritis and not interstitial nephritis.

In conclusion, although most evidence suggests that L. canicola is the cause of CIN, this is far from conclusive, and other organisms may well be able to initiate this nephropathy as well. Secondly, the mechanisms producing the progressive destruction of nephrons are unclear. Hypertension certainly occurs in association with interstitial nephritis, but its precise role in the progression of the disease is not known. There is conflicting evidence over the possible role of immune complexes, while the role of cell-mediated immunity has never been investigated.

Inherited Renal Disease

Certain cases of nephritis, which were chronic interstitial in type, have been classified separately in the literature from CIN as they appeared to have an inherited basis. Such cases were first described in the Cocker Spaniel (Krook 1957) and more recently in the Norwegian Elkhound (Finco et al. 1970, 1977, Finco 1975) and the Samoyed (Bernard and Valli, 1977). In addition, several case reports have described very similar nephropathies in the Alaskan Malamute (Kaufman et al. 1969, Smart and Fletch 1972, Burk and Barton 1978), the Keeshond (Klopper et al. 1975) and the Lhasa Apso (Osborne et al. 1972); however, the lack of information in these reports describing renal disease in related dogs, means an inherited disorder may not have been present.

Finally, on the basis of generalized renal disease occurring in young dogs, a similar inherited renal disease has been suspected in German Shepherd dogs (Alsatians), dachshunds, miniature schnauzers and shih-tzus (Osborne et al. 1972).

Although a different disorder may be present in each breed, the clinical and pathological descriptions in the literature are very similar. Affected animals develop chronic renal failure at a young age (several months to five years) and at necropsy a chronic interstitial nephritis (Fig. 4) is found (Krook 1957, Finco et al. 1970, 1977, Bernard and Valli 1977). There is widespread obliteration of nephrons by severe diffuse renal scarring of both cortex and medulla but there is no focal concentration around the cortico-medullary junction. Renal calcification is often severe while mononuclear cell infiltration is minimal. Glomerular scarring is severe, with obliteration of the tuft by excess mesangial matrix and basement membrane, thickening of Bowman's capsule, formation of capsular adhesions and fibrin deposition, prominent.

The lack of functional nephrons in young dogs prompted early authors to describe the nephropathy as renal cortical hypoplasia. However, a recent study of Norwegian Elkhound puppies from affected breeding lines has shown that, in this breed at least, the kidney is normal at birth and then undergoes progressive scarring, hence hypoplasia is an inaccurate term. Because of the familial relationship of affected animals, the nephropathy in Norwegian Elkhounds and Samoyeds has been termed familial renal disease in

preference to renal cortical hypoplasia. The genetic basis for these familial relationships has not yet been elucidated, but it is probably a recessive trait in the Norwegian Elkhound (Osborne et al. 1972).

The pathogenetic mechanisms operating in this type of nephritis are a complete mystery. Renal hypertension probably occurs although its role in the progression of the nephropathy is not known. Only Persson et al (1961b) have measured blood pressures (in 10 affected cocker spaniels) and although they judged the values obtained to be normal, others would regard them as abnormally high (Spangler et al. 1977). Two studies of 21 affected Norwegian Elkhound dogs failed to yield any positive results; there were no significant pathogenetic alterations in blood or urine biochemistry (Finco 1975) nor were immune complexes or auto-antibodies present in the kidneys (Finco et al. 1977).

PRIMARY GLOMERULAR DISEASE

Glomerulonephritis

Diseases of the kidney in which the primary and most significant lesion is an inflammatory reaction in the glomeruli are termed glomerulonephritis (GN) (Robbins 1967). It is now widely accepted that most if not all forms of GN are mediated by immunological mechanisms (McCluskey 1974).

GN was once thought to be rare in dogs. Monlux (1953) in a survey of 321 nephritic dogs classified only 9 cases as GN and Wettimuny (1963) reported only 3 cases in a survey of 178 dogs with renal disease. However, the advent of electron and immunofluorescence microscopy has led to a much greater knowledge and understanding of kidney pathology, and GN is now known to be a more common lesion.

Several case reports (Murray et al. 1971, Halliwell and Blakemore 1972, Larkin et al. 1972, Deschepper et al. 1974, Osborne et al. 1976) and five series reports (Kurtz et al. 1971, Murray and Wright 1974, Wright et al. 1976, Lewis 1976, Müller-Peddinghaus and Trautwein 1977a) have shown that GN is the cause of a significant amount of clinical renal disease although the actual incidence has not yet been established. GN also occurs in association with various systemic diseases in the dog: experimental bacterial endocarditis (Highman et al. 1959), pyometra (Obel et al. 1964), systemic lupus erythematosus (Lewis et al. 1965, Osborne et al. 1973), experimental and spontaneous canine adenovirus (CAV) infection (infectious canine hepatitis) (Wright et al. 1974, Morrison et al. 1975), experimental Dirofilaria immitis infection (Casey and

Splitter 1975), various malignant neoplasms (Hottendorf and Nielsen 1968, Murray and Wright 1974, Müller-Peddinghaus and Trautwein 1977b), and acute pancreatitis (Murray and Wright 1974, Lewis 1976). In most cases, however, GN appears to play a relatively minor part in these disease complexes.

Recently several reports have indicated that GN may be common in dogs that are clinically normal or just have a proteinuria (Stuart et al 1975, Rouse and Lewis 1975, Müller-Peddinghaus and Trautwein 1977a,b). However, the stated incidence varies considerably. Stuart et al. (1975) in the U.S.A. reported a 25% incidence of proteinuria associated with varying degrees of GN in a research colony of otherwise normal 4-6 year old Beagles. Rouse and Lewis (1975) working in Canada reported GN to be present in 23% (16) of 71 stray dogs examined; only 2 of the 16 showed proteinuria. In comparison the German workers Müller-Peddinghaus and Trautwein (1977a,b) reported a massive 90% incidence of GN in a group of 101 dogs, composed of 72 animals submitted for euthanasia (36 of which had slight proteinuria), 15 animals with biochemical evidence of a nephropathy and 14 control animals from various experiments. This wide variation in the incidence of GN may truly exist between North America and Germany, but it may just reflect the different types of environment from which the dogs were gathered. In addition, it is possible that Müller-Peddinghaus and Trautwein (1977a,b) overestimated the incidence, as it is not clear from their figures whether an immunologically mediated lesion was

present in every case. Moreover 71 (80%) of their 91 cases had a concomitant interstitial or pyelonephritis (Müller-Peddinghaus and Trautwein, 1977b). Although they judged that there was no correlation between the type of glomerular lesion and degree of interstitial or pyelonephritis, it is possible that in some instances the glomerular lesion was a secondary event. Such cases would not therefore be covered by the definition of GN (see above).

A detailed classification, comparable to that found in Human GN, is not possible at present. Compared to Man very few cases have been described and most of these have been diagnosed at necropsy. Thus, knowledge of the clinical aspects of GN is particularly sparse, but to date, three broad categories have emerged:-

Proliferative	Glomerulonephritis
Chronic (exudative)	Glomerulonephritis
Membranous	Nephropathy

Such a classification although made on purely morphological grounds appears to reflect distinct clinical differences as well. Recently Müller-Peddinghaus and Trautwein (1977a,b) further subdivided proliferative GN and membranous nephropathy on morphological grounds. However, further studies are needed to show if such groups do actually reflect different clinical entities, which would make this more complex classification worthwhile to retain.

Proliferative Glomerulonephritis

Most reports indicate that this is the commonest type of GN. Murray and Wright (1974) classified 37 of their 42 cases (88%) in this group, and Müller-Peddinghaus and

Trautwein (1977a), although using a more complex classification, described 46 cases (51%) characterized by some degree of proliferation out of a total of 91 cases of GN. Such figures do not take into account that it is also the type seen in association with pyometra (Obel et al.1964) and systemic CAV infection (Morrison et al. 1975).

Most reports are of necropsy material only, so the clinical picture is at present vague (Kutz et al. 1971, De Schepper et al. 1972, Murray and Wright 1974, Stuart et al. 1975). Proteinuria of varying amount is almost always present, and this can lead to hypoalbuminaemia. In only one reported case however, was this severe enough to produce oedema and ascites as well (De Schepper et al. 1972); this combination of proteinuria, hypoalbuminaemia and ascites and/or oedema is called the nephrotic syndrome. If the glomerular lesion is very extensive uraemia results.

The characteristic pathological feature of this group is glomerular hypercellularity resulting from mesangial proliferation; this may be augmented by the presence of polymorphonuclear leucocytes in the glomerular capillaries. Accompanying this proliferation is expansion of the mesangial matrix (Murray and Wright 1974). Depending on the extent and pattern of the hypercellularity three types, diffuse, segmental and focal occur (Morrison and Wright 1976a). In the diffuse type, mesangial proliferation is present in all parts of every glomerulus, while in the segmental form only a portion of every glomerulus is affected, and cases where only some glomeruli are involved form the focal group. What the clinical significance is of this variation in

lesion severity is not known. In some cases, there is thickening of the capillary loops as well and some authors have classified these separately as membrano-proliferative GN (Müller-Peddinghaus and Trautwein 1977a). Whether this variation reflects a particular aetiology has not been investigated. In Man such cases are associated with the presence of a particular complement-activating factor, the C_3 nephritic factor, in the serum (West 1976).

Unlike interstitial nephritis there have been both electron and immunofluorescence microscopic studies on GN in the dog. In proliferative GN the most striking and important ultrastructural features are a) electron-dense deposits in all parts of the glomerular basement membrane (GBM) and in the mesangium, b) proliferation of mesangial cells and production of excess mesangial matrix, c) thickening, wrinkling and splitting of the GBMs and d) "fusion" of the epithelial cell foot processes (Kurtz et al. 1971, Murray and Wright 1974, Stuart et al. 1975).

Immunofluorescence microscopy has shown that proliferative GN is associated with deposition of granules of immunoglobulin with bound complement, in the mesangium and along the GBMs (Kurtz et al 1971, Murray and Wright 1974, Stuart et al 1975, Rouse and Lewis 1975, Müller-Peddinghaus and Trautwein 1977a). Such granules appear to correspond to the electron dense deposits seen with electron microscopy and almost certainly represent immune complexes. Deposits of fibrin and albumin have also been identified in the glomeruli using immunofluorescence (Müller-Peddinghaus and Trautwein 1977a) but their significance was not commented on.

Membranous Nephropathy

Estimates of its incidence vary from 12% (Murray and Wright 1974) to 29% (Müller-Peddinghaus and Trautwein 1977a) of all GN cases.

Again, clinical details are sparse although several well documented individual case reports do exist (Halliwell and Blakemore 1972, Larkin et al. 1972, Osborne et al. 1976). When described the major clinical feature has been massive proteinuria and hypoalbuminaemia which often led to ascites and/or oedema (i.e. the nephrotic syndrome). Recovery has been reported (Osborne et al. 1976) whilst others progressed to renal failure (Halliwell and Blakemore 1972, Murray and Wright 1974). In America it has also been described in association with systemic lupus erythematosus (Lewis et al. 1966, Osborne et al. 1973) and Dirofilaria immitis infections (Casey and Splitter 1975).

The characteristic pathological feature is the diffuse thickening of the glomerular capillary walls, resulting in enlarged glomeruli which consequently may be prominent on the cut surface of the kidney (Fig. 5) (Murray and Wright 1974, Morrison and Wright 1976, Müller-Peddinghaus and Trautwein 1977a). Electron microscopy shows this thickening is caused by the presence of electron dense deposits on the subepithelial side of the GBMs, and the encircling of these by the formation of new basement membrane (Murray and Wright 1974). The other prominent ultrastructural lesion is the widespread "fusion" of the epithelial cell foot processes (Murray and Wright 1974).

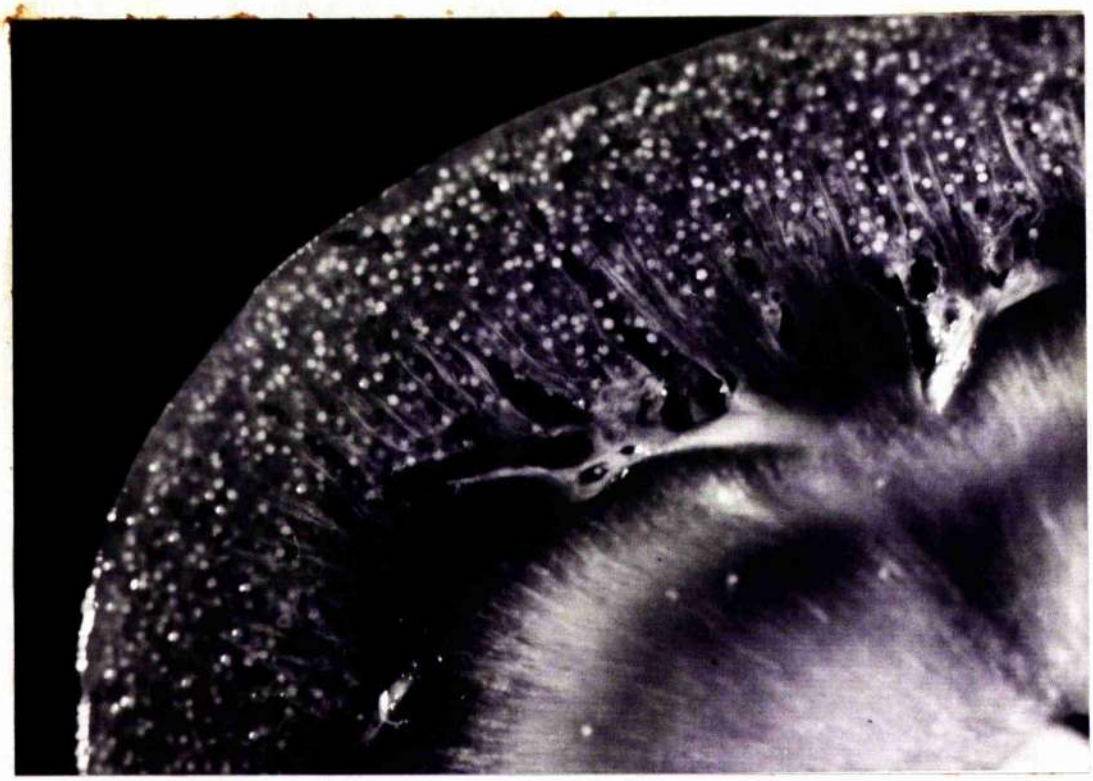
Immunofluorescence microscopy shows that this form of GN is also associated with the deposition of fine granules

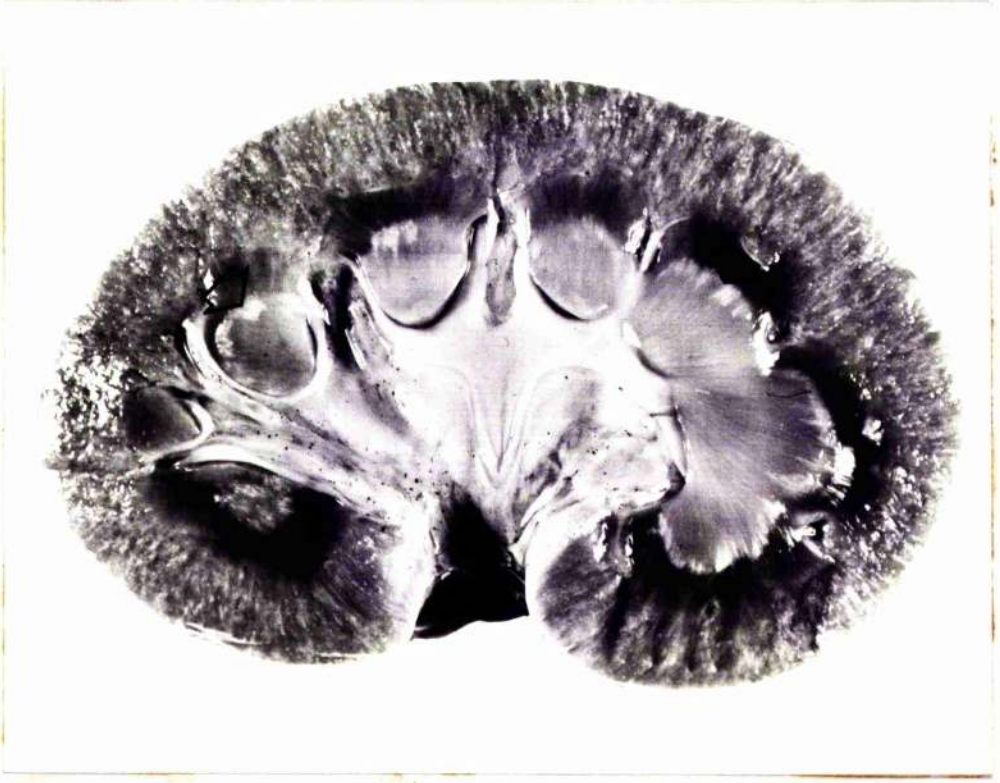
Fig. 5 Membranous Nephropathy

The enlarged glomeruli stand out very clearly on the cut surface of the kidney.

Fig. 6 Chronic Glomerulonephritis (CGN), Case 39

Typically, interstitial fibrosis is not as severe in CIN nor concentrated at the cortico-medullary junction, and the cortex is normal in width. N.B. the pale foci in the outer medulla (arrow) are areas of calcification.





of immunoglobulin with bound complement in the glomeruli particularly along the capillary walls (Murray and Wright 1974, Müller-Peddinghaus and Trautwein 1977a). The latter authors also found fibrin and albumin in the glomeruli in some cases of membranous nephropathy but their significance was not discussed.

Chronic Glomerulonephritis (CGN)

Recently Wright et al (1976) delineated this third group, which clinically and morphologically were very similar to CIN. Cases present in chronic renal failure with proteinuria and have widespread renal scarring resulting in pale, granular kidneys (Fig. 6). In contrast to CIN, the dominant microscopic feature is widespread and extensive glomerular scarring accompanied by extensive fibrin deposition. Although there is significant interstitial fibrosis it tends to be finely deposited as opposed to the heavy, irregular areas of scarring typical of CIN. Immunofluorescence again shows that this form of GN is associated with the deposition of immunoglobulin. Irregular granular and globular deposits of immunoglobulin with bound complement are present in the mesangium and less often in the capillary walls. Immunofluorescence also confirms the presence of widespread fibrin deposition in the glomeruli.

Recently Müller-Peddinghaus and Trautwein (1977a) described cases of "mesangial-sclerosing GN". Such cases would appear to be very similar to those of CGN as the major lesion was again glomerular scarring (synonymous with mesangial sclerosis).

Aetiology

Much evidence has now been produced to implicate immunological mechanisms in the aetiology of GN in Man and animals, including the dog. Two distinct mechanisms whereby antibodies cause glomerular injury are now known (Germuth and Rodriguez 1973, Wilson and Dixon 1974). Firstly, if antibodies combine with non-glomerular antigens in the blood, circulating immune complexes can form and these then deposit in the glomeruli. Secondly, antibodies can be produced which have specificity for GBM. Either type can cause injury by activating several interrelated mediator systems, including the complement cascade, polymorphonuclear leucocytes, kinins, vasoactive amines and the blood coagulation cascade (Wilson and Dixon 1974).

Since both mechanisms use similar mediators to cause tissue injury, similar morphological changes will be seen in each; differentiation therefore has to rely on other techniques such as immunofluorescence. Lesions mediated by immune complexes are characterized by the deposition of granules of immunoglobulin in the glomeruli, while lesions mediated by anti-GBM antibodies are characterized by the smooth linear deposition of immunoglobulin along the GBM. Although both types have been produced experimentally in the dog (Wright et al. 1973b, Barabas and Lannigan 1976) only immune complex mediated lesions have so far been positively identified in spontaneously occurring canine GN.

It is thought that, depending on their size and solubility, complexes will localize in different parts of the glomerulus and so give rise to different morphological types (Germuth and Rodriguez 1973). Small highly soluble complexes lodge in the subepithelial side of the GBM and

so produce a membranous nephropathy. Larger, less soluble complexes lodge in the mesangium as well as the GBM, and provoke a proliferative response or, if the coagulation cascade is activated, a scarring reaction.

A most important question still to be answered is what is the nature of the antigen in the immune complexes? Only in the case of the transient proliferative GN which follows CAV infection has this most important question been answered. CAV antigen has been identified by immunofluorescence in the glomeruli, in the same pattern as the IgG granules, and antibody eluted from such kidneys shows affinity for this antigen (Wright et al. 1974, Morrison et al. 1975). In addition, circulating complexes of virus antigen and antibody have been detected in sera of infected dogs (Morrison and Wright 1976c).

Amyloid Nephropathy

Amyloidosis is a disease characterized by the extracellular accumulation of an amorphous, eosinophilic protein material. Cases are classified either primary, where no underlying disease is present or secondary, where it complicates various chronic inflammatory lesions. In the dog, the primary form would appear to be the more common (Slauson et al. 1970).

In the dog amyloidosis is usually a disease of the older animal with no apparent sex or breed disposition (Slauson et al. 1970). Its incidence as a primary cause of clinical renal disease is low. Bloom (1939) reported it as the cause of renal failure in 1 out of 70 dogs (1.4%), while Wettimuny (1963) found amyloid or amyloid-like lesions

in 12 out of 178 nephritic dogs (7%). Although deposits are found throughout the body it is the renal deposits that give rise to the major clinical signs (Osborne et al. 1968, Slauson et al. 1970). The major feature is massive proteinuria which may be severe enough to produce the nephrotic syndrome. Other clinical signs often present are weight loss, weakness and depression, thirst and polyuria (reflecting progressive renal failure). In the most severe cases signs of uraemia (see above: CIN) may also be seen.

The major renal deposits are present in the glomeruli (Osborne et al. 1968, Slauson et al. 1970). There is a spectrum of involvement from mild focal deposition in the mesangium and GBM, to complete obliteration of the glomeruli. Where a glomerulus is so damaged, tubular atrophy and replacement-fibrosis occurs. Amyloid is also commonly found in the interlobular arteries and afferent arterioles whereas it is only occasionally present in the interstitium. This infiltration gives the kidney a pale-tan and waxy appearance often with abnormally prominent glomeruli. Additional impairment to renal function can occur due to infarction, as cases of amyloidosis have a tendency to thrombosis (Slauson et al. 1970).

The chemical nature and formation of amyloid is still obscure and the subject of much controversy despite a vast amount of research. However, it is generally accepted that amyloidosis results from a defective immune system and most of the protein in amyloid deposits is composed of fragments of immunoglobulin light chains (Osborne et al. 1972, Jones 1975).

SUPPURATIVE RENAL DISEASE

Pyelonephritis

Pyelonephritis is defined as a combined suppurative inflammation of the renal parenchyma and pelvis (Bloom 1954, Osborne et al, 1972).

Published work on canine pyelonephritis is meagre and confusion has occurred in two areas. Firstly, some authors (Monlux 1953) narrowed the definition only to cases where infection was judged to have ascended via the ureter from the lower genito-urinary tract. Others (Bloom 1954, Wettimuny 1963, 1967) included cases of possible haematogenous origin as well, although if the pelvis was not involved in such cases they were classified separately as suppurative or embolic nephritis (see below).

A second area of confusion has arisen because some authors (Monlux 1953, Bloom 1954, Wettimuny 1963, 1967) limit their description to cases where polymorphonuclear leucocyte infiltration is a prominent feature whilst others (Christie 1973; Crowell and Finco 1975; Müller-Peddinghaus et al. 1977) extend the definition to cover cases (presumably chronic) where plasma cells and lymphocytes are seen in absence of polymorphonuclear leucocytes. This latter viewpoint can lead to inaccuracies because plasma cell and lymphocyte infiltration are present in CIN as well as chronic pyelonephritis. In fact, it can be impossible to differentiate between a focal CIN and chronic pyelonephritis (Crowell and Finco 1975). However, a major distinguishing feature of most cases of pyelonephritis is the gross irregularity of the lesions. Firstly, only one kidney may be affected and where both are involved one is invariably more severely damaged than the other. Secondly, in an

affected kidney, lesions are focal so that segments of normal tissue are present between damaged areas.

Taking the above into account, it would appear that pyelonephritis is uncommon. Bloom (1954) reported an incidence of 5% in all necropsies with an increasing incidence with age and Wettimuny (1963, 1967) found an 8.5% incidence in 178 nephritic dogs again with a prevalence in older animals and with a female: male ratio of 2:1.

The clinical signs of pyelonephritis have only been scantily described (Bloom 1954, Wettimuny 1963, Osborne et al. 1972, Bush 1976). Often the degree of renal damage is mild so that pyelonephritis is first diagnosed as a chance finding at necropsy. In addition, clinical signs of renal disease can be obscured by associated lesions e.g. infection of the lower genito-urinary tract. Where suppurative lesions are extensive the following may be seen: fever, vomiting, lumbar and loin pain, bacteriuria and increased frequency of urination. If a sufficient amount of renal tissue is involved signs of renal failure (uraemia) will appear.

Pyelonephritis is classified either as acute or chronic (Monlux 1953, Bloom 1954, Wettimuny 1963, 1967). In acute cases the kidneys are mottled with small abscesses and sometimes haemorrhages and streaked by radial spread of the inflammatory reaction. Microscopically, this stage is characterized by a massive focal polymorphonuclear leucocyte infiltration with scattered colonies of bacteria, distorting and destroying the tubules and glomeruli. Many tubules are totally destroyed or show epithelial degeneration, while

others are compressed by the cellular reaction. Many are dilated with protein casts or foci of polymorphonuclear leucocytes. In contrast, the glomeruli are relatively spared. There may be a marked cellular infiltrate around them but only in a few is the structure itself infiltrated; Bowman's space is then filled with polymorphonuclear leucocytes and necrotic debris from the partial or total destruction of the tuft.

In chronic cases, areas damaged by the acute reaction have been replaced by fibrosis giving the kidney an irregular contracted appearance (Fig. 7). In these scarred areas, plasma cells and lymphocytes are the dominant infiltrating cells with few (if any) polymorphonuclear leucocytes. If however, infection is still present areas of acute inflammation are seen interspersed with these areas of scarring. Glomerular lesions are prominent in these areas of fibrosis and many glomeruli are reduced to shrunken, non-functional hypocellular, hypovascular masses composed of thickened basement membrane and mesangial matrix. Tubules remaining in the scarred areas are also affected; they are shrunken and distorted, lined by atrophic epithelium and often contain protein casts.

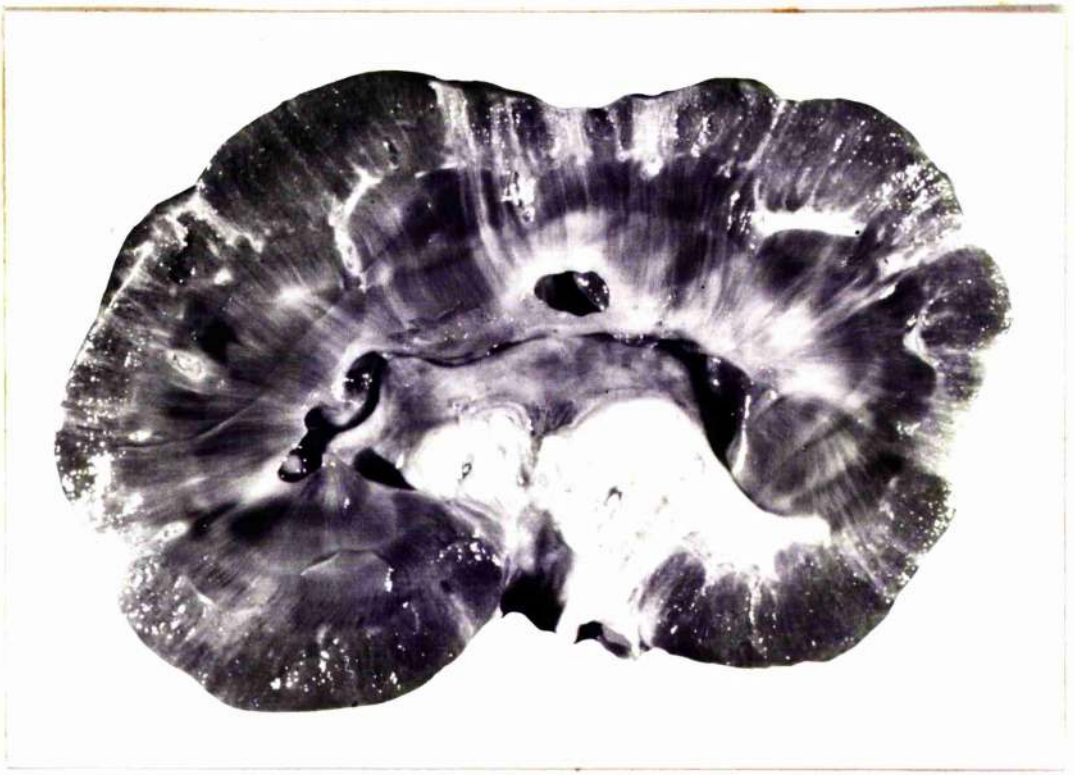
Pyelonephritis is caused by bacterial infection, the most commonly isolated organisms being Escherichia coli, Proteus vulgaris, Staphylococci and Streptococci (Bloom 1954, Osborne et al.1972). Four routes of infection are theoretically possible: via the blood stream, ascending via the ureter, direct invasion from adjacent tissues, and via the lymphatics. The last two are thought to be very

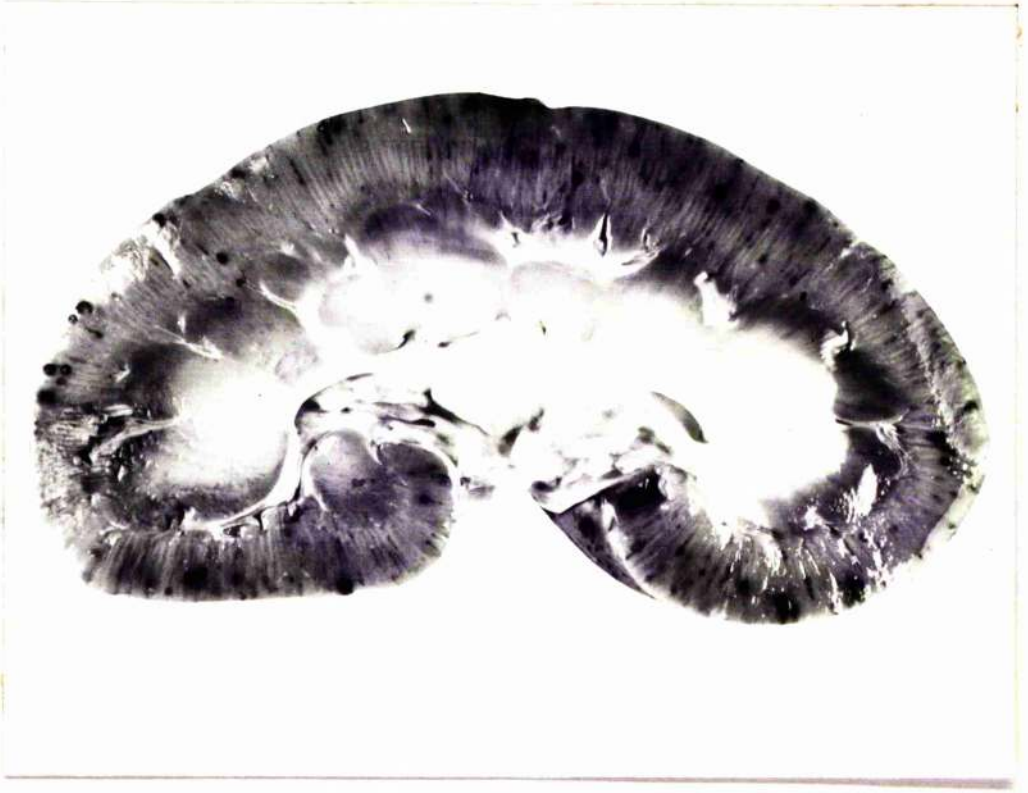
Fig. 7 Chronic Pyelonephritis

Note the irregularity resulting from focal radial scarring (pale areas) and streaks of acute inflammation (dark areas).

Fig. 8 Embolic Suppurative Nephritis

Small foci of inflammation are scattered through the cortex reflecting the haematogenous spread of infection.





uncommon and most cases are probably due to ascending infection (Bush 1976). This is known to be the case in Man (Belman 1976) and pyelonephritis in the dog is associated with conditions leading to urinary obstruction and stasis, and with bacterial infections of the lower genito-urinary tract (Bloom 1954, Wettimuny 1963, 1967).

In Man, vesicoureteral reflux caused by infection of the bladder is the most important method of producing this ascending infection (Belman 1976). In the dog, however, the role of vesicoureteral reflux is less clear. Several studies have shown that experimental infection of the dog's bladder can lead to reflux (Schoenberg et al. 1964, Somner and Roberts 1966). In addition, other studies have shown that when infection occurs in a bladder where unilateral reflux has previously been induced by surgery, pyelitis (inflammation of the renal pelvis) or pyelonephritis follows on the refluxing side (Scott 1964, King and Idriss 1967, Lenaghan et al. 1972). However, its role in spontaneously occurring pyelonephritis is not known. Only one study has been made into this but no clear correlation between bladder infection, reflux and pyelonephritis emerged (Christie 1973).

No immunofluorescence or electron microscopic studies of canine pyelonephritis have been reported, but such studies in Man indicate that in some cases of chronic pyelonephritis, glomerular scarring is associated with the presence of immune complexes and/or atypical forms of bacteria in the glomeruli (Beregi et al. 1974, Kincaid-Smith 1975a). In addition, chronic pyelonephritis in Man can be complicated by the generation of renal hypertension

which leads to further renal damage (Heptinstall 1974). It is probable that such a relationship also exists in the dog; Katz et al. (1954) and Weiser et al. (1977) found raised blood pressures in dogs with pyelonephritis.

Embolic Suppurative Nephritis

It is usual to distinguish from pyelonephritis cases where the suppurative lesions in the kidney results from a septicaemia (Bloom 1954, Wettimuny 1963). A variety of names have been applied to such lesions: suppurative nephritis (Monlux 1953, Bloom 1954), embolic or pyaemic nephritis (Wettimuny 1963), embolic suppurative nephritis (Jubb and Kennedy 1970).

Such cases are not common in the dog (Bloom 1954), with Wettimuny diagnosing the condition in 9 out of 178 nephritic dogs (5.1%). Renal symptoms are the same as those of pyelonephritis but are often overshadowed by those of the associated septicaemia (Bloom 1954, Wettimuny 1963).

Similarly, the kidneys are mottled and streaked with foci of acute inflammation, but infarcts both old and new may be present as well (Bloom 1954, Wettimuny 1963). The haematogenous route of infection is reflected in the consistent finding of acute inflammation in the glomeruli (glomerulitis) and the common finding of acute vasculitis (Wettimuny 1963). Affected glomeruli are infiltrated with polymorphonuclear leucocytes, and the tuft may be partly or totally destroyed filling Bowman's space with necrotic debris. Foci of bacteria may also be present in the glomeruli. The inflammatory reaction spreads from these glomeruli and vessels into the surrounding interstitial

tissue and tubules producing multiple small abscesses (Fig. 8) (Bloom 1954, Wettimuny 1963).

As stated before, such lesions are part of a septicaemia with staphylococci and streptococci being the most commonly involved bacteria (Bloom 1954). In those cases with infarction, infected emboli are also of importance in producing renal damage (Bloom 1954).

DISCUSSION

The above summary reveals many gaps in our knowledge of canine renal disease. Although the glomerular changes in GN have received attention from several research groups in the past few years, the equally important glomerular lesions in CIN have been virtually ignored. Furthermore neither of the modern investigative techniques, immunofluorescence or electron microscopy have been used to study in any detail the disease as it is recognized in Britain and three major questions still remain unanswered:-

1) What is the causal agent(s) of the initial AIN? L.canicola infection very likely leads to CIN but some cases possibly follow infection by other organisms.
2) What factor(s) produce the progressive renal scarring that leads to renal failure? There is some evidence to implicate renal hypertension but the possible role of immune mechanisms is not known.
3) What are the morphological details of this process? There have been several histological descriptions of CIN but glomerular morphology has received only scanty attention despite the prominence of glomerular fibrin deposition and scarring. Moreover, the ultrastructural features are not known.

Therefore, it was judged that a new study using a combination of light, electron and immunofluorescence microscopy, and elution techniques, was needed to attempt to answer these questions. In addition, a recent report has shown that cases of CGN are very similar to those of CIN and

it is possible that such cases were classified as CIN in the past. Therefore it was considered worthwhile to make a comparative study of these two nephropathies making use of these modern investigative techniques.

PART 2

A COMPARATIVE MORPHOLOGICAL STUDY OF CHRONIC INTERSTITIAL NEPHRITIS AND CHRONIC GLOMERULO-NEPHRITIS

MATERIALS AND METHODS

The 40 dogs studied all showed clinical and/or biochemical signs of chronic renal failure at the time of death or euthanasia. Some animals had additional extra-renal conditions but these were judged to be of minor importance compared with the renal lesions. Cases of renal failure from other causes e.g. nephrosis, amyloid nephropathy, proliferative GN, membranous nephropathy, AIN, pyelonephritis and "inherited" renal disease were excluded on clinical and pathological grounds. The dogs under study were subjected to detailed light, immunofluorescence and, when possible, electron microscopic investigations as described below. 6 normal puppies, which also acted as controls in the experiments in part 3 were studied for comparison of normal renal structure. In addition, elution studies were carried out in 28 cases. Controls for this latter study consisted of 22 animals, comprising 6 normal animals, 2 cases of amyloid nephropathy, 6 cases of GN, 3 cases of pyelonephritis, 2 cases of nephrosis, and 1 case each of diabetic glomerulosclerosis, focal interstitial nephritis following septicaemia and "inherited" renal disease in a cocker spaniel. On the basis of these studies, the 40 cases were classified as CIN (30 cases) or CGN (10 cases).

Light Microscopy

Pieces of kidney from each case were fixed for seven days in 10% neutral buffered formalin, post fixed for 24 hours in mercuric chloride formal, dehydrated, cleared and embedded in paraffin wax. Sections were routinely cut

at 4 μm and stained with haematoxylin and eosin (H & E). Selected sections from each case were then stained with periodic-acid Schiff (PAS) to show basement membranes, von Kossa to detect calcification, and the following stains to demonstrate fibrin: Gram Weigert, Martius-scarlet blue (MSB), Masson 44/41, Obadiah, Picro-Mallory V, phosphotungstic acid haematoxylin (PTAH) and yellowsolve. The respective staining reactions of fibrin and collagen are given in Table 3. Collagen is included as aging of fibrin deposits has been reported to be accompanied by a change in staining reaction to that of collagen (Lendrum et al 1962).

TABLE 3

STAIN	FIBRIN	COLLAGEN
Gram weigert	Purple	NSR
MSB	Red	Blue
Masson 44/41	Blue black	Pale blue
Obadiah	Blue black	Red brown
Picro-Mallory	Red	Blue
PTAH	Purple	Brown
Yellowsolve	Red	NSR

NSR no specific reaction.

At first all the fibrin stains were used, but as studies progressed in conjunction with the liquoid experiment (see part 3), it was found that MSB gave the most clear staining of fibrin and collagen coupled with ease of production and consistency of results. This was, therefore, used almost exclusively in the later cases. In some cases serial sections were cut and stained with MSB to study the progression of glomerular lesions, and the effect of

glomerular damage on the connected tubule.

In each case two semi-quantitative examinations of the glomeruli were carried out. Firstly, the total glomerular damage in a case was estimated by counting glomeruli from 2 or more sections so that at least 100 were examined. At least 2 sections were used in an attempt to compensate for focal variations in the pattern of glomerular scarring. Each glomerulus was classified as either normal (non-scarred), $< 50\%$, $> 50\%$ or 100% of the tuft scarred and non-functional. Scarring produces two types of glomeruli, cystic and contracted (described in detail below). To give an estimate of the incidence of each, the morphological type of the totally scarred obsolescent glomeruli was noted. Secondly, to investigate the various morphological alterations comprising the scarring process, the same sections were re-examined and the incidence found of all the various lesions in those glomeruli with less than 100% scarring.

To accurately convey the extent of renal lesions to the reader the following terminology is used:-

1) <u>with reference to a single glomerulus</u>

   Local - only part of a tuft involved

   Global- all of the tuft affected

2) <u>with reference to all the glomeruli</u>

   Focal - only a proportion of glomeruli affected with either a local or global lesion

   Segmental - a localized lesion in all glomeruli

   Diffuse - a global lesion in all glomeruli. The severity of the lesion may vary from tuft to tuft and between areas of the same tuft.

3) Extra-glomerular lesions

Lesions of tubules and collecting ducts, arteries and arterioles, and the interstitium are graded on a scale from 1+ to 4+ depending on incidence.

Electron Microscopy

When post mortem examination was carried out at the time of death, tissue was taken for electron microscopic examination. Small blocks of kidney less than 1 mm thick were fixed by immersion in a paraformaldehyde glutaraldehyde mixture at 4°C for 4 to 6 hours and post fixed for 1 hour in 1% osmium tetroxide (Karnovsky 1965). Following dehydration in an ethyl alcohol series the tissues were treated with propylene oxide and embedded in araldite Sections 1 µm thick were cut on L.K.B. Mark III Ultratome, mounted on glass slides and stained with Mallory's borax methylene blue. Areas on these slides of particular interest were marked and ultrathin sections (0.5 µm) cut. These were mounted on copper grids, stained with 20% uranyl acetate in methanol and lead citrate (Watson 1958), and examined with an A.E.I. 6B electron microscope. Where possible at least three glomeruli per case were examined.

Immunofluorescence Microscopy

In each case frozen sections of kidney 5 µm thick were cut, washed in phosphate buffered saline (PBS) at pH 7.2 for ½ hour, and fixed in acetone for ten minutes. These were then stained for ½ hour with antisera against canine IgG, IgM, β 1C globulin (C3) and fibrinogen (Cappel Laboratories, Downingtown, U.S.A.) and L. canicola (Difco

Laboratories, Detroit, U.S.A.) all prepared in rabbits, and against canine adenovirus (CAV) from infected dogs (Wright et al. 1973a). All antisera were conjugated with fluorescein isothiocyanate (FITC). After washing in PBS for another ½ hour, sections were examined by a Leitz "Orthoplan" microscope equipped for incident light fluorescence. Controls involved the testing of each new batch of sera against a known positive section:-

For IgG, IgM, C3 - kidney from a case of membranous nephropathy

For fibrinogen - kidney from a liquoid treated dog (see below)

For L. canicola - kidney from a case of AIN

For CAV - liver from a case of infectious canine hepatitis.

Elution Studies

The protein in one kidney was eluted following the method of Lambert and Dixon (1968). The capsule and medulla were removed, and the remaining renal cortex minced with scissors and washed repeatedly with cold (4°C) PBS (pH 7.2) to remove blood. The minced tissue was then homogenized using a Colworth Stomacher (Seward, London, England) followed by a homogenizer (Silverson, Chesham, England). The homogenate obtained was centrifuged in the cold at 3,500g for fifteen minutes and the packed sediment washed twice with PBS at 4°C. This was then suspended in 0.02M citrate buffer (pH 3.2) at a concentration of 20 ml of buffer per gram of washed sediment, and incubated at room temperature with constant stirring for 1½ hours. The sediment was removed by centrifugation in the cold at

3,500 g for fifteen minutes, and the eluate dialysed for two days with PBS (pH 7.2). This was then concentrated by surrounding the dialysis tubes with 20M carbowax (polyethylene glycol), (Searle Diagnostics, High Wycombe, Britain) and resuspended in PBS.

The protein concentration of the eluates was measured by Lowry's technique (1951) and the eluates were tested for anti-L.canicola, anti-L.icterohaemorrhagiae, anti-CAV and anti-kidney antibodies. An agglutination-lysis test using live antigen was used for the first two and an indirect immunofluorescence test for the second two. In this latter test, frozen sections 5 µm thick were cut of liver from a case of infectious canine hepatitis (confirmed by direct immunofluorescence), and of normal kidney (from a dog with no clinical or pathological signs of renal disease). These were washed for ½ hour in PBS (pH 7.2) and fixed in acetone for 10 minutes. Then they were exposed to the eluate, washed in PBS and stained with FITC conjugated antiserum produced in rabbits against dog immunoglobulin (Sera Services Ltd., Maidenhead, Britain), all for ½ hour. After a final wash in PBS the sections were examined as described in the immunofluorescence studies.

TABLE 4

CHRONIC INTERSTITIAL NEPHRITIS (CIN): CLINICAL FINDINGS

CASE	THIRST	ANOREXIA	WEIGHT LOSS	VOMITING	HALITOSIS	ORAL ULCERATION	MUCOSAE	INELASTIC PULSE
1	-	+	+	Frequent	-	+	Normal	-
2	NR	NR	NR	NR	NR	+	NR	NR
3	-	+	+	Occasional	+	+	Normal	+
4	-	+	NR	Frequent	+	+	Normal	-
5	+	+	+	Frequent	+	+	Normal	-
6	+	+	+	Frequent	+	+	Pale	+
7	+	-	-	Frequent	+	+	Pale	+
8	+	+	+	Occasional	+	+	Pale	+
9	+	+	+	Frequent	+	+	Normal	+
10	+	+	-	Occasional	+	+	Pale	+
11	+	+	+	Occasional	+	+	Normal	+
12	-	+	+	Frequent	-	-	Normal	-
13	-	+	+	Occasional	+	-	Normal	+
14	NR	NR	NR	NR	+	+	Pale	+
15	-	+	+	Frequent	+	+	Normal	-

CASE	THIRST	ANOREXIA	WEIGHT LOSS	VOMITING	HALITOSIS	ORAL ULCERATION	MUCOSAE	INELASTIC PULSE
16	-	-	+	-	+	+	Normal	+
17	+	-	+	Occasional	+	-	Normal	+
18	-	-	+	Frequent	-	+	Normal	-
19	-	-	-	-	+	+	Pale	-
20	-	+	+	Frequent	-	-	Normal	-
21	+	+	+	Frequent	+	+	Pale	+
22	-	+	+	Frequent	+	+	Pale	+
23	NR	NR	NR	NR	NR	-	NR	NR
24	-	+	-	Frequent	+	-	Pale	-
25	+	NR	NR	NR	NR	+	NR	NR
26	+	+	+	Occasional	+	+	Normal	+
27	+	+	-	Occasional	-	+	Normal	+
28	-	+	-	-	+	+	Pale	+
29	-	-	+	Frequent	-	-	Normal	-
30	+	+	-	Frequent	NR	-	NR	NR

NR Not recorded.

TABLE 5

CIN: GENERAL INFORMATION, BIOCHEMICAL AND SEROLOGICAL DATA[A]

CASE	AGE Years	SEX	BREED	BLOOD UREA $mmol.L^{-1}$	URINE PROTEIN $mg.100ml s^{-1}$	URINE UREA $mmol.L^{-1}$	SPECIFIC GRAVITY OF URINE	AGGLUTINATION LYSIS TITRE L. CANICOLA	AGGLUTINATION LYSIS TITRE L. ICTEROHAEMORRHAGIAE
1	3½	M	Alsatian	107	190	115	1.020	-	-
2	Aged	M	Labrador	NR	NR	NR	NR	NR	NR
3	3½	F	Alsatian	110	92	168	1.019	1:30,000	-
4	7½	M	Crossbred	>98	76	305	1.020	-	-
5	3½	M	Labrador	>100	323	230	1.019	1:300	1:30
6	7	M	West Highland Terrier	97	167	138	1.015	1:1,000	1:100
7	7	M	Boxer	104	140	233	1.020	1:10,000	1:300
8	8	M	Crossbred	106	250	483	1.015	1:300	1:100
9	10	M	Doberman	77	147	310	1.022	1:30	-
10	8	M	Crossbred	100	103	305	1.019	1:1,000	1:3,000
11	8	F	Alsatian	100	52	230	1.016	1:300	-
12	7	M	Crossbred	126	268	170	1.020	1:3,000	-
13	14	M	Crossbred	51	150	145	1.013	1:30	
14	7	M	Crossbred	148	129	310	1.020	NR	NR
15	10	M	Crossbred	118	137	240	1.019	-	-
16	4	M	Crossbred	149	420	227	1.021	1:30,000	-

CASE	AGE Years	SEX	BREED	BLOOD UREA mmol.l^{-1}	URINE PROTEIN mg.100mls^{-1}	URINE UREA mmol.l^{-1}	SPECIFIC GRAVITY OF URINE	AGGLUTINATION LYSIS TITRE L. CANICOLA	L. ICTEROHAEMORRHAGIAE
17	3	M	Shih-Tzu	71	70	170	1.015	-	-
18	10	M	Crossbred	101	310	260	1.020	-	-
19	11	M	Collie	71	NR	NR	NR	NR	NR
20	3	M	Boxer	108	NR	NR	NR	NR	NR
21	7½	M	Collie	97	294	212	1.020	1:1,000	-
22	3	M	Crossbred	131	164	215	1.017	1:10,000	-
23	7	M	Collie	61	230	190	1.015	-	-
24	6	M	Cairn Terrier	150	75	170	1.013	-	-
25	Adult	F	Beagle	NR	NR	NR	NR	NR	NR
26	1½	M	Doberman	100	600	265	1.020	-	-
27	5½	F	Elkhound	73	110	250	1.020	-	-
28	16	F	Crossbred	111	168	165	1.015	1:3,000	1:30
29	12	F	Poodle	69	60	124	1.015	NR	NR
30	12	F	Crossbred	92	500	225	1.020	NR	NR

A value of samples taken nearest to time of death.
NR not recorded
F female
M male

TABLE 6

CIN: EXTRA-RENAL PATHOLOGY

CASE	ORAL ULCERATION	GASTRITIS	INTERCOSTAL MYOSITIS/ CALCIFICATION	NECROTIZING ENDOCARDITIS	LEFT VENTRICULAR HYPERTROPHY	PARATHYROID HYPERPLASIA	OTHER LESIONS
1	+	-	+	-	+	+	
2	+	+	+	-	-	-	
3	+	+	-	-	-	-	Oesophageal Ulceration
4	+	-	-	-	-	-	
5	+	+	+	-	-	-	
6	+	+	-	-	-	-	Atrial fibrosis Intercostal fibrosis
7	+	-	-	-	+	+	
8	+	-	+	+	-	-	
9	+	+	+	-	-	-	Thyroid adenoma Prostatic hyperplasia
10	+	+	+	+	-	+*	
11	+	+	+	-	-	-	
12	-	+	-	-	+	-	
13	-	-	+	-	-	+	Endocardosis
14	+	+	-	-	-	-	

CASE	ORAL ULCERATION	GASTRITIS	INTERCOSTAL MYOSITIS/ CALCIFICATION	NECROTIZING ENDOCARDITIS	LEFT VENTRICULAR HYPERTROPHY	PARATHYROID HYPERPLASIA	OTHER LESIONS
15	+	+	-	-	+	+	Endocardosis Pancreatic hyperplasia
16	+	-	-	-	-	+	Tonsilitis Enteritis
17	-	NR	NR	NR	NR	NR	NR
18	+	+	+	-	-	+*	Endocardosis Splenic lymphoma
19	+	+	+	-	-	+	Hepatic adenoma Seminoma Pneumonia Endocardosis
20	-	+	+	+	-	+	Laryngeal ulceration Pulmonary arteritis Pulmonary calcification Epihyoid fracture
21	+	+	+	-	-	+*	Endocardosis Alveolitis
22	+	+	+	+	-	+*	Pulmonary arteritis
23	-	+	+	-	-	+*	Prostatitis Cystitis
24	-	+	-	-	+	-	Endocardosis Acute Pancreatitis

CASE	ORAL ULCERATION	GASTRITIS	INTERCOSTAL MYOSITIS/ CALCIFICATION	NECROTIZING ENDOCARDITIS	LEFT VENTRICULAR HYPERTROPHY	PARATHYROID HYPERPLASIA	OTHER LESIONS
25	+	+	+	-	-	-	Endocardosis Pancreatic hyperplasia
26	+	-	-	-	-	-	
27	+	-	-	+	-	-	Pulmonary arteritis
28	+	+	-	-	-	-	Hepatic adenocarcinoma Persistent ductus arteriosis
29	-	+	+	+	-	-	Endocardosis
30	-	+	+	-	+	+	Haemorragic enteritis Pancreatic hyperplasia Hepatic hyperplasia Adrenal hyperplasia Endocardosis

* Osteodystrophia fibrosa also present

NR Not recorded. Full post mortem not done due to owner's wishes.

TABLE 7 CIN: RENAL PATHOLOGY

CASE	RENAL FIBROSIS					ARTERIES AND ARTERIOLES				TUBULES			OTHERS	
	DEGREE	PATTERN			CORTEX WIDTH	SPIRALLING	MEDIAL HYPERTROPHY	PLASMATIC VASCULOSIS		ATROPHY	CYSTIC DILATION	CASTS	CELLULAR INFILTRATE	CALCIFICATION
		DIFFUSE	DEEP CORTICAL SCARS	RADIAL CORTICAL SCARS				FIBRIN STAINING	COLLAGEN STAINING					
1	4+	Yes	Yes		R	1+	-	-	-	4+	4+	1+	1+	4+
2	4+	Yes	Yes		R	1+	-	-	1+	3+	4+	1+	1+	4+
3	2+		Yes	Yes	R	-	-	-	-	3+	4+	1+	1+	2+
4	3+		Yes	Yes	R	3+	3+	2+	2+	4+	4+	3+	2+	-
5	4+		Yes	Yes	R	1+	-	-	1+	3+	4+	1+	1+	3+
6	4+		Yes	Yes	R	3+	3+	1+	1+	4+	3+	3+	1+P	1+
7	4+	Yes	Yes		R	3+	3+	-	1+	4+	3+	2+	1+	1+
8	4+	Yes	Yes		R	2+	-	-	-	2+	4+	2+	2+	1+
9	4+		Yes	Yes	R	2+	4+	-	4+	3+	4+	4+	1+	1+
10	4+	Yes	Yes		R	3+	2+	-	2+	4+	3+	3+	1+	4+
11	4+	Yes	Yes		R	1+	1+	-	-	3+	1+	1+	1+P	3+
12	2+	Yes	Yes		R	-	-	-	-	2+	1+	4+	1+	1+
13	3+		Yes	Yes	R	3+	-	-	-	2+	1+	1+	1+	2+
14	4+	Yes	Yes		R	1+	1+	1+	1+	3+	3+	4+	1+	1+
15	4+		Yes	Yes	R	4+	1+	2+	2+	3+	4+	1+	1+	-
16	3+		Yes	Yes	R	3+	2+	-	1+	2+	4+	2+	2+P	-
17	3+		Yes	Yes	R	2+	1+	-	-	2+	1+	1+	1+	3+
18	4+		Yes	Yes	R	3+	2+	-	3+	4+	3+	3+	1+	2+
19	3+		Yes	Yes	R	1+	2+	-	3+	2+	4+	1+	1+	2+
20	3+	Yes	Yes		R	1+	1+	-	1+	3+	4+	3+	1+	3+
21	3+		Yes	Yes	R	2+	2+	1+	2+	2+	4+	2+	1+	1+
22	2+		Yes	Yes	R	2+	1+	-	-	3+	3+	1+	1+	2+
23	4+		Yes	Yes	R	2+	2+	-	1+	4+	4+	4+	1+P	-
24	3+		Yes	Yes	R	2+	2+	2+	1+	2+	2+	4+	1+	-
25	3+	Yes			N	1+	2+	1+	1+	2+	-	1+	1+	4+
26	2+	Yes			N	-	-	-	-	2+	1+	2+	1+	-
27	3+	Yes			R	1+	-	-	1+	2+	-	1+	1+	4+
28	2+	Yes			N	-	-	-	-	2+	-	2+	1+	-
29	3+	Yes			R	2+	-	-	1+	4+	-	3+	1+	1+
30	2+	Yes			R	-	1+	2+	2+	3+	-	2+	1+	2+

P A few polymorphonuclear leucocytes present
R Reduced
N Normal

TABLE 8

CIN: GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	< 50% SCARRING	> 50% SCARRING	100% SCARRING CONTRACTED	CYSTIC
1	296	5.4	11.1	20.3	23.3	39.9
2	305	3.6	24.3	25.9	31.5	14.7
3	197	8.6	21.8	28.0	25.4	16.2
4	147	2.0	23.1	34.0	36.1	4.8
5	231	17.7	32.5	15.2	29.8	4.8
6	203	3.9	26.6	30.6	35.0	3.9
7	193	2.6	29.5	22.8	41.5	3.6
8	159	6.9	26.4	21.4	13.8	31.5
9	282	0.7	9.9	7.5	70.2	11.7
10	301	7.0	22.9	17.3	39.5	13.3
11	404	2.5	5.0	13.1	6.7	72.7
12	168	3.6	26.8	32.7	32.7	4.2
13	211	11.9	30.3	24.7	26.5	6.6
14	239	2.1	21.3	31.4	43.9	1.3
15	197	2.0	20.3	12.2	62.4	3.1

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	< 50% SCARRING	> 50% SCARRING	100% SCARRING CONTRACTED	CYSTIC
16	304	0	7.3	31.9	54.9	5.9
17	214	11.6	19.6	18.4	32.2	18.2
18	188	6.9	20.2	21.3	51.1	0.5
19	208	1.9	19.2	16.4	61.5	1.0
20	118	4.2	35.6	33.1	25.4	1.7
21	252	2.0	25.8	29.7	40.1	2.4
22	130	11.5	36.9	32.4	19.2	0
23	193	10.3	30.6	14.5	38.9	5.7
24	171	5.8	17.5	31.1	40.9	4.7
25	299	3.0	20.4	24.7	25.8	26.1
26	111	5.4	15.3	37.0	28.8	13.5
27	281	2.5	11.4	31.7	20.6	33.8
28	201	12.4	33.8	19.4	22.4	12.0
29	322	5.6	35.7	24.9	24.2	9.6
30	578	3.8	25.4	19.4	48.5	2.9

TABLE 9

CIN: GLOMERULAR MORPHOLOGY

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED											
		FIBRIN DEPOSITS[A]				HYPERCELLULARITY			GBM THICKENING			CAPSULAR	CAPSULAR
		TOTAL[B]	URINARY SPACE	CAPILLARY	ADHESIONS	TOTAL[B]	LOCAL	GLOBAL	TOTAL[B]	LOCAL	GLOBAL	ADHESIONS	THICKENING
1	112	14.3	0	10.7	3.6	19.6	13.4	6.2	85.7	27.7	58.0	73.2	65.2
2	160	1.3	0	1.3	0	10.0	8.7	1.3	91.9	47.5	44.4	60.0	65.0
3	111	23.5	0	17.2	6.3	11.7	8.1	3.6	83.8	35.1	48.7	59.5	63.1
4	88	72.7	0	46.6	26.1	23.9	18.2	5.7	92.0	25.0	67.0	92.0	88.6
5	153	0.7	0	0.7	0	5.9	5.9	0	46.4	26.1	20.3	35.3	69.3
6	124	1.6	0	0	1.6	8.9	8.9	0	97.6	26.6	71.0	75.0	93.5
7	107	8.4	0.9	2.8	4.7	7.5	7.5	0	91.5	30.8	60.7	53.3	99.1
8	87	19.5	0	11.5	8.0	16.0	12.6	3.4	83.9	35.6	48.3	62.1	50.6
9	52	3.8	0	3.8	0	13.4	11.5	1.9	90.4	32.7	57.7	90.4	98.1
10	148	0	0	0	0	4.1	4.1	0	72.3	27.0	45.3	41.2	82.4
11	84	1.2	0	1.2	0	8.3	7.1	1.2	88.1	25.0	63.1	57.1	63.1
12	103	13.7	1.0	4.9	7.8	7.8	7.8	0	88.4	44.7	43.7	59.2	79.6
13	141	2.1	0	1.4	0.7	2.8	2.8	0	58.1	32.6	25.5	54.6	68.1
14	132	59.2	0.8	39.4	19.0	16.7	15.2	1.5	84.9	41.7	43.2	79.5	97.0
15	69	26.0	2.9	21.7	1.4	11.6	8.7	2.9	75.3	50.7	24.6	27.5	82.6
16	112	28.6	0.9	22.3	5.4	31.3	27.7	3.6	98.2	14.3	83.9	89.3	88.4

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED											
		FIBRIN DEPOSITS[A]				HYPERCELLULARITY			GBM THICKENING			CAPSULAR	CAPSULA
		TOTAL[B]	URINARY SPACE	CAPILLARY	ADHESIONS	TOTAL[B]	LOCAL	GLOBAL	TOTAL[B]	LOCAL	GLOBAL	ADHESIONS	THICK-ENING
17	88	2.3	0	2.3	0	9.1	8.0	1.1	88.7	30.7	58.0	35.2	54.5
18	81	9.9	0	6.2	3.7	4.9	4.9	0	86.5	34.6	51.9	50.6	84.0
19	78	27.0	0	16.7	10.3	9.0	9.0	0	80.8	59.0	21.8	75.6	80.8
20	88	4.5	0	1.1	3.4	22.7	19.3	3.4	82.9	40.9	42.0	56.8	81.8
21	145	60.1	0	44.2	15.9	13.1	11.7	1.4	87.6	23.4	64.2	82.8	74.5
22	108	34.2	0	22.2	12.0	7.4	7.4	0	91.6	43.5	48.1	54.6	88.9
23	105	8.6	0	4.8	3.8	23.8	20.0	3.8	84.8	42.9	41.9	50.5	62.9
24	85	32.9	0	7.0	25.9	27.1	24.7	2.4	85.9	42.4	43.5	67.1	74.1
25	139	7.9	0	4.3	3.6	3.6	3.6	0	78.4	43.9	34.5	64.0	74.8
26	61	36.1	3.3	21.3	11.5	24.6	21.3	3.3	78.7	37.7	41.0	85.2	93.4
27	130	70.8	0	42.3	28.5	6.2	6.2	0	93.1	28.5	64.6	90.0	83.8
28	140	10.0	0.7	0.7	8.6	7.9	7.9	0	96.4	35.0	61.4	45.0	74.3
29	225	8.8	0	8.4	0.4	4.0	4.0	0	97.3	12.0	85.3	40.9	35.1
30	285	15.1	0	7.4	7.7	9.1	8.4	0.7	95.4	39.3	56.1	64.2	63.9

A Position of largest deposit
B Total percentage of glomeruli affected
GBM Glomerular basement membrane

RESULTS 1. CHRONIC INTERSTITIAL NEPHRITIS (CIN)

All 30 dogs had a history of terminal chronic renal failure. The various clinical signs shown in Table 4, viz: weight loss, thirst, anorexia, vomiting, halitosis, oral ulceration, inelastic pulse and pale mucosae, were typical of those described in detail by McIntyre (1954) and Wettimuny (1963). The details of age, sex, and breed, with pertinent biochemical and serological findings, are given in Table 5. The age range and male predisposition were in agreement with previous reports (McIntyre 1954, Wettimuny 1963). Thus, the disease was one of adulthood, the youngest case being 1½ years old, and approximately four times as many males were affected than females. Although a range of breeds was noted, including some relatively uncommon ones such as Elkhound and Shih-tzu which indicated any breed could be affected, there was a great preponderance (40%) of crossbreds.

Gross Pathology

1) Extra-renal lesions (Table 6)

Uraemic lesions were found in all the 29 dogs where a full post mortem examination was done. The most common lesions were necrosis and ulceration of the oral and gastric mucosae, (22 and 21 cases respectively), and calcification and necrosis of the intercostal muscles and parietal pleura (17 cases).

Oral lesions ranged from a brown discolouration of the tip of the tongue to ulceration of its anterior and lateral borders. This was often accompanied by ulceration of the

gums and cheeks, especially where these made contact with the canine teeth. In the stomach, lesions ranged from small localized pin-head sized ulcers to multiple confluent areas of ulceration. Ulceration was invariably accompanied by haemorrhage, with the stomach often filled with sanguineous fluid. Less often (6 cases), necrotic lesions were present in the endocardium of the left atrium; similar lesions were also found in the pulmonary artery (cases 20, 22, 27), right atrium (cases 10, 29) and left and right ventricles (case 20). Grossly, these areas appeared as pale yellow crumbling deposits on the vessel or heart wall. In addition, scar tissue was seen in the right atrium of case 6 suggesting a healed stage of this lesion.

Although left ventricular hypertrophy secondary to hypertension has been reported to be fairly common in CIN (Platt 1962), this lesion was only seen in 6 cases. However, only a visual assessment was made and quantitative measurements may have revealed a much greater incidence.

Parathyroid hyperplasia was present in 13 cases but only 5 of these had gross evidence of osteodystrophia fibrosa (i.e. "rubber jaw").

2) Renal lesions

In all cases the kidneys were severely scarred (Figs. 2, 3). Characteristically they were shrunken, pale and firm with an irregular granular surface. The capsule, although thickened, stripped off easily. In all but 3 cases the cortices were reduced in width (Table 7). Cystic changes were present in all cases, ranging from an occasional small cyst in the cortex and medulla, to wide-

spread cystic dilations in the outer medulla. One case (11) was notable in this respect, having very many small cysts in both cortex and medulla.

Light Microscopy

The histopathological findings, excluding glomerular lesions, are summarized in Table 7. Because of the particular interest in glomerular morphology this is dealt with separately in greater detail. The most prominent extra-glomerular lesion was marked interstitial fibrosis, and two groups could be distinguished based on its pattern and degree. 24 dogs (cases 1-24) had the "classical" distribution with severe focal or diffuse scarring in a band around the cortico-medullary junction (Fig. 9). In addition in these animals, there was usually diffuse fibrosis of the medulla, and either diffuse or radial strands of fibrosis in the cortex. In the remaining 6 dogs (cases 25-30) scarring was less severe, and fibrous tissue was spread in fine strands diffusely through cortex and medulla with no obvious focal concentrations; the 3 dogs with cortices of normal width formed part of this group. This pattern and degree of scarring was similar to that seen in most cases of CGN (see below). In every case, scattered through the cortical interstitium particularly in areas of fibrosis, were small foci of lymphocytes, plasma cells and macrophages. In 4 cases (marked P in Table 7) a very few polymorphonuclear leucocytes were also present, usually around the pelvis. In all cases such infiltration was very small and probably reflected a pyelitis or pyelonephritis secondary

to an interstitial nephritis. Gross irregularity of the kidneys as found in primary pyelonephritis was not seen in any of these 4 animals.

Tubular lesions were related to the degree and pattern of fibrosis. In the cortex the tubules that still remained in the scarred areas were compressed, atrophied and partially or completely denuded of epithelial cells, and had thickened, wrinkled basement membranes. Outwith areas of scarring the tubules were often hypertrophic, but variable numbers of them also showed degenerative changes with the lining epithelium reduced to a low atrophic layer. Cystic dilation of the collecting ducts was a common and prominent feature. The normal cuboidal lining cells were sometimes replaced by flattened, atrophic epithelium but more often there was hyperplasia, with columnar cells piled up into several layers. Only very rarely was such hyperplasia seen in the cortex. This cystic lesion of the collecting ducts was most prominent in those cases with heavy focal or diffuse fibrosis at the cortico-medullary junction. Those 6 cases (25-30) which had diffuse fibrosis throughout the kidney had little or no cystic change in the collecting ducts. Many surviving tubules and collecting ducts contained hyaline and occasionally granular protein casts.

26 dogs had lesions of the arcuate and interlobular arteries, and afferent arterioles. The most common lesion was an apparent spiralling or tortuous passage of the vessels where they passed through areas of scarring. This change was absent or mild in those 6 animals (cases 25-30)

where there was diffuse but relatively mild fibrosis. In 20 dogs lesions of plasmatic vasculosis were present. In these animals plaques of featureless eosinophilic material were found in the intima and media of the affected vessels. In the mildest lesion, foci of this material were present in the intima with the internal elastic lamina intact. In more severe lesions the elastic lamina had ruptured and the media was also affected. Rupture of the external elastic lamina with extrusion of the plasmatic material into the adventitia was not seen. The staining characteristics of this material varied. The majority of deposits stained solely for collagen but some deposits stained wholly for fibrin or had a mixed fibrin/collagen reaction. Finally, arterial walls were thickened in 19 cases due to hypertrophy of the smooth muscle of the media and hyperplasia of the adventitial connective tissue.

Calcification of the kidney was common (23 cases). In the majority of cases the most widespread deposits were in the basement membranes of the proximal convoluted tubules, with smaller deposits free in the tubular lumina and in Bowman's capsules. Only occasionally were deposits present in the collecting tubules and interstitium.

Glomerular Lesions (Tables 8, 9)

In every case of CIN virtually all glomeruli were damaged to some extent and all parts - glomerular tuft, urinary space, and Bowman's capsule could be affected. In any section a range of glomerular morphology form normal to complete obsolescence was seen and a comparison of the range allowed the dynamic process of glomerular scarring to be

visualized. This range of morphology will first be briefly described and then the individual lesions of each part of the glomerulus will be discussed in detail.

Only a small percentage of glomeruli were not scarred (Table 8) but these were abnormal in that they were hypertrophied to as much as three times normal size. This was interpreted as an adaptation of the nephron to attempt to compensate for those that had ceased to function.

In a variable number of glomeruli scarring was mild with $<$ 50% of the tuft obliterated (Fig. 13). These were still functionally viable, serial sections revealing a normal or hypertrophic proximal convoluted tubule attached. These glomeruli also often showed a degree of compensatory hypertrophy. In the capillary tufts there were varying degrees of mesangial expansion and hypercellularity, thickening, wrinkling and duplication of the GBMs, capsular adhesions and sometimes fibrin deposits. Accompanying these tuft changes were varying degrees of distortion, thickening and duplication of the capsular basement membranes (CBM).

Once the scarring process had obliterated more than about half of the capillaries there was often evidence of functional derangement. Serial sections showed that the glomeruli were often shrunken and smaller than normal and that there was degeneration of the epithelium of the proximal convoluted tubules. In the majority of instances the CBM had shrunk with the tuft but in others it remained dilated producing a cystic appearance (Fig. 20).

Many glomeruli were completely obliterated by the scarring process (Table 8). Serial sections showed that

such glomeruli were non-functional, the related tubule being obliterated by fibrous tissue. The capillary tufts of these obsolescent glomeruli were reduced to a nodule of collapsed, wrinkled, thickened GBM and mesangial matrix, which contained few, if any, cells or patent capillaries; occasionally however, an arteriole appeared to persist (Figs. 16, 17). Usually the CBM had collapsed around this mass and such glomeruli appeared to progressively shrink and disintegrate as some very small knots of GBM, matrix and portions of CBM were seen. These were hard to distinguish from the surrounding fibrous tissue with H and E but PAS highlighted them well (Fig. 17). Less often the CBM remained intact and distended with pale eosinophilic fluid, thus forming a cyst in which the remnants of the tuft could completely disappear (Fig. 12). In most cases only a small percentage of glomeruli were so affected but in one case (11) this was the predominant morphological type. It is possible that such glomeruli result from occlusion of a still functioning nephron by the interstitial reaction.

The severity of glomerular damage in any one area was related to the degree of interstitial fibrosis, and most glomeruli in the radial cortical scars were often obsolescent (Fig. 11). In contrast outwith such areas, a few glomeruli would be normal, most were scarred but still functional and only a few obsolescent. In those cases with diffuse fibrosis of the cortex, there were no focal variations in the concentrations of the various morphological forms.

1) The Glomerular Tuft

(a) The mesangium

A constant part of glomerular scarring was the formation of excess mesangial matrix, a material which was PAS positive and also stained for collagen (Figs. 13, 14, 18, 19). In its mildest form the mesangium was locally or globally thickened so accentuating the tuft lobulation. A more severe stage was the localized occlusion of capillaries by the expansion of the mesangium, while in some glomeruli virtually all the capillaries were obliterated. In some glomeruli the formation of mesangial matrix seemed to follow fibrin deposition in the tuft (see below).

Changes in the cellularity of the mesangium always accompanied the changes in the matrix. Focal hypercellularity was present in every case, usually involving localized areas in the less severely damaged glomeruli (Fig. 18, 19) (Table 9). In some glomeruli, localized areas of hypercellularity appeared to be produced by tuft collapse but in others a genuine overall increase in the number of cells was present. The position and morphology of the extra cells appeared to be mesangial. However, mitoses were never seen and this raised the possibility that they may have originated from circulating mononuclear cells rather than by proliferation of fixed mesangial cells.

In contrast, in the more severely scarred glomeruli the number of cells was often reduced and, in totally obliterated glomeruli, few and occasionally no cells remained.

(b) The glomerular capillaries

Lesions of the GBMs, best shown by PAS, were very common (Table 9). In the mildest lesion, a single or a few peripheral capillaries were thickened (Fig. 13). In more severely affected glomeruli not only were more capillaries involved but some were obliterated by this process. This obliteration was accompanied by loss of the lining endothelial cells. Where many capillaries had been obliterated and collapsed, the glomerulus was left with a "simplified" appearance, composed of a few large distorted capillaries with very thickened, wrinkled walls but patent lumina (Fig. 15). In the severest form the glomerulus was reduced to a mass of collapsed, wrinkled and thickened GBM with few, if any, patent capillaries (Figs. 16, 17). Occasionally some capillary loops had doubled layered GBMs, a change which could have reflected either splitting or the formation of a new separate membrane. In some capillaries, particularly at the periphery of the tuft, GBM thickening appeared to be a result of fibrin deposition. Along with expansion of the mesangial matrix, thickening, wrinkling and duplication of the GBMs followed by their collapse, were the major factors leading to glomerular obliteration. Most glomeruli had a combination of both but the relative proportions varied; in some mesangial expansion was the more prominent in others abnormalities of the GBMs were the main features.

(c) Visceral epithelium

The obliteration of the tuft by the aforementioned processes was accompanied by loss of visceral epithelial

cells. In some severely damaged glomeruli these cells occasionally became cuboidal and orientated close together producing a hypercellular ring around the tuft. Hypercellularity due to proliferation of these cells was never seen.

2) Bowman's Capsule

(a) The capsular basement membrane (CBM)

Thickening of Bowman's capsule due to formation of new basement membrane was a very common feature; lesions were best shown with PAS. Most commonly, the CBM was locally or globally thickened and its normal spherical shape distorted (Fig. 15). Less often, distinct new layers of CBM formed internal to the original (Fig. 14). These were usually present in the more severely scarred glomeruli and tended to form towards and around the junction with the proximal convoluted tubule. It is possible that, in some instances, these extra layers were formed by a process of splitting of the original CBM rather than by synthesis of new separate layers. Between the layers there was often faintly PAS positive material containing occasional cells. Where many layers had built up in the more severely scarred glomeruli, the urinary space was reduced in volume. In such glomeruli the original CBM appeared to be disintegrating as breaks and missing segments were often seen. In obsolescent glomeruli the capsule could remain intact and distended forming a cyst (Fig. 12). In most instances, however, the capsule had collapsed around the shrunken tuft and had partially or totally disintegrated (Figs. 16, 17).

Two lesions which had to be distinguished from this were, firstly, thickening of the capsule due to the deposition of calcium salts (not common), and secondly, apparent thickening and duplication of the capsule due to concentration of interstitial fibrous tissue around it (common). This fibrosis was always external to the original CBM and only faintly PAS positive.

(b) Capsular (parietal) epithelium

A common feature was hypertrophy of the parietal epithelial cells. The cells, closely packed together, were swollen and occasionally cuboidal, and had prominent nuclei. Proliferation of the parietal epithelial cells was never present, although crescent shaped hypercellular areas were seen in the capsule and urinary space of a few glomeruli. Close examination revealed these to be the result of tuft scarring. Where a whole lobule had become adhesed to the tuft, the parietal epithelium extended to cover the mass. This left a reduced but functional portion of the tuft, and a crescent shaped mass of mesangial matrix and GBM, containing occasional cells, attached to Bowman's capsule. In addition, tuft scarring could distort the hilar region in such a way as to produce a hypercellular crescent shaped mass. In obsolescent glomeruli parietal epithelial cells were decreased in number or completely absent.

3) Urinary Space

Partial or complete obliteration of the urinary space was a common sequel to glomerular damage. Where scarring of the tuft was mild there were localized areas of

obliteration due to the thickening of the CBM and the formation of capsular adhesions. In severely scarred and obsolescent glomeruli the urinary space was filled, in the majority of instances, with eosinophilic material which was faintly PAS positive and stained for collagen (Fig. 16). In some, this material formed a thin layer internal to Bowman's capsule but usually it completely occluded the urinary space. An occasional cell could be seen embedded in this material. In those obsolescent glomeruli where the capsule had disintegrated this material merged imperceptibly into the surrounding interstitial fibrous tissue. On the other hand, in those glomeruli where tuft shrinkage was accompanied by a cystic dilation of Bowman's capsule, the urinary space was filled with pale eosinophilic fluid.

4) Fibrin Deposits

The process of glomerular fibrin deposition is dealt with separately as all parts of the glomerulus, viz; tuft, urinary space and Bowman's capsule, could be involved. Although positive results were obtained with all stains, those which depict "old" fibrin e.g. MSB and Obadiah's methods gave the best and most consistent results.

Fibrin was present in a focal distribution except in case 10 where it was absent (Table 9). The degree of deposition varied from a small globule in a very few glomeruli (cases 5, 11) to large multiple deposits in many glomeruli (cases 4, 14, 21, 27). In addition to these deposits in functional glomeruli, obsolescent glomeruli occasionally contained fibrin. Glomeruli where tuft

collapse was accompanied by cystic dilation of Bowman's capsule rarely contained fibrin.

As stated above all parts of the glomerulus could contain fibrin. The most common site was the capillaries, particularly those at the periphery of the tuft where globular or crescent shaped masses were present (Fig. 21). Although deposits often appeared to be intracapillary, when serial sections were cut it was found that often a mass of fibrin lay partly within a capillary and partly liberated into the urinary space. Such exudation appeared to rapidly lead to the formation of capsular adhesions. In most instances deposits were associated with adhesions and only very rarely was fibrin seen lying apparently free in the urinary space. Occasionally such liberated fibrin penetrated between the layers of the CBM, and in a few instances, into the periglomerular tissue as well. In a few glomeruli collections of globules staining for fibrin were seen apparently inside epithelial cells (usually visceral); this was taken to indicate phagocytosis and degradation of liberated fibrin by these cells.

The change in staining of deposits from fibrin to collagen, previously noted with regard to the arterial and arteriolar deposits, was seen in the glomeruli. Often fibrin was surrounded by or confluent with collagen staining material (Fig. 21), and serial sections through fibrin deposits showed a gradual change of staining reaction from fibrin to that of collagen. In some areas this transfor-

mation appeared to be associated with a non-staining phase, with areas of fibrin separated from the surrounding "collagen" by a clear zone (Fig. 21). The final result of this transformation was that many of the adhesions present stained solely for collagen, while in the tuft itself fibrin deposits were replaced by material indistinguishable (with the stains used here) from mesangial matrix and basement membrane. Thus the impression was gained that, in some instances at least, mesangial expansion and GBM thickening were the result of fibrin deposition.

Electron Microscopy

A) The Normal Canine Glomerulus

There are only a few descriptions of the normal ultnastructure of the canine glomerulus (Movat and Steiner 1961, Crowell et al. 1974). Consequently it was considered important to establish the normal morphology using the same fixation and embedding procedures that were applied to the cases of chronic nephritis. Therefore 6 normal control dogs from the liquoid experiment (see Part 3) were examined with the electron microscope and a typical glomerulus is shown in Fig. 22.

1) The Glomerular Tuft

a) Epithelial cells

The visceral epithelial cells were composed of a main cytoplasmic mass, cytoplasmic processes (trabeculae) and terminal foot processes (pedicels). The nucleus and

most of the cell organelles were present in the main cytoplasmic mass. The nucleus, marginally the largest in the glomeruli, was usually round but could be indented. Much of the chromatin was condensed at the nuclear borders and both nucleoli and nuclear pores were prominent. The following organelles were present; mitochondria (swollen and distorted by paraglutaraldehyde fixation), a small Golgi apparatus, smooth and rough endoplasmic reticulum, free ribosomes and occasional vacuoles. A few microvilli could also be present and microfibrils and microtubules were scattered randomly through the cytoplasm. The scanning electron microscope has revealed that the trabeculae are arranged in primary, secondary and tertiary orders (Arakawa and Tokunaga 1972). However, this could not be appreciated with the transmission electron microscope, and depending on the plane of section, many apparently haphazard arrangements of the trabeculae were seen. All the organelles mentioned above, with the exception of the Golgi apparatus, were found in small numbers in the trabeculae. The cells terminated in bulb shaped foot processes which usually arose from the tertiary trabeculae. The foot processes were placed on the lamina rara externa of the GBM and were separated from their neighbours by a small space bridged by a thin filtration slit membrane. Scanning electron microscopy has also revealed that neighbouring foot processes arise from different epithelial cells (Arakawa and Tokunaga 1972). The foot processes lacked any organelles with the exception of numerous microfibrils and a few vacuoles. The microfibrils were

concentrated at the periphery of the foot processes resulting in a greater electron density of the cytoplasm here than in the rest of the cell. Occasionally, as a result of plane of section, the foot processes, instead of being bulb shaped, were much broader. This was easily distinguishable from "fusion" of the foot processes, which was present in nephritic glomeruli, where virtually all foot processes encompassing a capillary loop were apparently replaced by long segments of cytoplasm (see below).

b) Glomerular basement membrane (GBM)

The GBM formed a continuous band which separated the epithelial cells from the mesangium and the endothelium. It was composed of fine fibrillar material which formed three distinct layers; the inner and outer relatively electron translucent areas, the lamina rara interna and the lamina rara externa, and the middle relatively electron dense layer, the lamina densa. The lamina interna and externa were of approximately equal width and were three or four times thinner than the lamina densa. However, in a few capillaries portions of the lamina rara interna could be irregularly expanded to two or three times their normal width. Very occasionally the GBM appeared to be double with two laminae densae separated by an electron translucent area. Such areas were usually present at mesangium/capillary borders near the glomerular hilus and were possibly artifacts.

c) Endothelial cells

The endothelial cells were composed of a main cytoplasmic mass positioned in the axial region of the

capillary near the mesangium, and a layer of attenuated fenestrated cytoplasm which lined the rest of the capillary wall. The nucleus and virtually all the cell organelles were placed in the main cytoplasmic mass. The nucleus was round and very like that of an epithelial cell except that it was smaller. Less mitochondria were present than in epithelial cells and free ribosomes were more prominent than rough endoplasmic reticulum. In addition, smooth endoplasmic reticulum, Golgi apparatus, pinocytotic vesicles, and scattered microfilaments were present. Tangential sections revealed the "sieve" like structure produced by the fenestrae in the peripheral cytoplasm. This cytoplasm usually lay flat on the lamina rara externa but in places it could be curled up and positioned slightly distant from the GBM. This latter finding was also considered to be an artifact.

d) The mesangium

The mesangium consisted of stellate shaped cells embedded in a fibrillar matrix. The cells were morphologically very similar to the endothelial cells except that the nucleus tended to be larger and more irregular in outline, while the peripheral cytoplasm was very filamentous and occasionally contained electron dense bodies resembling lysosomes. The matrix was composed of many fine non-banded fibrils, and was similar in electron density and structure to the lamina rara interna with which it was continuous. Collagen fibrils were absent from the mesangium. The mesangium was separated from the epithelial cells by the GBM, and from the capillary lumina by the endothelial cells.

Occasionally however, segments of mesangial cytoplasm extended between adjacent endothelial cells making direct contact with the capillary lumen. Usually two capillaries were separated by a mesangium containing only one cell, but occasionally two and very rarely three cells were present.

e) Capillary lumina

The capillary lumina usually contained a fine granular precipitate of plasma and in some instances a variable number of red blood cells. Occasionally a single lymphocyte, polymorphonuclear leucocyte or monocyte was also present in the lumen.

2. Bowman's Capsule and Urinary Space

Bowman's capsule was formed by a basement membrane (CBM) and a single layer of epithelial cells. The CBM consisted of a single moderately dense band of a variable thickness, composed of fine and fibrillar material. Usually it was homogeneous but there were a few areas where slight layering or splitting could be discerned. Collagen fibrils were never present in the CBM or between it and the capsular epithelium.

The capsular (parietal) epithelial cells were narrow elongated cells. The oval nucleus was placed in the middle and widest part of the cell, and had much of its chromatin clumped at the nuclear border. Mitochondria, Golgi apparatus, smooth and rough endoplasmic reticulum, free ribosomes and occasional vesicles were present in the cytoplasm. The cytolplasm was very filamentous particularly near the border with the CBM. Microvilli were sometimes

formed by these cells.

The urinary space was always patent and free from debris.

B) Chronic Interstitial Nephritis

Glomeruli from 8 cases (4,10,11,16,23,24,26,28) were examined with the electron microscope. An attempt was made to examine glomeruli in varying degrees of obsolescence so that an overall ultrastructural picture of the scarring process could be built up.

1) The Glomerular Tuft

Electron microscopy confirmed the histological finding that the major tuft changes were mesangial expansion, and GBM thickening and wrinkling. Varying amounts of excess mesangial matrix were present in all glomeruli examined, resulting in narrowing of the capillaries (Figs. 23,24). In most instances the matrix was similar in texture to that in normal glomeruli but less homogenous in character (compare Figs. 22 with 23,24). In addition, in the more severely scarred glomeruli it could also contain occasional collagen fibres and/or collections of myelin figures, electron dense bodies, vesicles and granules. Changes in the mesangial cell was also present. Although unequivocal mesangial hypercellularity (more than 3 cells) was never seen, it was more common to find 2 or 3 mesangial cells present in one area than in a normal glomerulus. In several capillaries there had also been circumferential interposition of these cells between the GBM and endothelium (Fig. 24,32). This resulted in a thickened capillary wall

containing segments of mesangial cytoplasm and matrix. In contrast, in the more severely scarred areas mesangial cells were often atrophic and reduced in number. Those remaining were composed of a small amount of abnormally dense cytoplasm containing myelin bodies, electron dense lysosome-like bodies, and increased numbers of vacuoles but reduced numbers of other organelles. Presumably death of these cells leaves the collections of cellular debris that were seen embedded in the matrix.

Thickening of the GBM took a variety of forms. In some the lamina densa was particularly thickened, in others the lamina rara interna was markedly increased in width. Another common lesion was an increase in thickness of the GBM accompanied by obliteration of distinction into lamina densa and laminae rarae (Fig. 25). Sometimes these thickened GBMs were very vacuolated (Fig. 26), or contained collections of myelin figures and granules presumably resulting from cell necrosis. Wrinkling often accompanied the thickening and in general the greater the thickening the more marked the wrinkling. Occasionally this irregularity was accentuated by the formation of nodules of basement membrane on the epithelial side of the GBM. Sometimes areas of duplication were present with a double lamina densa separated by a relatively electron translucent zone. Finally, the capillary walls could also be thickened by mesangial cell interposition, described above, and in a few instances by fibrin deposition (see below). No breaks in the GBM were ever seen in the glomeruli examined.

Abnormalities were also present in the epithelial and

endothelial cells. Degenerative changes in the endothelial cells were very like those seen in mesangial cells, and such degenerate cells would often lie partially detached from the capillary wall and have very irregular cell borders. Changes in the epithelial cells were more complex. Along stretches of thickened GBM virtually all foot processes were replaced by broader segments of cytoplasm, which were usually abnormally dense (Figs.25, 26). Scanning electron microscopy has revealed that this apparent "fusion" of foot processes is, in fact, a reflection of their retraction and swelling, and is an indication of a glomerular protein leak (Arakawa and Tokunaga 1972). The epithelial cells often possessed excess numbers of vacuoles which could contain dense material, and increased numbers of microvilli could be formed at the cell surface. As degeneration advanced some GBMs were covered in just a thin sliver of featureless cytoplasm (Fig. 26). Finally, in the more severely scarred capillaries the epithelial cytoplasm had become completely detached from the GBM, the space being filled with pale amorphous granular and fibrillar material (Fig. 27). No evidence of proliferation of either epithelial or endothelial cells was seen, nor was infiltration with any circulating mononuclear cell present.

In the obsolescent glomeruli examined many of these changes in tuft morphology could no longer be discerned. All that remained was a small knot of collapsed, wrinkled lamina densa enclosing a mass of pale, granular and fibrillar material (Fig. 28). No distinct mesangial area could be

seen nor could lamina rara interna or externa be distinguished. The very few glomerular cells left were atrophic and patent capillaries were absent.

2. Bowman's capsule

The CBM was abnormal in every glomerulus examined. Typically it was irregularly thickened and divided into many layers composed of dark fibrillar material (Figs. 27,29a,30). Embedded in these layers were many small dense granules (Fig. 29b) possibly the ultrastructural equivalent of the calcium deposits seen with the light microscope. In two glomeruli examined gaps were found in the CBM. These were now filled with the same material as that present between different layers of CBM. This material was composed of pale granular and fibrillar elements. A variety of banded and non-banded fibrils of varying diameters were seen including collagen fibres. Thin strands of cytoplasm were also present between the layers of CBM. These were usually composed of fibrillar cytoplasm containing vacuoles of varying sizes, electron dense granules, occasional mitochondria and small amounts of endoplasmic reticulum. The fibrillar nature of the cytoplasm suggested a parietal epithelial cell origin. The electron dense granules in the cells and those in the CBM appeared identical. Occasionally a fibroblast-like cell containing prominent endoplasmic reticulum was also seen.

3. Urinary Space

Electron microscopy revealed that the eosinophilic material seen obliterating the urinary spaces of obsolescent

glomeruli was largely collagen (Fig. 28). In addition, other banded and non-banded fibrils and granular elements were also present. Segments of atrophic cytoplasm were scattered through this material. Usually these were composed of fibrillar cytoplasm containing myelin figures, electron dense bodies, vacuoles and occasional swollen mitochondria. Presumably these were effete epithelial cells. Although collagen fibres were found in the urinary space no fibroblasts were seen in the glomeruli examined. Scattered through these obliterated urinary spaces were collections of myelin figures, granules and vesicles. No glomeruli with cystic dilation of Bowman's capsule were present in the samples examined with the electron microscope.

Several capsular adhesions were seen, formed either by the GBMs and CBMs attaching directly to each other, or via a "bridge" of fibrillar basement membrane-like material (Figs. 30, 31). Both visceral and parietal epithelial cells were absent from the area but foci of myelin figures, granules and vesicles could be present in the "bridge". In several instances in case 24, the capillaries involved in the adhesions were expanded with an electron dense granular material (see below).

4. Fibrin Deposits

The "characteristic" fibres of fibrin with a periodicity of about 230^{o}A (Kay and Cuddigan 1967) were never seen. However, in two cases (23,24) deposits with different characteristics were present in the capillaries and associated with capsular adhesions, and it is possible that

these were fibrin or a derivative of fibrin (see discussion). In case 23, there was obliteration of several capillaries and thickening of other capillary walls by a predominantly electron translucent granular material (Figs. 32,33). Usually small foci of dense granular and non-banded fibrillar material were present in these areas as well. In addition, occasional banded elements of unknown composition were also present (Fig. 33). Mesangial cells were seen invading these materials leading to circumferential interposition between GBM and endothelium. In case 24 a different picture was seen (Figs. 30, 31). Several peripheral capillary loops associated with capsular adhesions were engorged with electron dense granular material. The lamina densa could still be distinguished surrounding this material, unlike the lamina rara interna and matrix. Only small segments of atrophic endothelial and mesangial cytoplasm remained embedded in this granular material.

Immunofluorescence Microscopy (Table 10)

1. Glomeruli

Deposits of fibrin were the most prominent finding. Small fluorescing granules and globules were present in just a few glomeruli from 15 cases. Most deposits were associated with capsular adhesions but intracapillary fluorescence was also seen (Fig. 35). The amount identified by immunofluorescence was never as great as that suggested by the light microscopic fibrin stains. Indeed certain cases e.g. 21, 26, which had fairly extensive fibrin deposition in histological sections were negative using immunofluorescence.

TABLE 10

CIN: IMMUNOFLUORESCENCE FINDINGS[1]

CASE	GLOMERULI				ARTERIES AND ARTERIOLES				TUBULES[2]	INTERSTITIUM[3]	
	IgG	IgM	C3	FIBRIN	IgG	IgM	C3	FIBRIN	C3	IgG	IgM
1	-	NR	-	-	-	NR	-	-	-	-	-
2	-	-	-	-	+	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-
4	+	NR	-	+	+	NR	-	+	-	-	-
5	-	NR	-	+	+	NR	-	+	-	-	-
6	-	NR	-	+	+	NR	-	+	-	+	-
7	-	NR	-	+	+	NR	-	+	-	-	-
8	+	NR	-	-	+	NR	-	+	-	-	-
9	-	NR	-	-	-	NR	-	-	-	-	-
10	-	NR	-	-	+	NR	-	-	-	-	-
11	-	NR	-	-	-	NR	-	-	-	-	-
12	-	NR	-	-	-	NR	-	-	-	-	-
13	-	-	-	+	-	-	-	-	-	+	-
14	-	+	-	-	-	-	-	-	-	-	-
15	-	-	-	+	-	-	-	-	-	-	-
16	-	+	+	+	-	-	-	-	+	-	-

CASE	GLOMERULI				ARTERIES AND ARTERIOLES				TUBULES[2]	INTERSTITIUM[3]	
	IgG	IgM	C3	FIBRIN	IgG	IgM	C3	FIBRIN	C3	IgG	IgM
17	-	-	-	-	-	-	-	-	+	+	-
18	-	-	-	+	-	-	-	-	+	+	-
19	-	-	-	+	-	-	-	-	+	-	-
20	-	+	+	+	-	-	-	-	+	-	-
21	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	+	-	+	-	-	+	+	+
23	-	-	-	-	-	-	+	-	+	-	-
24	+	+	+	+	+	-	+	+	+	-	-
25	-	-	-	+	-	-	-	-	+	-	-
26	-	NR	-	-	-	NR	-	-	-	-	-
27	+	NR	-	+	+	NR	-	+	-	-	-
28	+	NR	-	-	+	NR	-	-	-	-	-
29	-	-	-	-	-	-	-	-	+	-	-
30	-	+	-	+	-	-	-	+	+	-	-

1 No CAV or L.canicola antigens found in any case
2 No deposits of IgG, IgM or fibrin found.
3 Plasma cells
NR Not recorded

TABLE 11

CIN: ELUTION STUDIES[1]

CASE	WEIGHT ELUTED g	VOLUME OF ELUATE ml	CONCENTRATION OF ELUATE $\mu g.ml^{-1}$	ANTI-*L. CANICOLA* ANTIBODIES ELUATE	SERUM
1	8.2	15	168	-	-
2	8.5	10	110	1:3.000	NR
3	16.0	11	1,375	1:100	1:30,000
8	3.9	2	388	1:3,000	1:300
9	5.0	10	115	-	1:30
10	9.6	4	1,375	1:3,000	1:1,000
13	16.2	19	250	-	1:30
14	13.6	14	355	1:300	NR
15	18.3	19.5	268	-	-
16	11.2	24.5	235	1:100	1:30,000
17	6.0	3	875	1:10	-
18	16.8	25	775	1:3,000	-

CASE	WEIGHT ELUTED g	VOLUME OF ELUATE ml	CONCENTRATION OF ELUATE $\mu g.ml^{-1}$	ANTI-*L. CANICOLA* ANTIBODIES	
				ELUATE	SERUM
19	16.7	25.5	505	1:1,000	NR
20	8.7	25	230	1:300	NR
21	19.9	26	400	1:3,000	1:1,000
22	6.4	24	255	-	1:10,000
23	15.3	22	1,475	1:10,000	-
24	9.6	14	340	-	-
25	12.4	23	1,050	-	NR
27	9.8	10.5	370	-	-
29	11.2	25	330	-	NR
30	11.4	17	480	-	NR

1. All eluates negative for anti-CAV, anti-kidney and anti-*L. icterohaemorrhagiae* antibodies.

TABLE 12

ELUTION STUDIES - CONTROLS[1]

CASE	RENAL PATHOLOGY	WEIGHT ELUTED (g)	VOLUME OF ELUATE (ml)	CONCENTRATION OF ELUATE $\mu g.ml^{-1}$	ANTI-L. CANICOLA ANTIBODIES	
					ELUATE	SERUM
C1	Normal	7.3	9	480	-	1:30
C2	Normal	23.7	17.5	1385	-	1:3000
C3	Normal	43.7	26	770	-	NR
C4	Normal	5.2	13	1550	1:10	NR
C5	Normal	22.9	24	1005	-	NR
C6	Normal	9.2	11	510	-	1:100
C7	Amyloid Nephropathy	1.8	2	775	-	NR
C8	Amyloid Nephropathy	29.5	20	1950	1:300	1:30,000
C9	Glomerulonephritis	4.7	11	240	-	-
C10	Glomerulonephritis	67.5	25	1300	1:10	1:1,000
C11	Glomerulonephritis	30.2	20	1570	-	-
C12	Glomerulonephritis	3.8	3	390	-	NR
C13	Glomerulonephritis	9.7	10	875	-	-
C14	Glomerulonephritis	23.7	21	1100	-	1:30
C15	Pyelonephritis	25.4	13	1175	1:30	-
C16	Pyelonephritis	5.8	15	170	-	-
C17	Pyelonephritis	6.7	10	425	-	-
C18	Nephrosis	5.6	7.5	278	-	-
C19	Nephrosis	16.8	22	975	-	-
C20	Diabetic sclerosis	4.6	8	1025	-	-
C21	Septicaemia	19.3	15	240	-	-
C22	"Inherited" Renal disease	7.4	12.5	243	-	-

1. All eluates negative for anti-CAV, anti-kidney and anti-*L. icterohaemorrhagiae* antibodies.

In 9 cases small granules of immunoglobulin (IgM and/or IgG) were found; again only a few glomeruli were involved. Usually this seemed to result from non-specific trapping of serum proteins in the exudative lesions, the deposits being in the same position as fibrin. In three cases (16, 20, 24) complement (C_3) was also present in these areas and granules of immunoglobulin and complement (C_3) granules were occasionally found distant from fibrin deposits as well (Fig. 36).

2. Arteries and Arterioles

Foci of fibrin were present in the intima and media of these blood vessels in 8 cases (Fig. 37). Usually only one or two vessels per section were affected. Although confirming the light microscope findings there was, however, a poor correlation between the two. Only in 5 cases (4,6, 24,27,30) was fibrin identified by both methods. Other cases were positive only with immunofluorescence (5,7,8) or only with light microscopic stains (14,15,21,25). This apparent lack of correlation probably reflected the very focal nature of these lesions.

In 7 of the 8 cases positive with immunofluorescence IgG was also present in the same lesions, while in another 4 cases either IgM or IgG was found in the absence of fibrin. C_3 was present in two instances, alone in case 23 and accompanying fibrin and IgG in case 24.

3. Tubules

In 11 cases there were areas, virtually always in the cortex, where the tubular basement membranes stained in a

linear pattern for C_3 (Fig. 38). Usually the total number affected was small. No IgG, IgM or fibrin were ever found in this location.

4. Interstitium

Although some plasma cells were seen with light microscopy in the interstitium in every case, only in 5 were such cells identified by immunofluorescence.

5. Canine adenovirus and L. canicola

These antigens were not found in the glomeruli, tubules or interstitium of any case.

Elution Studies (Tables 11, 12)

Elution studies were carried out on tissue from 22 CIN cases. Anti-L. canicola antibodies were present in 12 of these eluates compared to only 4 out of the 22 controls. Moreover, the levels were usually much higher in the CIN eluates than in the controls. All 12 positives came from cases with the "classical" pattern of scarring (i.e. heavy focal or diffuse scarring around the cortico-medullary junction). No correlation between serum and eluate titres of anti-L.canicola antibodies was evident in either CIN or control animals. Anti-L. icterohaemorrhagiae, anti-CAV, and anti-kidney antibodies were not found in any CIN or control eluate.

Fig. 9 Chronic Interstitial Nephritis (CIN), Case 19

Severe renal scarring is present around the cortico-medullary junction, with many tubules obliterated by large areas of fibrous tissue (*). Co. Cortex, Me. Medulla.

(MSB x 35)

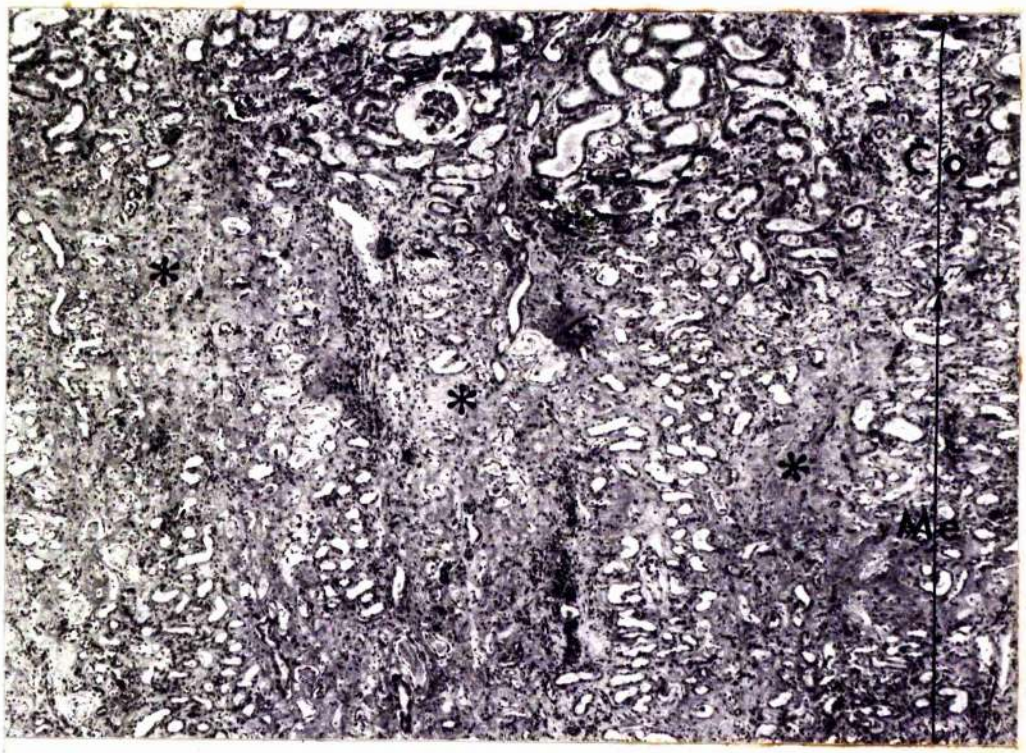

C
Me

Fig. 10 Normal Canine Glomerulus, Case 84

This puppy glomerulus is slightly more cellular than a normal adult glomerulus. Note the patent capillary loops with thin walls, the obvious urinary space and the thin even Bowman's capsule.

(H & E x 250)

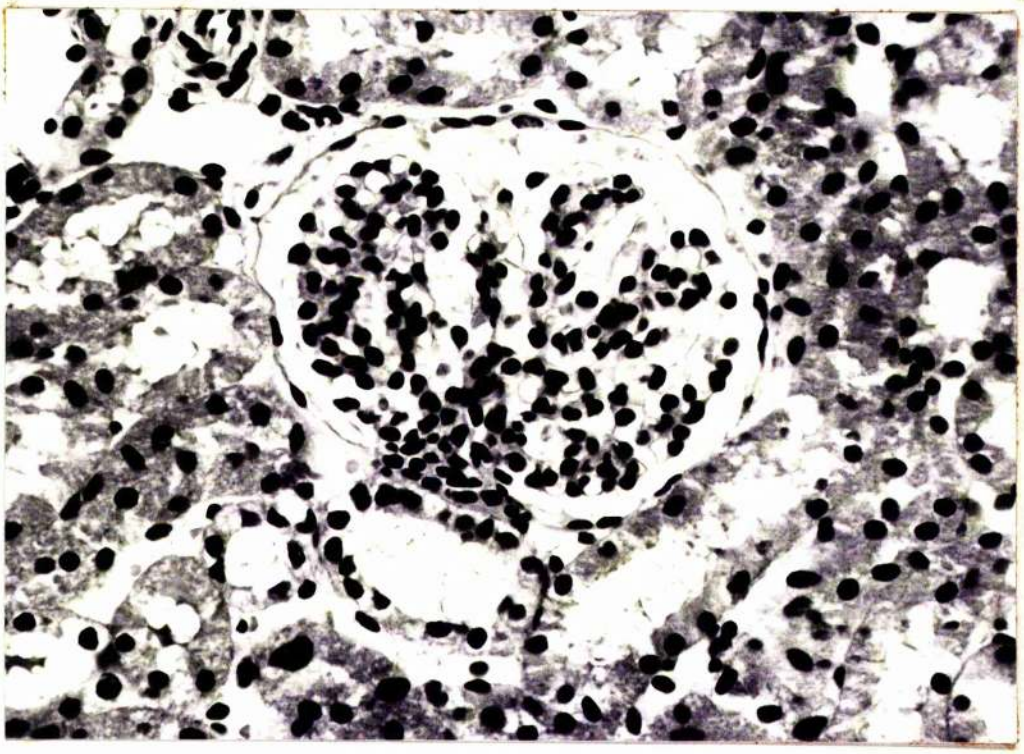


Fig. 11 CIN, Case 3

9 obsolescent glomeruli of the "contracted" type are shown. The tufts are reduced to collapsed, shrunken masses of GBM and mesangial matrix, and patent capillary loops are virtually absent. The capsular basement membranes (CBM) have collapsed around the tufts, and in most instances are disintegrating.

(PAS x 110)

Fig. 12 CIN, Case 11

Obsolescent glomeruli of the "cystic" type are present (*). Bowman's capsule remains dilated whilst the tuft collapses. Note also the cystic dilation of certain tubules (arrow).

(H & E x 35).

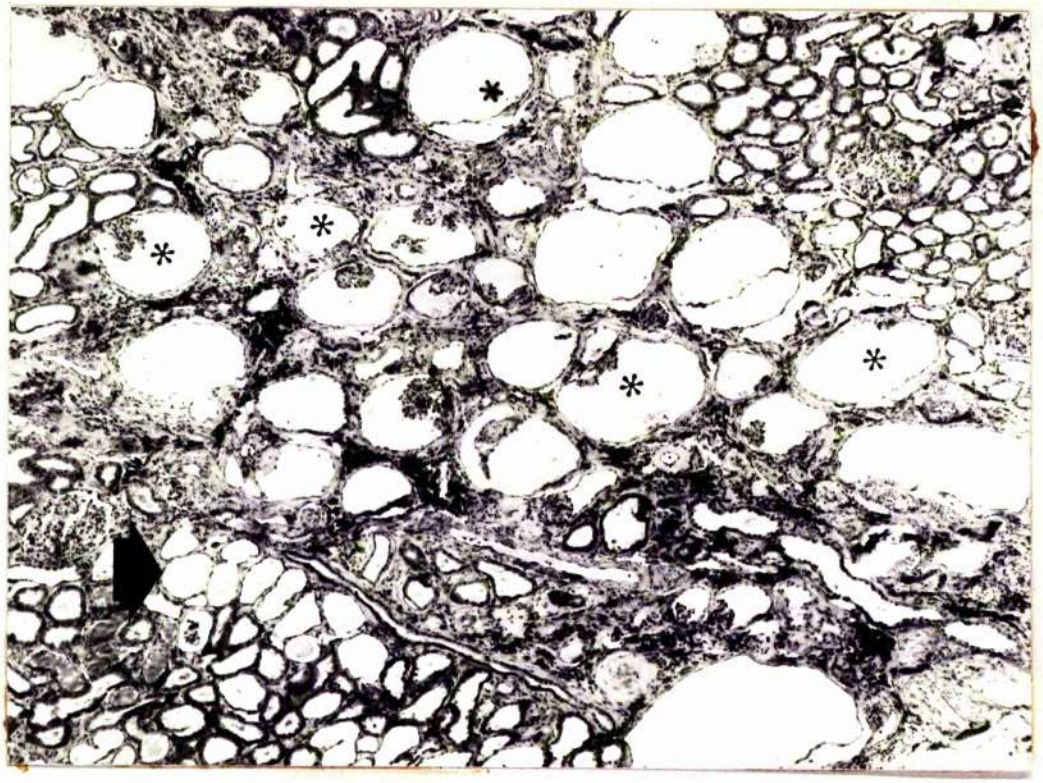


Fig. 13 CIN, Case 22

Glomerulus with < 50% of the tuft scarred obliterated. Some glomerular basement membranes (GBMs) are irregularly thickened (open arrow) and there is localized mesangial expansion. A capsular adhesion is also present (shaded arrow).

(PAS x 250).

Fig. 14 CIN, Case 21

Glomerulus with > 50% of the tuft scarred. All GBMs are thickened and there is widespread areas of mesangial expansion. Note that one part of the tuft has been completely obliterated by the scarring process, and is reduced to a hypocellular mass of PAS positive material (*). The CBM is also irregularly thickened and duplicated (arrow).

(PAS x 250)

Fig. 15 CIN, Case 29

Scarring in this glomerulus involves the global thickening, wrinkling and collapse of the GBMs. A reduced number of distorted capillaries remain giving the tuft a "simplified" appearance.

(PAS x 250)

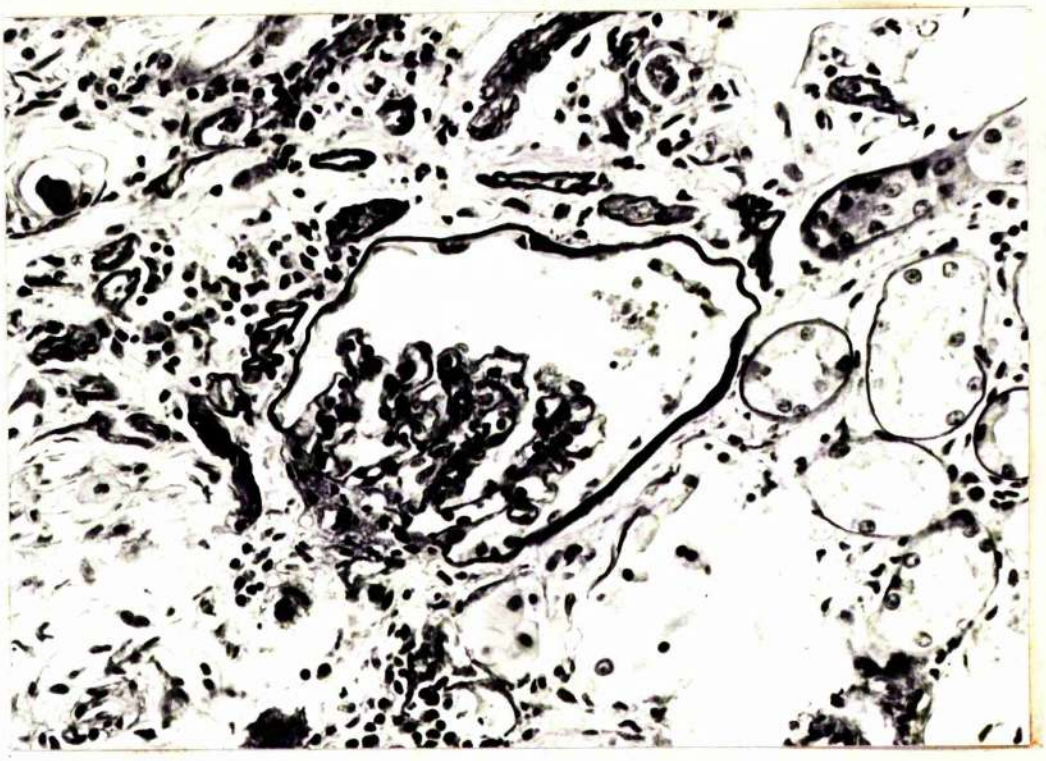


Fig. 16 CIN, Case 3

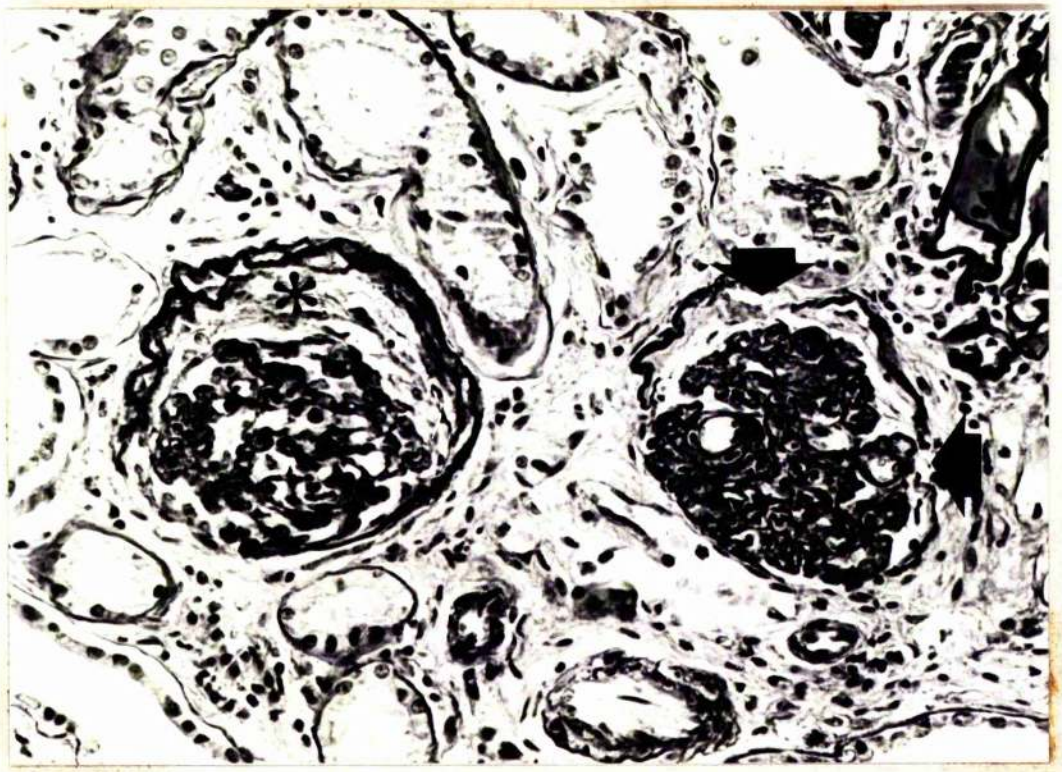
Two obsolescent, 100% scarred glomeruli are seen; the tufts are reduced to a knot of GBM and mesangial matrix and only a few capillary loops remain open. The CBMs, collapsed around the tufts, are irregularly thickened and duplicated and are starting to disintegrate (arrows). The remains of the urinary space are filled with faintly PAS positive material (*).

(PAS x 250)

Fig. 17 CIN, Case 15

Obsolescent glomeruli appear to progressively shrink and disintegrate. This glomerulus is very small and only a small segment of the CBM can still be clearly distinguished (arrow). An arteriole-like structure (*) appears to be persisting in the tuft.

(PAS x 400)



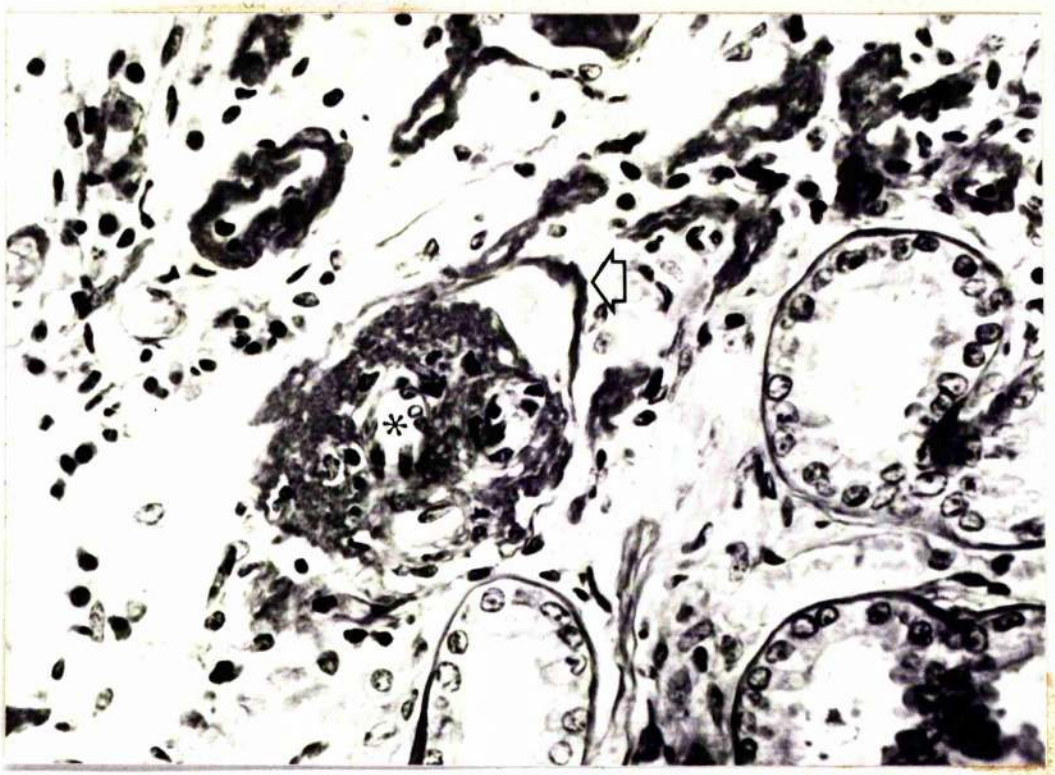


Fig. 18 CIN, Case 29

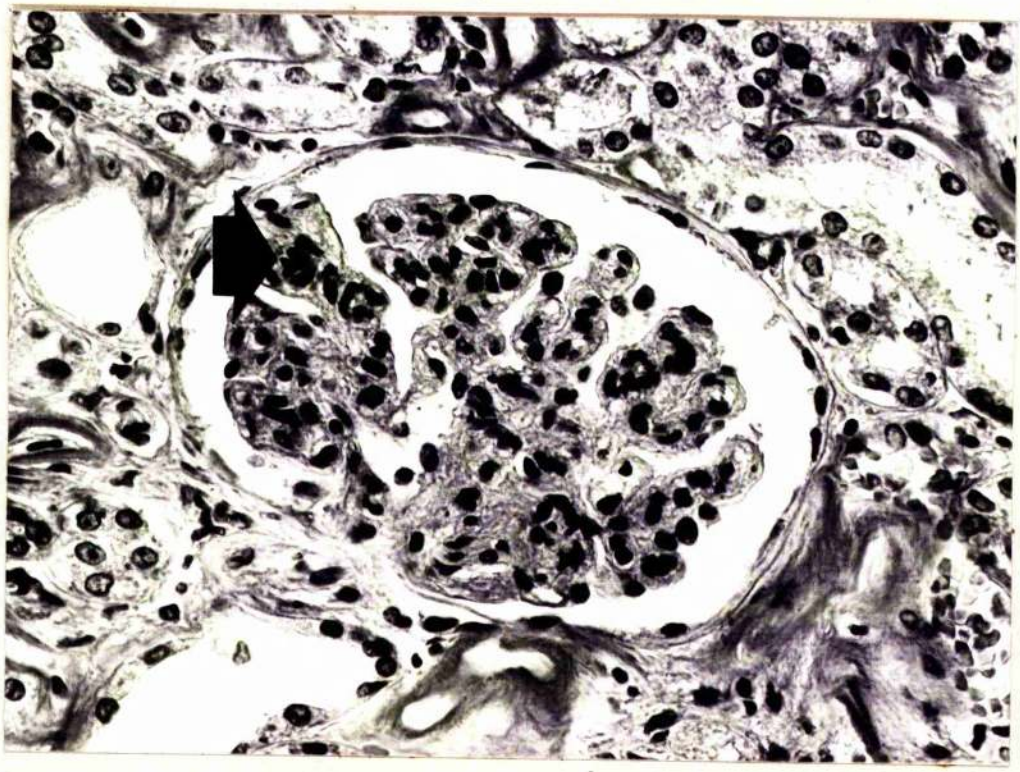
Global mesangial expansion and local hypercellularity (arrow) are present in this glomerulus.

(MSB x 400)

Fig. 19 CIN, Case 16

Global mesangial expansion and hypercellularity are present accompanied by the widespread formation of capsular adhesions.

(H & E x 250)



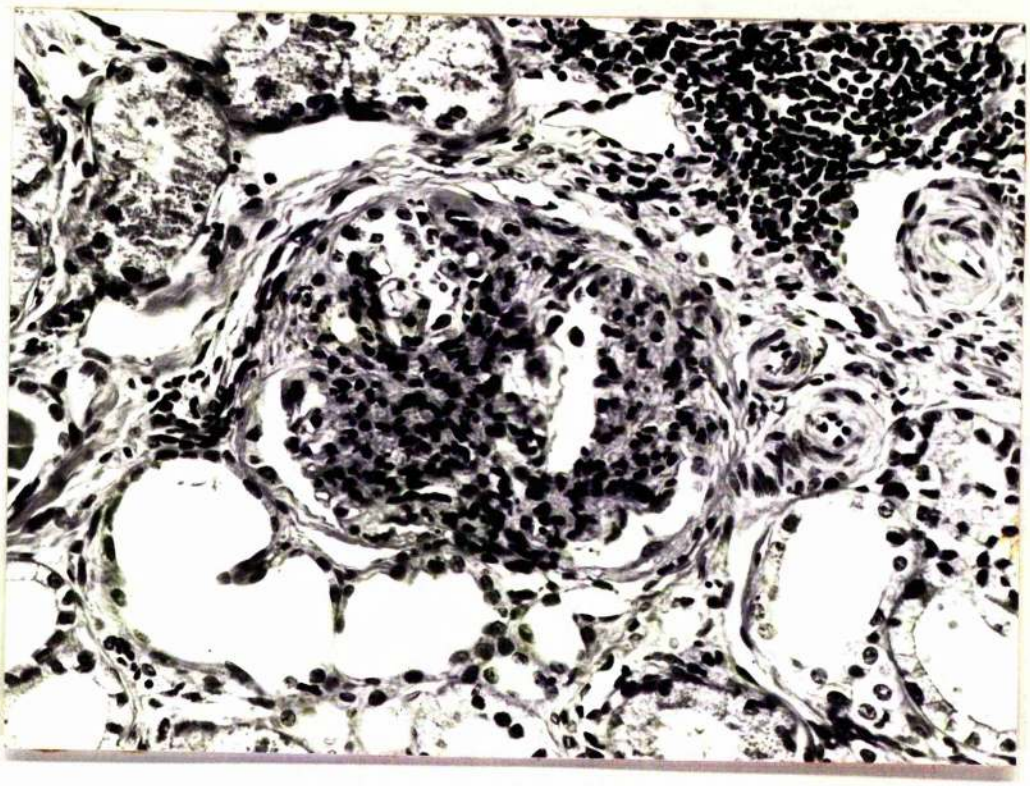


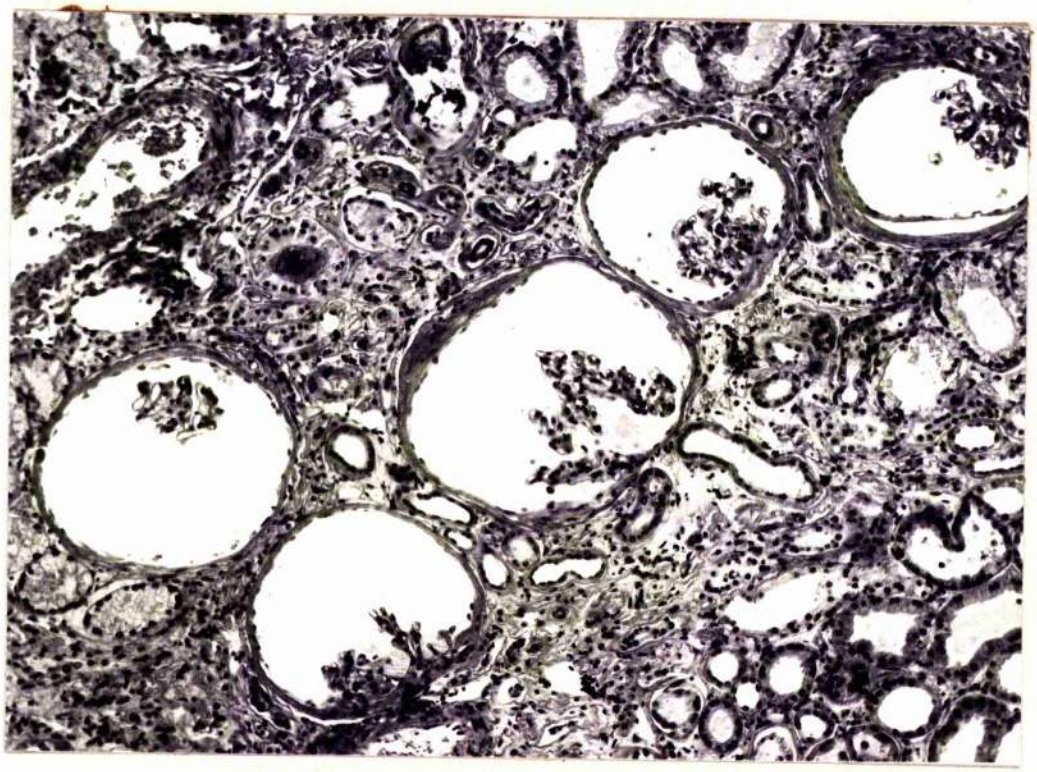
Fig. 20 CIN, Case 25

The CBMs of these 5 glomeruli are all thickened. Note that this thickening is not accompanied by any increase in cellularity of the CBM or parietal epithelium.

Fig. 21 CIN, Case 14

A typical pattern of fibrin deposition (red material). Most fibrin is present as globular deposits in peripheral capillaries associated with capsular adhesions. Note that most deposits are surrounded by a pale staining zone before merging into areas of collagen (blue) staining material.

(MSB x 400)



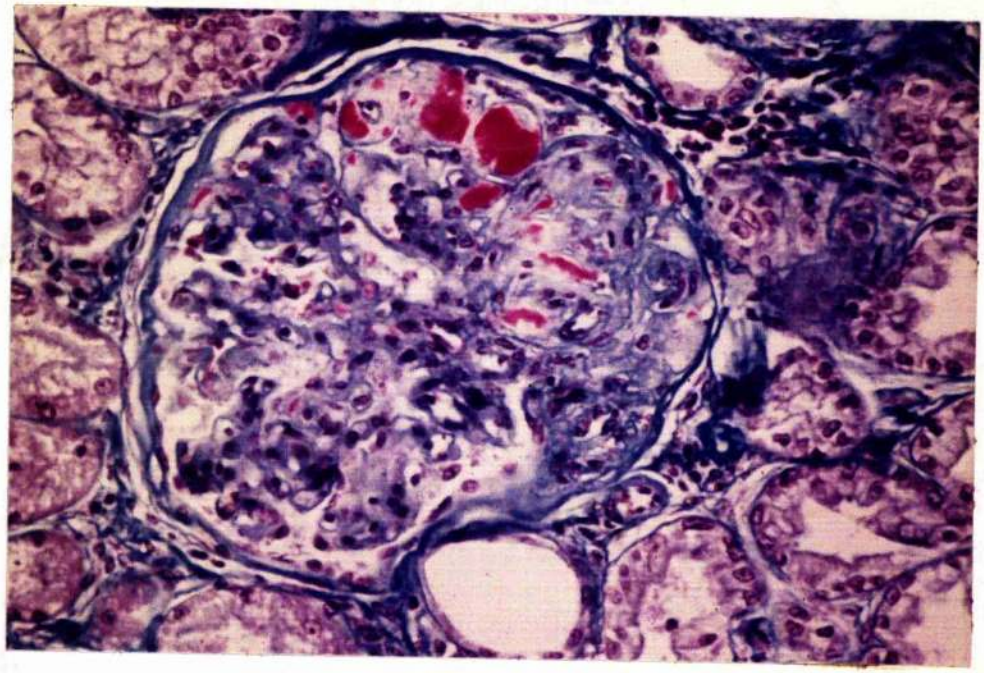


Fig. 22 Normal Canine Glomerulus, Case 83

4 capillary loops (C) are shown in this field, all of which contain a fine granular precipitate of plasma. Capillaries are separated from each other by the mesangium which is composed of mesangial cells (M) and matrix (m). The capillary walls are comprised of an inner layer of fenestrated endothelium, a continuous basement membrane and an outer layer of epithelium. The basement membrane is usually of even thickness but irregularities (*) can be present near the mesangial/capillary border. The epithelial cytoplasm forms distinct bulb shaped foot processes that rest on the basement membrane (open arrow), but due to plane of section much broader segments of cytoplasm may be seen (shaded arrow). E. Endothelial Cell, Ep. Epithelial Cell, U. Urinary space.

(Electron microscopy x 10,000)

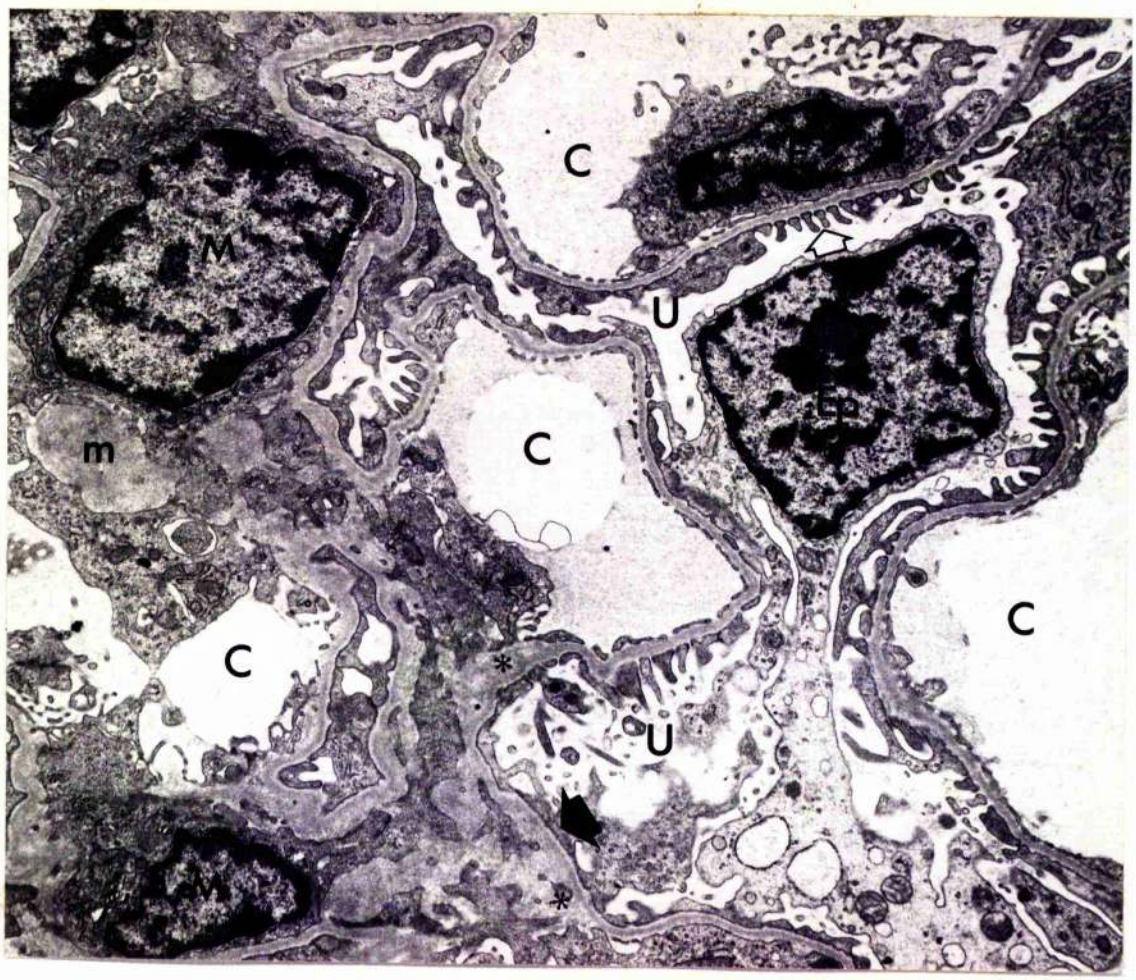

C
M
U
m
C
C
C
U
M

Fig. 23 CIN, Case 23

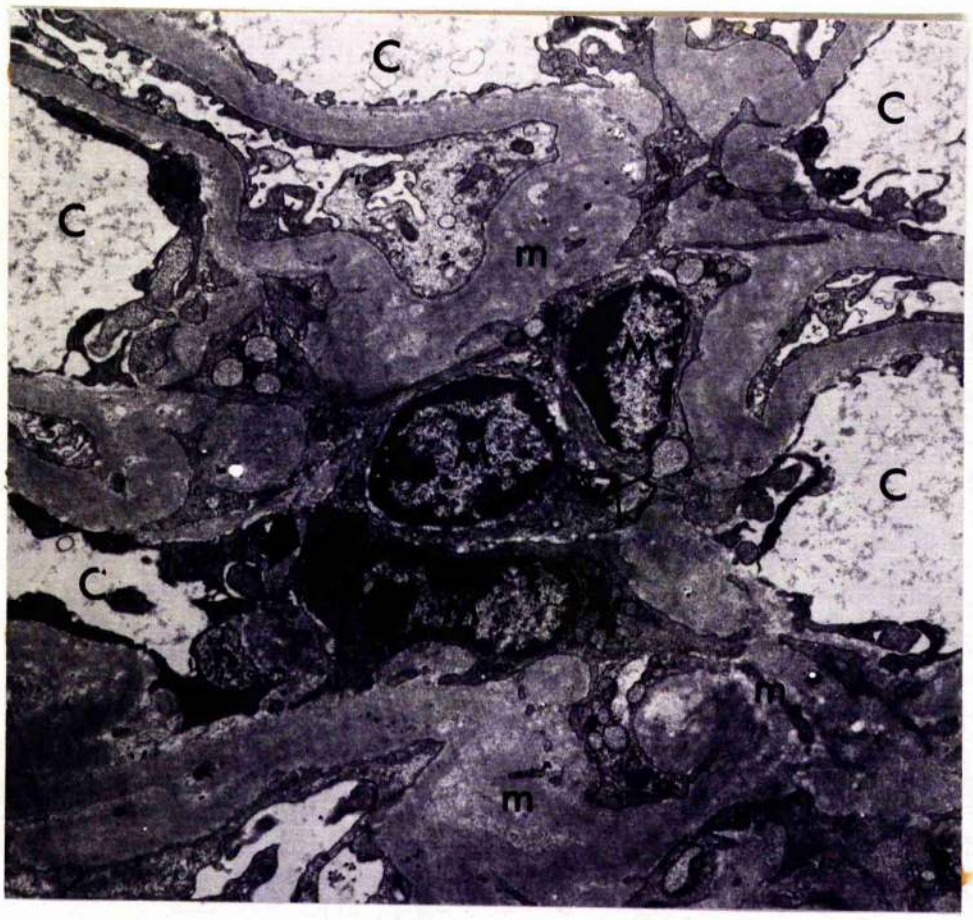
Three mesangial cells (M) are present in one area. The cells show signs of degeneration with myelin bodies (open arrow), vacuoles and dense lysosome-like bodies (shaded arrow) present. Excess mesangial matrix (m) is also present. C. capillaries.

(Electron microscopy x 10,000)

Fig. 24 CIN, Case 23

A capillary loop (C) is narrowed due to a combination of axial (arrow) and circumferential expansion of 2 mesangial cells (M) and matrix (m).

(Electron microscopy x 20,000)


C
C
C
m
M
C
C
C
m
m


C
M
m
M

Fig. 25 CIN, Case 24

The glomerular basement membrane (GBM) of this capillary (C) is irregularly thickened and a clear distinction of the lamina densa and lamina rara interna cannot be made. Epithelial cell foot processes (*) are fused indicating a glomerular protein leak. U. Urinary space.

(Electron microscopy x 30,000)


U
G
B
M
C

Fig. 26 CIN, Case 28

Thickening and vacuolation of the glomerular basement membranes (GBM) are very prominent in these three capillaries (C). Note also that the epithelial covering is reduced to a continuous, dark, atrophic layer (arrow), U. Urinary space.

(Electron microscopy x 10,000)


C
U
C
GBM
U

Fig. 27 CIN, Case 28

Part of an obsolescent lobule from the same glomerulus shown in Fig. 26. The capillary lumina (C) are completely obliterated by vacuolated, granular basement membrane-like material, surrounded by a collapsed, wrinkled lamina densa. Endothelial and mesangial cells are absent but distorted epithelial cells (Ep) still remain. The epithelial cytoplasm no longer rests on the GBM, the space between the 2 being filled with a fine granular and fibrillar material (*). The capsular basement membrane (CBM) is also thickened.

(Electron microscopy x 10,000)

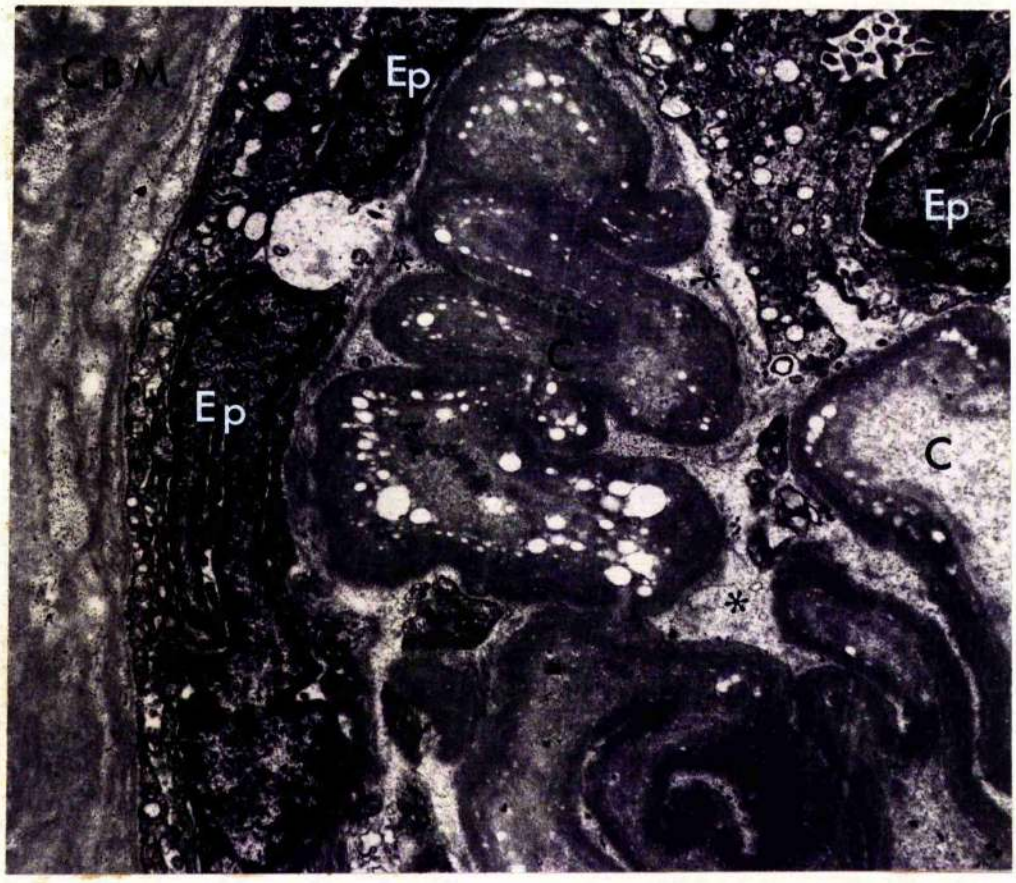

CBM
Ep
Ep
Ep
C
C
C

Fig. 28 CIN, Case 16

Ultrastructural appearance of a glomerulus similar to that shown in Fig. 16. The tuft is reduced to a collapsed, thickened GBM which encloses pale, granular and fibrillar material. A distinct mesangial region can no longer be distinguished but occasional strands of atrophic mesangial cytoplasm (M) can be seen. Collagen fibres are seen in the urinary space (open arrow) and occasionally in the tuft remnants (shaded arrow). Atrophic epithelial cells, containing many dense bodies, are also present (Ep).

(Electron microscopy x 15,000)

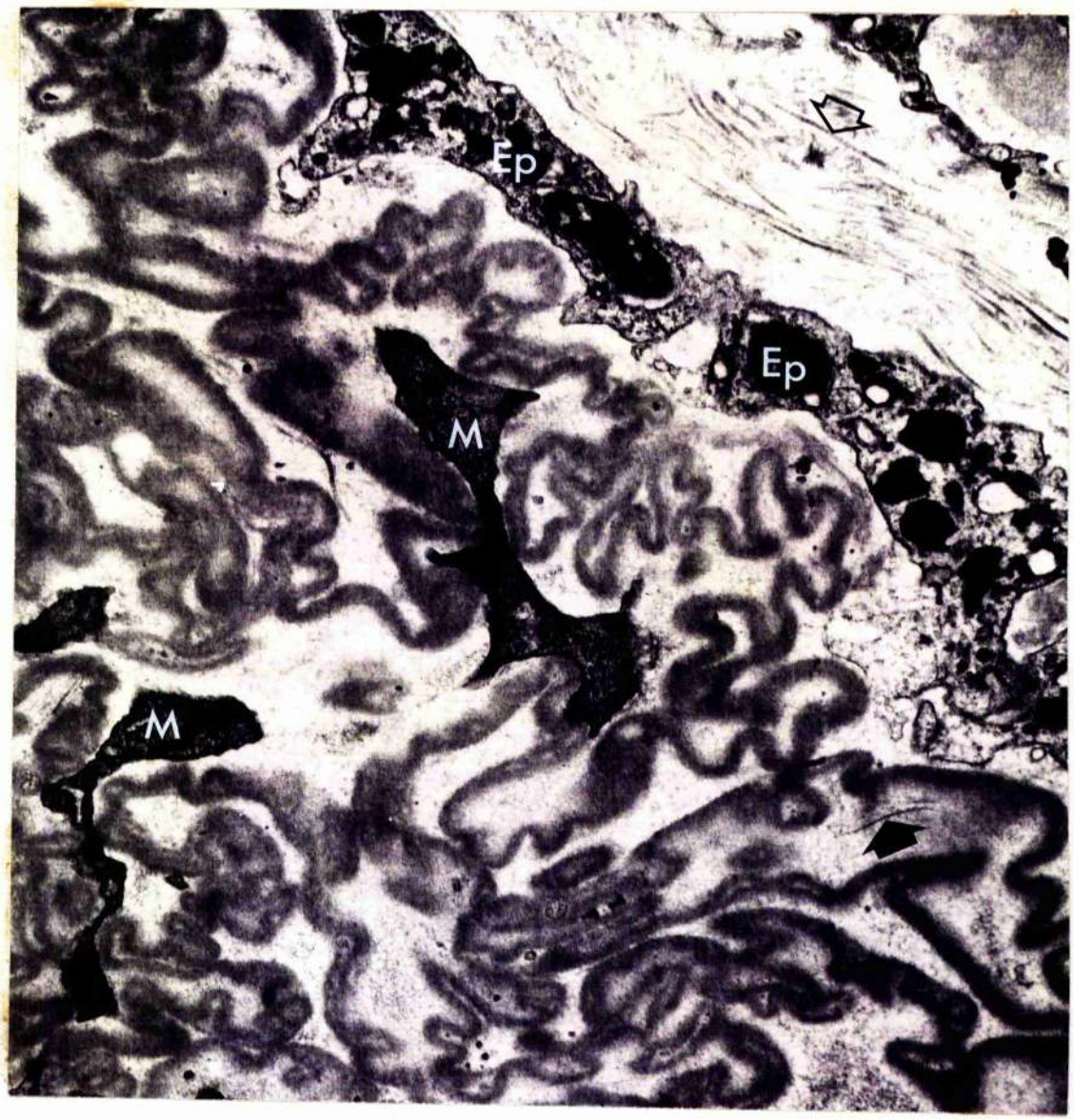

Ep
Ep
M
M

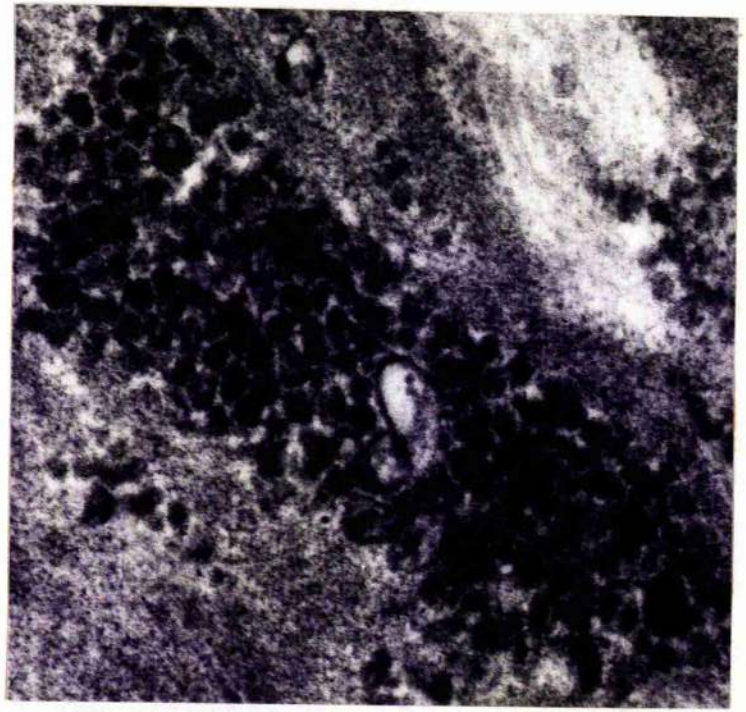
Fig. 29(a) CIN, Case 24

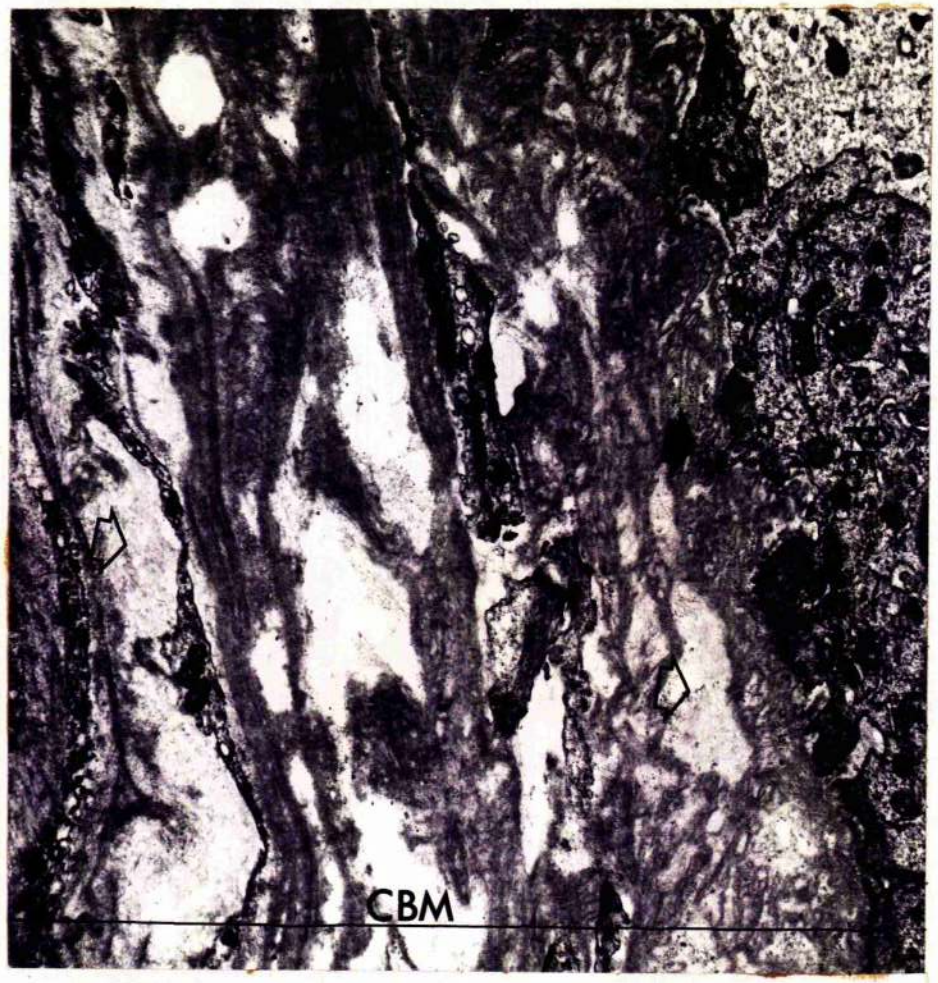
A typical thickened capsular basement membrane (CBM) is shown. Irregular dark strands of basement membrane lie in a haphazard arrangement interspersed with occasional strands of atrophic fibrillar cytoplasm and paler granular and fibrillar material (open arrow). Collections of dense granules (shaded arrow) are also seen. Pep. Parietal epithelial cell.

(Electron microscopy x 10,000)

Fig. 29(b) High power electron micrograph of the dense granules in the CBM. No obvious structural organization is visible. It is possible that these are small deposits of calcium salts.

(Electron microcsopy x 80,000)

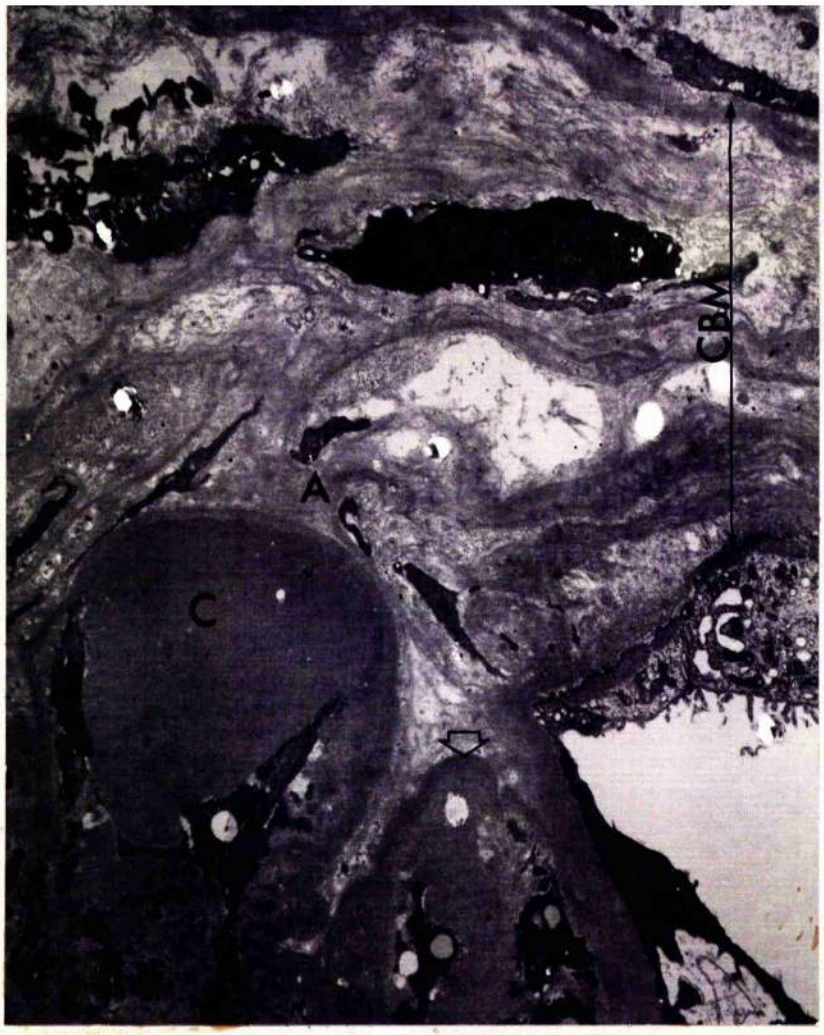



CBM

Figs.30,31 CIN, Case 24

Two areas from one glomerulus are shown. In both a capsular adhesion (A) formed by strands of CBM and other fibrillar elements is present. The capillaries (C) involved are engorged with a dense granular material obliterating everything except occasional strands of atrophic cytoplasm and the surrounding remains of the GBM (arrow). The position of this material in a peripheral capillary associated with a capsular adhesion suggests it is or is derived from fibrin. M. mesangial cell, E. Endothelial cell, CBM capsular basement membrane.

(Electron microscopy x 6,000)


CBM
A
C


A
M
E

Fig. 32 CIN, Case 23

Two capillaries (C_2, C_2) have been completely obliterated and the wall of another (C_1) thickened by a mass of predominantly pale granular material. Dense granular and fibrillar elements are also present (shaded arrow) as are occasional banded fibrils (open arrow). This type of appearance has been reported to be a feature of nephropathies where active deposition and lysis of fibrin is occurring (Kincaid-Smith 1972). Endothelium can no longer be identified in capillaries C_2 and C_3, while in C_1 the endothelial cell (E) is dark and atrophic and appears to be involved in the phagocytosis of granular material(*). Mesangial cells (M) are seen to be invading these areas. Ep. Epithelial cell.

(Electron microscopy x 10,000)


C1
E
*
M
C3
M
C2
M

Fig. 33 CIN, Case 23

High power electron micrograph of an area similar to that shown in Fig. 32. Dense granular material (shaded arrow) and faint banded fibrils (open arrow) are clearly seen at this magnification. The nature of these fibrils was not determined. Segments of mesangial cells (M) identifiable by the fibrillar cytoplasm are again present.

(Electron microscopy x 30,000)

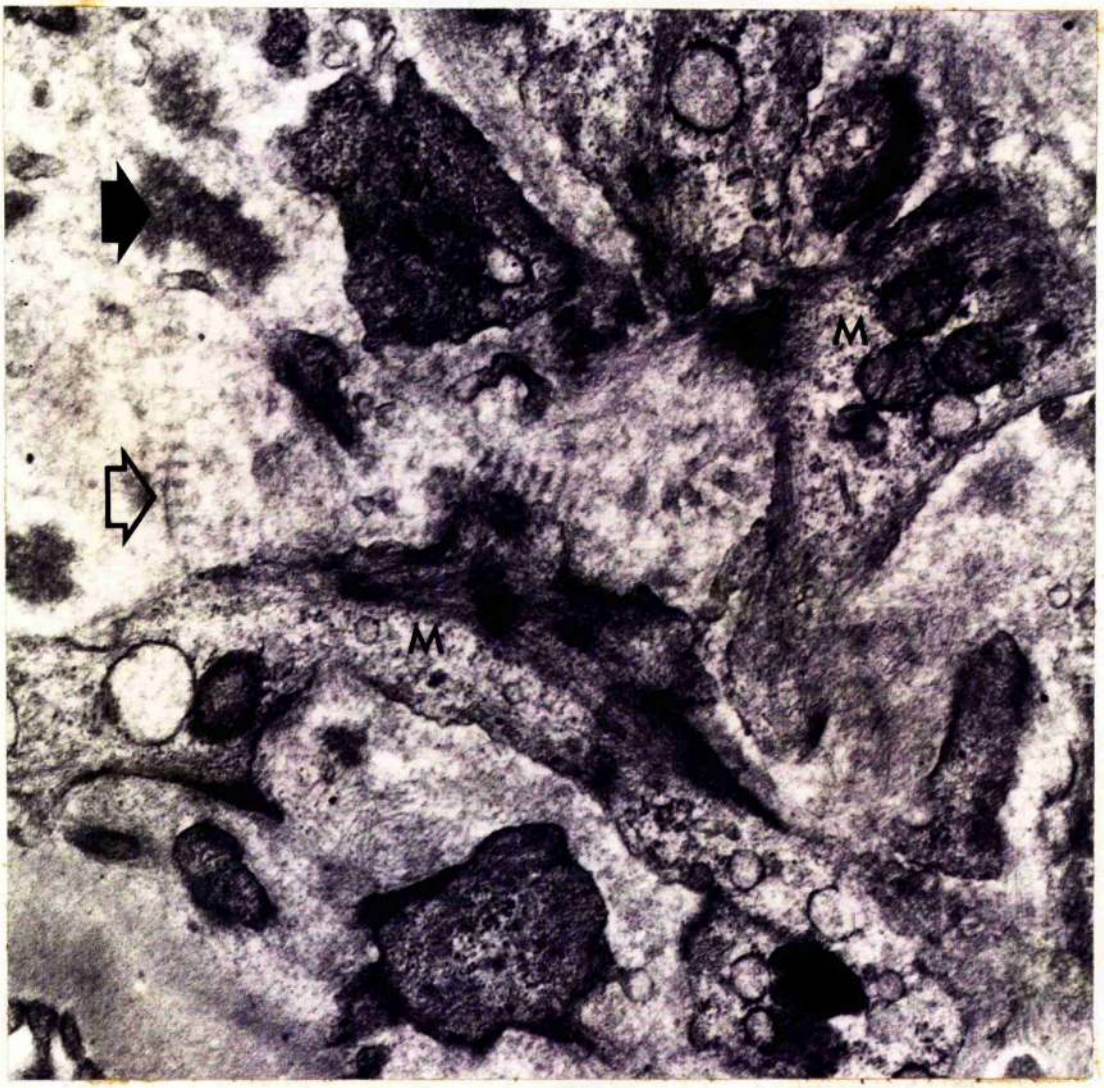

M
M

Fig. 34 CIN, Case 11

An obsolescent tubule is shown. Atrophic tubular epithelial cells (TEp) remain surrounded by a mass of thickened, wrinkled basement membrane. Many small dense granules, similar to those present in the CBM (Fig. 29), are present in basement membrane.
L. Interstitial Lymphocyte.

(Electron microscopy x 6,000)

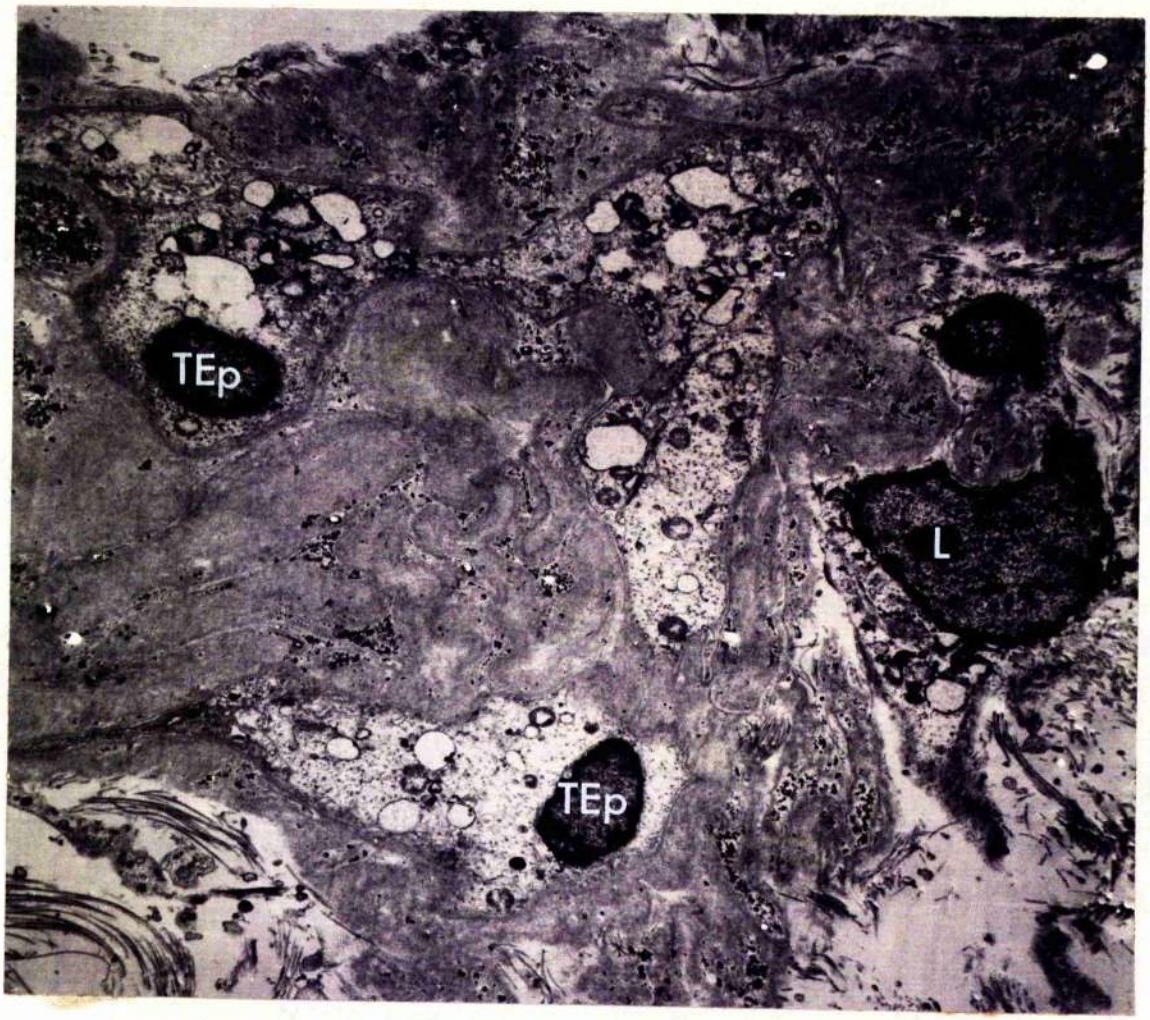

TEp
L
TEp

Fig. 35 CIN, Case 24

Large amounts of fibrin are present in this glomerulus concentrated particularly in the peripheral capillaries.

(Immunofluorescence x 450).

Fig. 36 CIN, Case 16

Globules of complement (C_3) are present in the peripheral capillaries. Fibrin was identified in the same positions suggesting non-specific trapping of C_3 in areas of tissue damage. In addition, occasional granules of C_3 are present elsewhere in the tuft. This, however, is minimal compared to the global deposition typical of chronic glomerulonephritis (Fig.44)

(Immunofluorescence x 300)


3

Fig. 37 CIN, Case 24

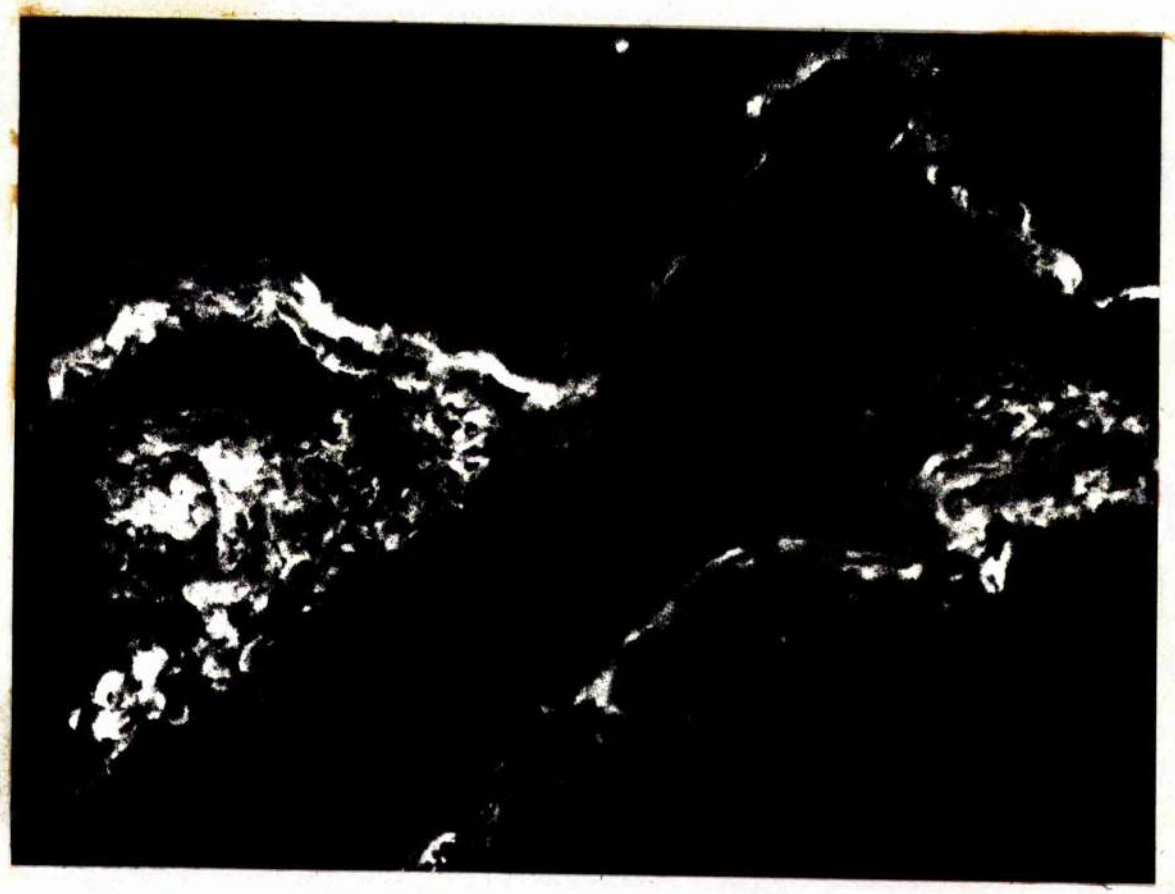
Fibrin is present in the tunica intima and media of an interlobular artery.

(Immunofluorescence x 300)

Fig. 38 CIN, Case 23

Irregular linear staining for C_3 is present on these tubular basement membranes. The significance of this was not ascertained in this study.

(Immunofluorescence x 450)



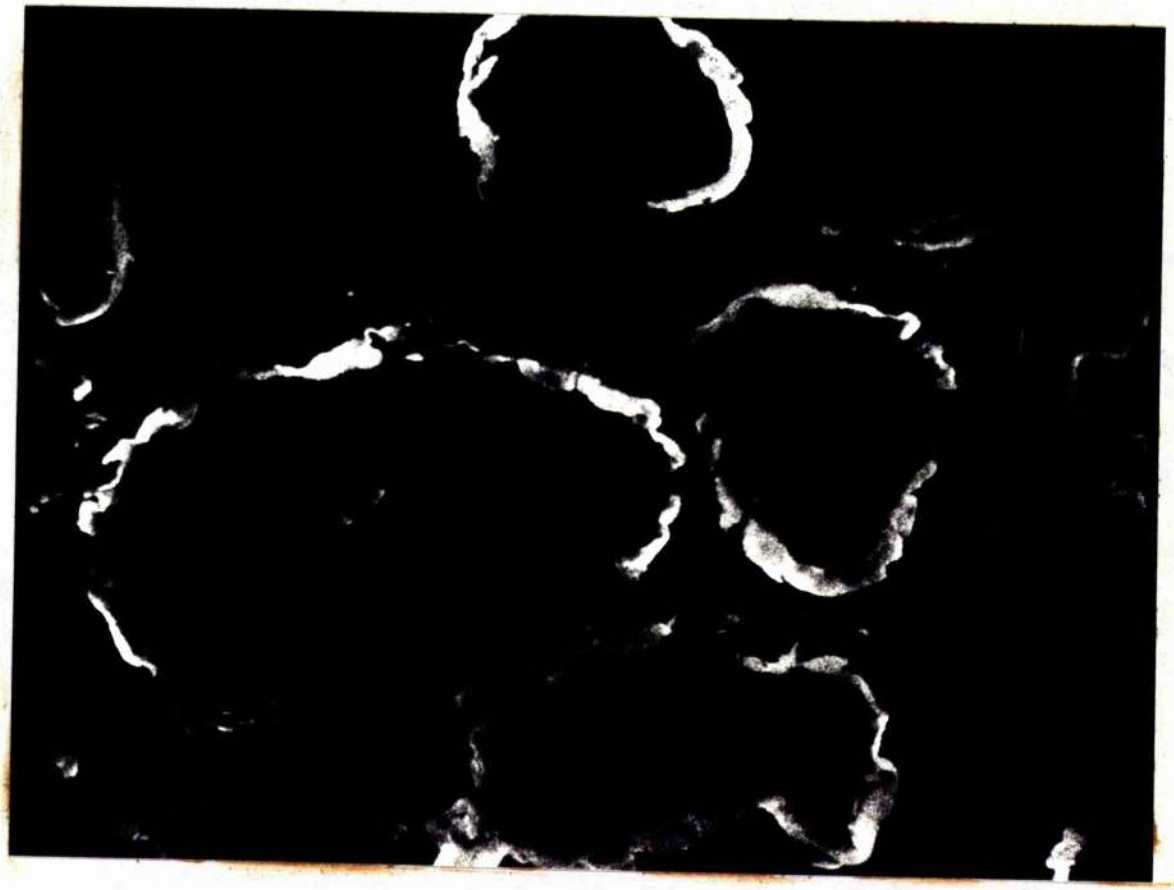


TABLE 13

CHRONIC GLOMERULONEPHRITIS (CGN): CLINICAL FINDINGS

CASE	THIRST	ANOREXIA	WEIGHT LOSS	VOMITING	HALITOSIS	ORAL ULCERATION	MUCOSAE	INELASTIC PULSE
31	+	+	NR	NR	NR	-	NR	NR
32	+	+	NR	NR	NR	-	NR	NR
33	+	+	+	Occasional	+	+	NR	NR
34	NR	NR	NR	NR	+	+	Cyanotic	-
35	-	+	+	Occasional	-	+	Normal	+
36	+	-	NR	Occasional	+	+	Pale	-
37	+	+	NR	Occasional	NR	-	NR	NR
38	+	+	+	Frequent	+	+	Normal	+
39	-	-	+	Occasional	+	+	Normal	+
40	+	+	+	Occasional	+	+	Normal	-

TABLE 14

CGN: GENERAL INFORMATION, BIOCHEMICAL AND SEROLOGICAL DATA[A]

CASE	AGE Years	SEX	BREED	BLOOD UREA mmol.L^{-1}	URINE PROTEIN mg.100mls^{-1}	URINE UREA mmol.L^{-1}	SPECIFIC GRAVITY OF URINE	AGGLUTINATION LYSIS TITRE L.CANICOLA	AGGLUTINATION LYSIS TITRE L.ICTERO-HAEMORRHAGIAE
31	Adult	M	West Highland Terrier	NR	1104	265	1.029	NR	NR
32	Adult	F	Cairn Terrier	NR	NR	NR	NR	NR	NR
33	4½	F	Labrador	98	348	200	1.021	NR	NR
34	11	F	Corgi	157	207	303	1.021	-	-
35	4½	M	Dalmatian	92	1060	172	1.020	-	-
36	Adult	M	Boxer	18	33	167	1.029	1.300	-
37	10½	F	Crossbred	102	NR	NR	NR	NR	NR
38	8	F	Crossbred	62	767	290	1.019	-	-
39	7	F	Dalmatian	75	292	233	1.020	-	-
40	9	F	Shetland Sheepdog	46	NR	NR	NR	-	-

A value of samples taken nearest to time of death
NR Not Recorded
M male
F female

TABLE 15

CGN: EXTRA-RENAL PATHOLOGY

CASE	ORAL ULCER-ATION	GASTRITIS	INTERCOSTAL MYOSITIS/ CALCIFICATION	NECROTIZING ENDOCARDITIS	LEFT VENTRICULAR HYPERTROPHY	PARATHYROID HYPERPLASIA	OTHER LESIONS
31	-	-	-	-	-	-	
32	-	-	-	-	-	-	
33	+	-	-	-	-	-	
34	+	+	+	-	-	-	
35	+	+	-	-	+	-	
36	+	+	-	-	-	-	Intestinal ulceration Urolithiasis Pancreatic hyperplasia
37	-	-	-	-	-	-	Pyometra Endocardosis
38	+	+	-	-	-	-	
39	+	-	-	-	-	-	
40	+	+	-	-	+	+	Enteritis Endocardosis Healed rib fractures

TABLE 16 CGN: RENAL PATHOLOGY

CASE	RENAL FIBROSIS					ARTERIES AND ARTERIOLES				TUBULES			OTHERS	
	DEGREE	PATTERN			CORTEX WIDTH	SPIRALLING	MEDIAL HYPERTROPHY	PLASMATIC VASCULOSIS		ATROPHY	CYSTIC DILATION	CASTS	CELLULAR INFILTRATE	CALCIFICATION
		DIFFUSE	DEEP CORTICAL SCARS	RADIAL CORTICAL SCARS				FIBRIN STAINING	COLLAGEN STAINING					
31	2+	Yes			N	-	-	1+	1+	2+	-	2+	1+	-
32	1+	Yes			N	-	-	-	1+	1+	-	3+	1+	-
33	2+	Yes			N	-	-	-	-	2+	1+	4+	1+	-
34	4+		Yes	Yes	R	1+	-	-	-	2+	4+	3+	2+ P	-
35	3+	Yes			N	-	-	-	-	3+	1+	1+	1+	2+
36	3+			Yes	R	-	-	1+	1+	1+	1+	2+	1+	-
37	2+	Yes			N	-	-	-	-	2+	-	1+	1+	-
38	2+	Yes			N	-	-	-	-	3+	-	1+	1+	2+
39	3+	Yes			N	2+	-	1+	-	2+	1+	2+	1+	1+
40	3+			Yes	R	1+	1+	3+	1+	3+	-	1+	1+	-

P Foci of polymorphonuclear leucocytes present

N Normal

R Reduced

TABLE 17

CGN: GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	< 50% SCARRING	≥ 50% SCARRING	100% SCARRING CONTRACTED	100% SCARRING CYSTIC
31	181	2.8	11.0	45.4	40.8	0
32	219	10.0	21.5	31.5	33.8	3.2
33	260	3.5	32.7	51.1	11.5	1.2
34	166	0	19.3	28.9	50.6	1.2
35	214	8.4	13.1	28.0	19.2	31.3
36	240	29.1	35.0	12.9	19.6	3.4
37	158	0.6	10.1	60.8	28.5	0
38	127	0	5.5	47.2	45.7	1.6
39	209	2.4	13.4	37.8	38.3	8.1
40	395	0	10.6	32.7	56.7	0

TABLE 18

CGN: GLOMERULAR MORPHOLOGY

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED											
		FIBRIN DEPOSITS[A]				HYPERCELLULARITY			GBM THICKENING			CAPSULAR	CAPS
		TOTAL[B]	URINARY SPACE	CAPILLARY	ADHESIONS	TOTAL[B]	LOCAL	GLOBAL	TOTAL[B]	LOCAL	GLOBAL	ADHESIONS	THI ENI
31	108	81.5	20.4	31.5	29.6	17.6	14.8	2.8	100	1.8	98.2	84.3	96.
32	139	26.6	0	7.9	18.7	14.4	11.5	2.9	79.1	35.2	43.9	69.8	72.
33	218	14.7	0	3.7	11.0	6.0	5.5	0.5	76.6	50.0	26.6	78.9	83.
34	86	74.4	2.3	45.4	26.7	16.3	12.8	3.5	94.2	23.3	70.9	95.3	93.
35	103	33.0	1.9	13.6	17.5	15.6	14.6	1.0	78.6	53.4	25.2	76.7	79.
36	186	21.0	1.6	12.9	6.5	8.6	7.0	1.6	52.7	33.9	18.8	41.4	60.
37	115	74.8	2.6	51.3	20.9	16.5	16.5	0	94.8	22.6	72.2	96.7	88.
38	59	76.3	5.1	39.0	32.2	18.6	18.6	0	96.6	18.6	78.0	96.6	100
39	108	48.1	4.6	18.5	25.0	26.9	20.4	6.5	98.2	20.4	77.8	91.7	96.
40	185	64.3	0.5	18.4	45.4	10.8	10.8	0	96.2	35.1	61.1	88.1	79.

A Position of largest deposit
B Total percentage of glomeruli affected
GBM Glomerular basement membrane.

RESULTS 2. CHRONIC GLOMERULONEPHRITIS (CGN)

All 10 dogs had a history and clinical signs of chronic renal failure similar to those of the cases of CIN (Table 13). The details of age, sex, and breed, with pertinent biochemical and serological findings, are given in Table 14. All animals were adults with females more commonly affected than males (7 to 3). No breed susceptibility was present.

Gross Pathology

1) Extra-renal lesions (Table 15)

The most common lesions were those of uraemia, particularly oral ulceration (7 cases) and gastritis (5 cases). Calcification and necrosis of the intercostal muscles and parietal pleura was only seen in one case, while necrotizing endocarditis was never seen. Such lesions were similar in character to those seen in cases of CIN. Gross hypertrophy of the left ventricle was found in two animals. Only one case had parathyroid hyperplasia but osteodystrophia fibrosa ("rubber jaw") was never seen.

2) Renal lesions

In all cases the kidneys were scarred. Typically they were normal in size, firm and pale with a finely granular surface. The cortices were usually normal in width but in 2 cases they were narrow (Table 16). Cysts were rarely seen. Case 34, however, was atypical. Again the kidneys were firm and pale but, in addition, they were cystic and irregular, with variable narrowing of the cortices.

Light Microscopy

The histopathological findings excluding glomerular lesions are summarized in Table 16. Except for case 34, which will be described separately, all cases were similar to the "atypical" form of CIN (cases 25-30). Thus significant but relatively mild scarring was present, with fine strands of fibrous tissue scattered diffusely (7 cases) or in radial streaks (2 cases) through both cortex and medulla. There was no particular concentration of scarring around the cortico-medullary junction. Small foci of plasma cells, lymphocytes and macrophages were scattered through the cortical interstitium.

Both tubular hypertrophy and atrophy were less marked compared to CIN cases. In addition, cystic distension of the collecting ducts was also much reduced, and hyperplasia of the lining epithelia was present in only 2 cases (35,36) and then limited to just a few ducts. Both hyaline and granular tubular casts were always present.

Lesions of the renal vasculature were less prominent than in cases of CIN but were similar in type. Interlobular arteries and afferent arterioles were affected. Plasmatic vasculosis was the most common lesion, present in 5 animals. Like CIN only a few vessels per section were affected. Reflecting the less severe renal scarring, only in 2 cases was there any twisting and spiralling of the arteries. Thickening of the arteries and arterioles due to medial hypertrophy and advential hyperplasia, was present in 1 case only.

Corresponding to its different gross appearance, the

histopathology of case 34 was also atypical. Renal scarring was more severe, with large focal areas of fibrosis around the cortico-medullary junction and radial scars in the cortex. In addition, cystic distension of the collecting ducts with epithelial hyperplasia was very prominent. The most significant difference was the presence of a more extensive cellular infiltrate, with several foci containing large numbers of polymorphonuclear leucocytes (Fig. 39). Such a picture indicates chronic pyelonephritis to be present but because of the immuno-fluorescence findings (see below) it was included in this series.

Glomerular Lesions (Tables 17,18)

Glomerular scarring was the most prominent histological feature in CGN (Fig. 40). The details of the process were very similar to those noted in CIN. The tufts were progressively obliterated by expansion of the mesangium, and thickening, wrinkling and duplication of the GBMs. In the early stages of scarring local or global mesangial hypercellularity was present, but as the process advanced the tuft became hypocellular with a loss of all types of glomerular cell. Changes in Bowman's capsule and the parietal epithelium were also identical to those seen in CIN, involving thickening and duplication of the CBM and hypertrophy of the cells.

One major difference between CGN and CIN did emerge; in general the degree of fibrin deposition was much greater in the former. Not only were more glomeruli usually affected (compare Tables 9 and 18) but deposits in an

individual glomerulus tended to be larger and more often multiple (Fig. 41). The pattern of deposition, however, was the same; all parts of the glomerulus could be affected, but most fibrin was present in the peripheral capillaries and associated with capsular adhesions. Consequently capsular adhesions were a very prominent feature of CGN. Reflecting the greater severity of fibrin deposition, crescent shaped lakes of fibrin, apparently unassociated with capsular adhesions, were more commonly present in the urinary spaces than in cases of CIN.

As in CIN where there were focal radial scars in the cortex (cases 34, 36, 40), the glomeruli were often severely scarred and reduced to obsolescent collapsed masses, while outwith these areas glomerular damage was less severe. In the other cases, where there was diffuse fibrosis of the cortex, no focal variations in the severity of glomerular scarring were seen.

The glomeruli of case 31 showed an additional feature. All GBMs were markedly thickened, a change generally considered typical of membranous nephropathy (Fig. 42). However, as glomerular scarring and fibrin deposition were also widespread in this case, it was classified as CGN.

Electron Microscopy

Tissue from only one case (39) was examined with the electron microscope (Fig. 49). Many features of the glomerular morphology in this case were similar to those already described in CIN, viz: obliteration of the tuft by excess mesangial matrix and GBM, derangement and atrophy of

glomerular cells, and thickening and duplication of the CBM. However, one major difference did exist, mesangial expansion and GBM thickening were associated with the presence of many electron dense granular deposits of varying sizes. Most deposits were present in the GBM, where they took up an intramembranous or less often a subepithelial position. A comparison of deposits in this latter position suggested that new GBM material progressively built up around and eventually encircled them. Thus, these deposits came to lie more in an intramembranous position in a thickened irregular GBM. Some intramembranous and mesangial deposits had ragged borders with an electron translucent halo suggesting their degradation and removal. The pattern of deposits corresponded to the granules of immunoglobulin and complement identified by immunofluorescence (see below). Along these altered GBMs there was almost total "fusion" of the foot processes indicating a glomerular protein leak.

In some capillaries floccules composed of fine granular and fibrillar material were present in the lumen, in endothelial cell vacuoles and lying between the endothelium and GBM. Such masses were usually larger and slightly less dense than those embedded in the mesangium or GBM. Although an unequivocal identification as fibrin cannot be made (because fibres with the "characteristic" periodicity were never seen) it is possible that this material was the ultrastructural equivalent of the widespread deposits identified by light and immunofluorescence microscopy.

TABLE 19

CGN: IMMUNOFLUORESCENCE FINDINGS[1]

CASE	GLOMERULI				ARTERIES AND ARTERIOLES				TUBULES[2]	INTERSTITIUM[3]	
	IgG	IgM	C3	FIBRIN	IgG	IgM	C3	FIBRIN	C3	IgG	IgM
31	+	NR	+	+	-	NR	-	-	-	-	-
32	+	NR	+	+	+	NR	-	+	-	-	-
33	+	NR	+	+	-	NR	-	-	-	-	-
34	+	+	+	+	-	+	+	+	-	-	-
35	+	NR	+	+	-	NR	-	-	-	+	-
36	+	NR	+	+	+	NR	-	+	-	-	-
37	+	NR	+	+	+	NR	-	+	-	-	-
38	+	NR	+	+	-	NR	-	-	-	-	-
39	+	NR	+	+	+	NR	-	+	-	-	-
40	+	+	+	+	+	+	+	+	+	-	-

1. No CAV or L.canicola antigens found in any case.
2. No deposits of IgG, IgM or Fibrin found.
3. Plasma cells.

NR Not recorded.

TABLE 20

CGN: ELUTION STUDIES[1]

CASE	WEIGHT ELUTED g	VOLUME OF ELUATE ml	CONCENTRATION OF ELUATE $\mu g.ml^{-1}$	ANTI-L.CANICOLA ANTIBODIES	
				ELUATE	SERUM
31	16.5	4	2300	-	NR
33	10	11	410	-	NR
34	9.3	29	378	-	-
35	3.5	3	1100	1:100	-
38	10.0	5.5	625	-	-
40	15.5	22	410	-	NR

1 All eluates negative for anti-CAV, anti-kidney and anti-L. icterohaemorrhagiae antibodies.

Immunofluorescence Microscopy (Table 19)

1) Glomeruli

Immunofluorescence findings in the glomeruli proved to be the major diagnostic criteria in differentiating CGN from CIN.

In all 10 cases there was deposition of immunoglobulin with bound complement (C_3). In 9 cases granules and globules of these proteins were present in the capillary walls and mesangium, a pattern which is usually taken to indicate immune complex deposition (Fig. 44). In case 31, the pattern was different with linear deposits present along the capillary walls only, a pattern which suggests the deposition of anti-GBM antibody. Usually (8 cases) there was diffuse or segmental involvement but in 2 cases (33 and 40) it was focal with about 75% of the glomeruli affected. IgG was present in all cases and in 2 cases IgM was also found; in case 40 roughly equal amounts of each were present while in case 34 IgM was more extensive.

Fibrin deposits were seen in all cases, confirming the light microscopic picture. Large masses of fibrin were seen occluding capillaries, in capsular adhesions, and occasionally as crescentric lakes in the urinary spaces. In some instances, the large globules of immunoglobulin and complement were present in exactly the same positions as fibrin. Not surprisingly much more fibrin was seen than in the cases of CIN.

2) Arteries and arterioles

As in CIN fibrin was the most prominent deposit in the arterial and arteriolar walls. Again, it was present in

lakes in the intima and media beneath a broken internal elastic lamina and again, immunofluorescence and light microscopic findings correlated poorly. Three cases (32, 34, 37) were positive only with immunofluorescence and one (case 31) only with light microscopic stains. Only three cases (36, 39, 40) were positive with both. This apparent lack of correlation probably reflected the very focal nature of these lesions. Immunoglobulins (IgG and/or IgM) were present in the same areas in all 6 cases, and in 2 of these (cases 34, 40), C_3 was found as well.

3) Tubular deposits

Only in 1 case did the tubular basement membranes have a linear staining for C_3. IgG, IgM and fibrin were not found in this position in any case.

4) Interstitium

Only in 1 case were plasma cells identified in the interstitium.

5) Canine Adenovirus and L. canicola

Antigens of these two micro-organisms were not found in glomeruli, tubules or interstitium of any case.

Elution Studies (Table 20)

Elution studies were carried out on 6 cases. Only 1 eluate contained anti-L.canicola antibodies, at a low titre. Neither anti-L.icterohaemorrhagiae, anti-CAV nor anti-kidney antibodies were eluted from any case. No difference is apparent between CGN and the control group (Table 12).

Fig. 39 Chronic Glomerulonephritis (CGN), Case 34

Glomerulonephritis and pyelonephritis were both present in this case. Local hypercellularity and thickening of the mesangium are present in the glomeruli. The most prominent lesion is pyelonophritis; mononuclear cells are present in the interstitium and casts of polymorphonuclear leucocytes are seen in several tubules (shaded arrow).

(H & E x 110)

Fig. 40 CGN, Case 37

Global scarring is seen, which is characterized by mesangial expansion and the presence of eosinophilic globular deposits of fibrin (arrows) obliterating many of the capillaries. Many glomeruli in this case had a similar morphology.

(H & E x 250)

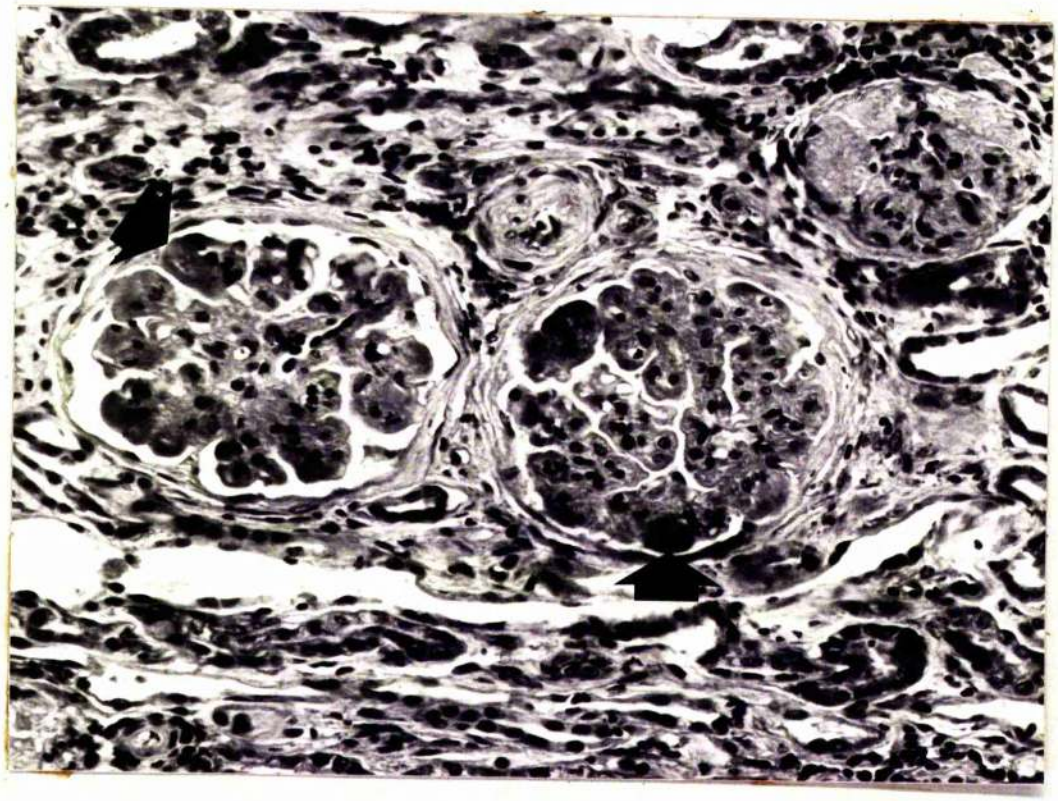


Fig. 41 CGN, Case 40

All 5 functional glomeruli in this field contain large globules of fibrin (staining black). Most fibrin is present in the peripheral capillaries associated with capsular adhesions.

(Obadiah x 110)

Fig. 42 CGN, Case 31

Global scarring, with $>$ 50% of the tuft obliterated, is present in these three glomeruli. In addition, this case was notable in having diffuse, marked thickening of the GBMs. This is best appreciated in the peripheral capillaries (arrows).

(PAS x 250)


5
2
3
4

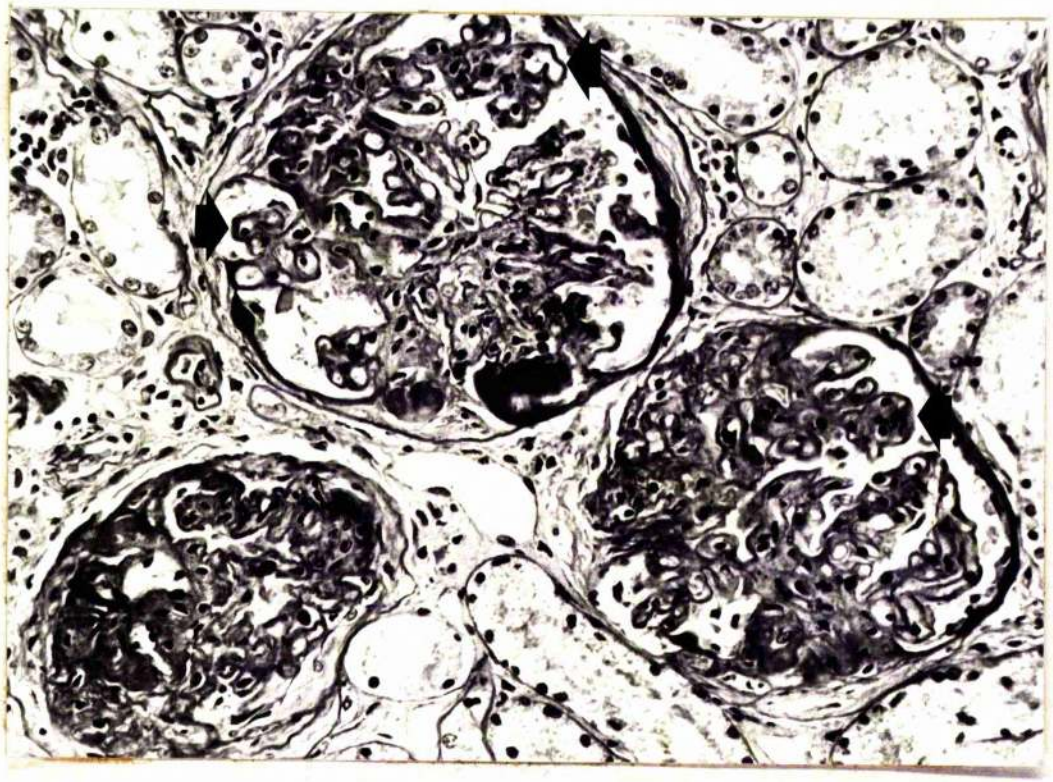


Fig. 43 CGN, Case 39

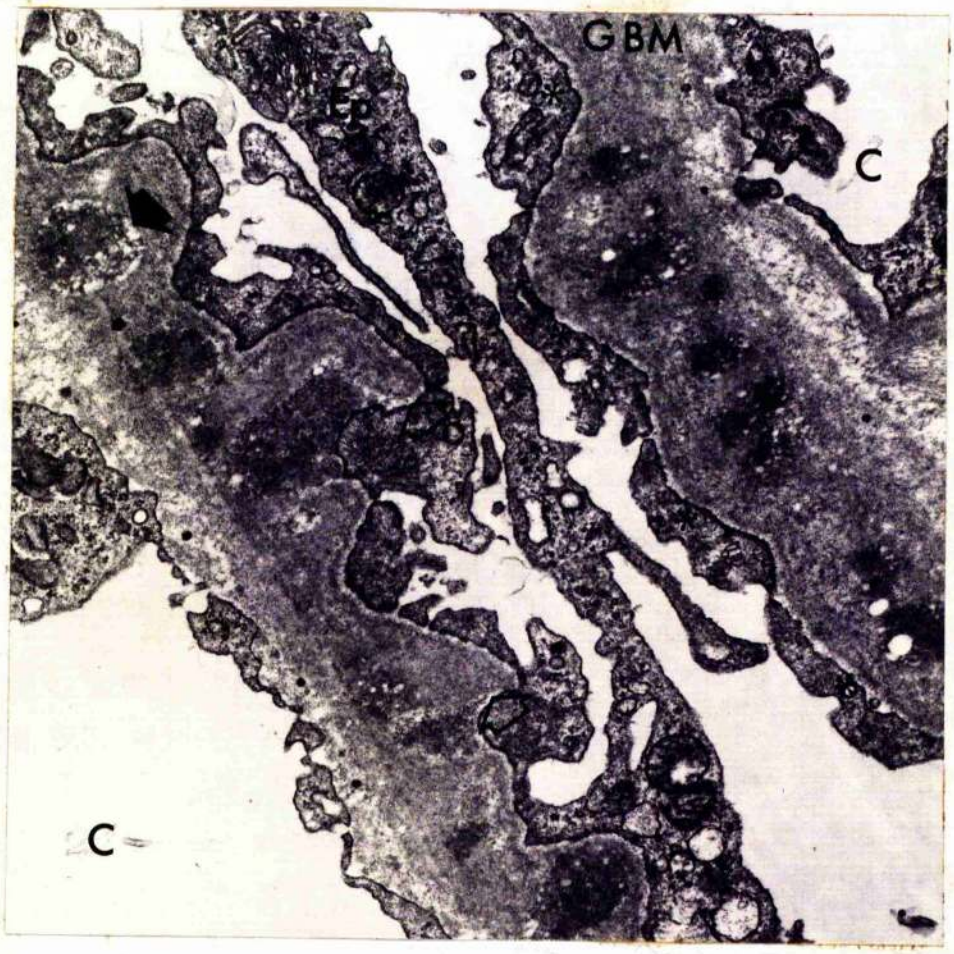
Electron dense deposits are situated in the glomerular basement membranes (GBM) in an intramembranous and a subepithelial position. These immune complexes were not found in any case of CIN. The electron micrograph suggests that GBM forms over the subepithelial deposits (open arrow) so that they eventually take up an intramembranous position. Some deposits (shaded arrow) have a translucent halo and ragged border suggesting their degradation. Epithelial cell foot processes (*) are fused indicating a glomerular protein leak.
C. capillary. Ep. Epithelial cell.

(Electron microscopy x 20,000)

Fig. 44 CGN, Case 32

Immune complex deposition is shown. Granular and globular deposits of IgG are present throughout the tuft in both mesangium and capillary walls. Staining for complement (C_3) was very similar. This was typical of the pattern of immunoglobulin and C_3 deposits seen in all cases of CGN, and was very different from the sparse local staining seen in a few cases of CIN (Fig. 36).

(Immunofluorescence x 450)


GBM
C
C

DISCUSSION

1) Pathogenetic Mechanisms in Chronic Nephritis

In the past canine nephropathies characterized by diffuse renal fibrosis without the marked irregular scarring of pyelonephritis have been classified as CIN (Bloom 1954, Wettimuny 1963). This study has shown that in some instances the primary lesion is an immunological reaction in the glomerulus; such cases, therefore, are correctly classified as GN. However, even when these are distinguished, the diagnosis of CIN in the dog still covers a variety of renal pathology which may reflect differing pathogenetic mechanisms.

Most evidence indicates CIN to be a result of an episode of AIN associated with L. canicola infection (see Introduction). Part of the evidence for this is that the heavy irregular fibrosis around the cortico-medullary junction corresponds exactly in position to the interstitial infiltrate present in the acute lesion. Of the 30 cases described here 24 had this pattern of fibrosis. Elution studies provided more evidence of a previous L. canicola infection in these cases. Antibodies to it were eluted from 12 out of 18 kidneys with this pattern of fibrosis. Antibodies obtained by this process will have been fixed in the kidney either in plasma cells or in immune complexes, as 3 PBS washings prior to acid elution removes most unbound protein including serum antibodies (Morrison and Wright 1976b, Woodroffe and Curtis, 1977). Presumably in these cases the antibody was from plasma cells as immune

complexes containing L.canicola antigen were never identified with immunofluorescence. Indeed, the organism itself was never seen in any of the 24 cases, although other workers have found occasional leptospirae in cases of CIN using silver stains (McIntyre and Montgomery 1952). Thus, it appears that the organism had been cleared from the kidney (if indeed it was present originally) before chronic renal failure developed in the cases described here.

The remaining 6 cases had diffuse renal fibrosis with no concentration around the cortico-medullary junction. Such a pattern would appear to be a less likely result of L. canicola infection. Indeed, none of the 4 eluates from such kidneys contained antibodies to this organism, but this may not be meaningful because of the small numbers tested. Are there then, more likely causes of the CIN in these 6 cases?

Canine adenovirus (CAV) and L. icterohaemorrhagiae have both been cited in this respect (see Introduction). Both elution and immunofluorescence studies gave no evidence for the involvement in these 6 cases (or indeed in the other 24). However, neither test proves that past infection had never occurred.

A second possibility is that these were cases of "inherited" renal disease (see Introduction). One dog (case 27) was of a breed (Elkhound) in which this disease has been described but its age ($5\frac{1}{2}$ years) makes this unlikely. Case 26 was young enough for this diagnosis but the disease has not been described in the Doberman. Therefore, it is very unlikely that any of the 6 were cases of "inherited" renal disease.

The renal pathology of this group was very similar to that of CGN. It is possible that immune complex deposition had occurred in the glomeruli for a period of time in the past, but deposits had since been degraded, so that immunoglobulin was no longer identifiable by immunofluorescence. This loss of staining has been reported to occur in some types of transient GN in Man (Germuth and Rodriguez 1973, Torunoth 1976), and in cases of Human CGN severely scarred glomeruli may lack identifiable immunoglobulin deposits (Heptinstall 1974). Therefore, there is the possibility that some of these animals were in fact cases of CGN.

Whatever the agent causing the initial AIN, it thus appears to have been eliminated, and the areas damaged in the acute reaction replaced by scars, by the time chronic renal failure had evolved. As fatal renal failure did not occur with the degree of damage present in the acute stage, there must have been mechanisms brought into action to produce the necessary extra destruction of nephrons. Three possibilities have been suggested: a) the deposition of immune complexes in the glomeruli (Krohn et al. 1971) b) release of renal antigens into the circulation resulting in the formation of autoantibodies (Torten et al. 1967) and c) the induction of renal hypertension (Mackey 1965, Anderson and Fisher 1968).

Although it is theoretically possible that a significant but transient deposition of immune complexes occurred at some period earlier in the disease, immunofluorescence failed to identify them in 27 of the 30 cases of CIN. In

the remaining three cases (16, 20, 24) granules of IgG and C_3 were present in small segments of a few glomeruli, at a distance from the deposits of fibrin, and it is possible that these were immune complexes. This finding is similar to that of Velosa et al. (1976) who discovered immune complexes to be present in scarred glomeruli in a wide variety of Human nephropathies. This they suggested, could indicate that immune complex deposition was an important mediator of glomerular scarring irrespective of the aetiology of the nephropathy. This may be true in certain instances in the dog as well (cases 16,20,24), but the findings from this study indicate that immune complex deposition plays little and usually no role in the progressive glomerular scarring in CIN in the dog.

There are two possible mechanisms by which auto-antibodies may damage the kidney. In either case renal antigen (s) could be released into the circulation during the initial episode of AIN and stimulate antibody production. The antibodies would then either a) combine with circulating antigen to form immune complexes which deposit in the glomeruli or b) attach directly to tubular and/or glomerular antigens. In the present study immunofluorescence failed to show the widespread granular or linear deposition of immunoglobulin that would be seen with a) and b) respectively. Neither did any eluate contain anti-kidney antibody. Again it must be stressed that neither test proves that such mechanisms were not operative some time earlier in the disease.

Although measurements of arterial pressure were not

made in this study, renal induced hypertension is likely to have played a role in the progression of the disease. In Man chronic renal diseases often lead to hypertension which then causes further renal damage (Heptinstall 1974). Moreover, it is already known that many cases of CIN in the dog are hypertensive and have arterial lesions (plasmatic vasculosis, medial hypertrophy) similar to those associated with hypertension in Man (Mackey 1965, Anderson 1968a). Dogs with CIN may also have left ventricular hypertrophy, which can be caused by hypertension (Platt 1952). In the 30 cases described here, 23 had such lesions in the renal arterial system and 6 had left ventricular hypertrophy.

The detailed examination of the glomeruli in this study revealed similarities to the process of glomerular scarring in primary (essential) hypertension in Man at both light (McGregor 1930, McManus and Lupton 1960) and electron microscopic level (Nagle et al. 1969, Jones 1974). In the benign form the major glomerular lesion, particularly well described by McManus and Lupton, is progressive wrinkling, thickening and duplication of the GBMs, with subsequent collapse and simplification of the tuft. Similar glomeruli were seen in this study (Fig. 15) and are thought to be a result of the ischaemia produced by arterial stenosis. Moreover, in Human malignant hypertension, where the blood pressure is very high, there is acceleration of the glomerular scarring process due to widespread fibrin deposition, itself caused by intravascular coagulation (Heptinstall 1974, Jones 1974,

Kincaid-Smith 1975b).

The glomerular lesions of CIN are also similar to those seen in experimental Goldblatt-type hypertension produced by unilateral renal artery ligation (Ben-Ishay et al. 1965, Okita 1971). These authors described various glomerular lesions including thickening of Bowman's capsule with collagen and excess CBM, thickening of the GBM often with vacuolated material, mesangial expansion and proliferation, and fibrin deposition in the capillaries, mesangium and urinary space.

A further area of similarity with primary malignant hypertension in Man is in the immunofluorescence findings of both arteries and glomeruli. Paronetto (1965) and Burkholder (1965) found foci of fibrin usually in association with IgG and complement in arteries, arterioles and glomeruli; occasionally IgG and/or complement were present in the absence of fibrin. Moreover, Heptinstall (1974) reported that fibrin could be identified by immunofluorescence in arteries which showed only cellular thickening under the light microscope. Paronetto and Burkholder both suggested that these findings indicated immune complex mediated arterial and glomerular lesions. However, Paronetto also found albumin to be present along with the other serum proteins and this many regard as an indication of non-specific seepage of plasma proteins into the vessel wall (Heptinstall 1974).

These similarities with primary hypertension in Man and experimental animal models are described here to show that certain renal lesions can be initiated by hypertension.

Thus, although the hypertension in CIN is secondary to renal damage, it is likely to cause further irreversible damage. It must be stressed that this comparison is not meant to indicate that CIN is the result of primary hypertension. In fact, this condition appears to be very uncommon in the dog and has never been associated with renal damage (Katz et al. 1957, Weiser et al. 1977).

CGN and CIN in the dog emerged from this study to be two very similar nephropathies. Both were characterised by widespread renal fibrosis, and glomerular scarring associated with fibrin deposition. However, the degree of each varied between CGN (except case 34 where pyelonephritis was also present) and CIN. In CGN renal fibrosis was less severe and lacked the focal concentrations around the cortico-medullary junction. The less severe scarring meant that the cortices were usually of normal width in CGN whereas shrunken cortices were a constant feature of "typical" CIN. However, those cases (25-30) of "atypical" CIN had a very similar pattern and degree of scarring to that of CGN. Thus, differences in renal fibrosis were not diagnostic. Similarly, glomerular fibrin deposition was usually, but not always, more widespread and severe in CGN than in CIN. Thus histological criteria could not be used to accurately differentiate CGN and CIN; this relied on immunofluorescence showing the presence of immunologically mediated lesions in CGN but not in CIN.

In 9 cases of CGN granules and globules of immunoglobulin and complement were deposited in the glomeruli, a pattern which is taken to indicate the presence of immune

complexes. In the remaining animal (case 31) the pattern was linear, which suggests the presence of anti-GBM antibody. However, such antibody was not eluted from this case. Indeed, the identity of the antigen involved in every case remains unknown. Anti-kidney antibodies were absent from all 6 eluates obtained showing renal antigens not to be involved in these cases. Furthermore neither did elution nor immunofluorescence studies provide any evidence for CAV, L.canicola or L.icterohaemorrhagiae as the antigen. Immunofluorescence failed to show up the first two organisms in any of the 10 cases but this is not conclusive as antigens can be masked by excess antibody in immune complexes (Wilson and Dixon 1974). In the 6 cases where elution was carried out only 1 gave a positive result; the eluate from case 35 had a low titre of anti-L.canicola antibody. This very low titre coupled with the negative result with direct immunofluorescence would suggest that this antibody was not from the immune complexes. Case 34 requires further mention, as chronic pyelonephritis was present in addition to CGN. These two lesions may have been unrelated but it is possible that circulating immune complexes were formed as a result of the pyelonephritis and subsequently deposited in the glomeruli causing a GN. A similar finding has been reported in some cases of Human pyelonephritis (see Introduction). No association was found in this study between CGN and any particular extra-renal lesion. However, case 37 also had a pyometra which has been associated with proliferative GN (Obel et al. 1964).

The severe fibrin deposition in CGN was probably a result of this immune complex deposition. The exact mechanisms by which they activate the coagulation cascade are not known. In-vitro experiments have shown a variety of possibilities (Josso et al. 1972, Zimmerman 1976). Immune complexes can directly produce changes in platelets comparable to those caused by thrombin. The activation of the complement cascade by immune complexes may trigger coagulation by a) causing cell lysis and hence activating the extrinsic pathway, b) causing cell lysis and exposing the GBM to the circulation which then activates platelets and/or Hageman Factor (Cochrane et al. 1972), c) acting directly on platelets and d) attracting polymorphonuclear leucocytes which may activate the extrinsic pathway. There is some evidence from both human and experimental animal renal disease that activation of platelets may be the most important route (George et al. 1975).

In addition, a degree of renal damage was probably a result of renal hypertension. CGN in man may be complicated by it (Heptinstall 1974), and 50% of the cases described in this study had lesions of plasmatic vasculosis and/or medial hypertrophy which are considered to be associated with hypertension (see above). The glomerular lesions were similar to those of CIN and hence to the glomerular lesions seen in primary Human hypertension and experimental animal hypertension (see above). In addition, the immunofluorescence picture of the arterial and arteriolar lesions was similar to those in primary Human hypertension.

The possible relationship of CGN to the other forms of

canine GN, viz proliferative GN and membranous nephropathy, is not clear. Focal hypercellularity, similar in degree to that seen in CIN, was present in all cases but the segmental or diffuse hypercellularity of the proliferative form was never seen. In contrast, case 31 had the diffuse thickening of GBM that characterizes membranous nephropathy. This form has been consistently associated with immune complex deposition in both dog and Man, but the immunofluorescence picture in case 31 did not conform with an immune complex aetiology. Although this does not prove that immune complexes were not involved, it indicates it may not be a typical case of membranous nephropathy. Therefore, the possibility that proliferative GN and membranous nephropathy may in time progress to the chronic form receives no support from these 10 cases.

Finally a feature of as yet unknown significance was present in both CIN (11 cases) and CGN (1 case). This was the presence of linear staining for complement (C_3) on a variable, but usually small, number of tubular basement membranes in the cortex, and very occasionally in the medulla. Immunoglobulin (IgG and IgM) were never present in these sites, and their absence could indicate activation of the complement cascade by the alternate pathway. However, no inflammatory lesions affecting the tubules were seen under the light microscope to support the concept of complement activation. On the other hand, a similar pattern of staining was seen when calcium deposits were highlighted by Von Kossa and PAS stains. However, comparison of Tables 7 and 10, and 16 and 19 shows that

there was a poor correlation between calcification and C_3 staining. Obviously further research is needed to determine whether or not this staining for C_3 is an artifact.

2) Glomerular Lesions

Before discussing various aspects of the process of glomerular scarring, certain aspects of the methods used warrant further explanation. The benefit of using serial sections should be stressed. It was only by using these that the association between the degree of glomerular scarring and the degeneration and eventual obliteration of the rest of the nephron was seen. In addition, they highlighted the process of fibrin deposition in the capillaries with its subsequent liberation into the urinary space where it led to the formation of capsular adhesions. It must also be pointed out that the figures calculated for the severity of glomerular scarring, and the incidence of the separate lesions in the glomerulus, are in reality only estimations. Firstly, in cases with focal radial scars in the cortex, the consequent variations in the degree of glomerular damage meant that different figures could be obtained if different sections were used. Secondly, only a few hundred glomeruli were actually examined in detail, which is a small percentage of the total number present in a dog (Finco and Duncan 1972). Thirdly, scarred glomeruli progressively shrink and eventually seem to disappear or at least become so small that they are overlooked (Moritz and Hayman 1934).

The process of glomerular scarring emerged from this study to be very similar in both CIN and CGN in the dog.

Moreover, a study of papers by McGregor (1930), McManus and Lupton (1960), Nagle et al. (1969), Jones (1974), and Thoenes and Rumpelt (1977), reveals great similarities to the process of glomerular scarring in a wide range of Human nephropathies e.g. primary hypertension, pyelonephritis, various forms of GN and diabetic glomerulosclerosis. The similarities tend to be obscured, however, by variations in terminology and interpretation. In this study it was found that up to four materials could be involved in the process of glomerular scarring: basement membrane (both capsular and glomerular), mesangial matrix, collagen, and fibrin or material derived from fibrin.

Two changes in particular characterize the scarring process: changes in the GBMs and expansion of the mesangial matrix. In any scarred glomerulus one, or in the vast majority of cases, both were present. Often, however, one dominated the picture. Thus, some tufts were composed of a mass of wrinkled, thickened and duplicated GBMs, with subsequent collapse and simplification of the tuft, but little mesangial expansion. This type of lesion has been associated with ischaemia resulting from arterial lesions in the kidney (McGregor 1930, McManus and Lupton 1960). The latter authors suggested that the simplification of the tuft was the result of "splitting open" of the mesangium to form a lumen as the capillary walls progressively contracted. However, this process could not be identified in this study, and it is possible that the tuft only appears to be composed of larger simplified capillaries due to spatial rearrangement following obliteration of other capillaries. It would be interesting

to see whether the three dimensional picture obtained with a scanning electron microscope would shed further light on the process.

In other scarred glomeruli expansion of the mesangial matrix was the dominant lesion. Occasionally such areas were also hypercellular. The position and morphology of these cells was mesangial but mitoses were never seen. This raised a doubt as to their origin, and it may possibly be that some cells were derived from an extra-glomerular source e.g. circulating mononuclear cells; this is true of certain experimental canine nephropathies (see Part 3: nephrotoxic serum nephritis).

Scarring of the tuft is often accompanied by changes in the capsule involving thickening, wrinkling and duplication of the CBM, and the formation of capsular adhesions. Occasionally small crescent shaped collections of cells could be found in association with the capsule. These were either formed by the adhesion of a whole tuft lobule to the capsule, or by severe distortion of the tuft's hilar region. These must be distinguished from lesions termed crescents, which were never seen. Crescents are collections of cells, which may be mixed with fibrous tissue, that are formed in the urinary spaces in certain Human (Churg et al. 1973, Meadows 1973, Min et al. 1974, Atkins et al. 1976) and experimental animal nephropathies (Kondo et al. 1972, Holdsworth et al. 1978); both infiltration by circulating mononuclear cells and proliferation of epithelial (mainly parietal) cells, are probably involved in this process. This is of comparative interest

as crescents characterize nephropathies where there is severe fibrin deposition with exudation into the urinary space (Churg et al. 1973, Meadows 1973, Min et al. 1974). Moreover, crescents are present in a proportion of the glomeruli in cases of CGN in Man (Heptinstall 1974).

Collagen was mainly found in two sites: between layers of the CBM and in the urinary space of severely or completely scarred glomeruli. In both places collagen fibrils were mixed with other banded and non-banded fibrils of unknown composition. The cells synthesizing this material probably originated from fibroblasts which penetrated from the interstitium through breaks in the CBM, although it is possible that the visceral and parietal epithelial cells also played a role (Thoenes and Rumpelt 1977). Occasional collagen fibres were also seen embedded in the mesangial matrix. Mesangial cells can, under certain stimuli, react as fibroblasts and produce collagen (Vassalli et al. 1963a) and its presence in the tuft is indicative of serious damage (Kincaid-Smith 1973a).

3) The Significance of Fibrin

The presence of fibrin in both nephropathies merits extensive discussion. Firstly, there are particular drawbacks to light, electron and immunofluorescence microscopy in the identification of fibrin (Davison et al. 1973a). Secondly, there is now much evidence to show that fibrin deposits play a major pathogenentic role in a range of Human and experimental animal nephropathies (Vassalli and McCluskey 1971, Kincaid-Smith 1972, 1975b, Kincaid-Smith et al. 1973).

The formation and subsequent breakdown of fibrin are dynamic processes involving several intermediate stages with changes in molecular size, shape and "framework". Thus, it is unlikely that a light microscope stain, which depends on the trapping of dye molecules in this "framework", will identify all stages. It must also be appreciated that material staining for fibrin may contain other "substances" derived from tissue necrosis or plasma which are trapped in the fibrin deposit. Moreover, the resulting staining of fibrin may actually depend on these "substances" or at least be altered by their presence. This has been shown to be true of fibrin clots formed in-vitro, and the necessary conditions vary from stain to stain (Gitlin and Craig 1957, Moe and Abildgaard 1969). These two points may explain the variations seen between the different stains. It was because of these variations that only one stain (MSB) was used in the assessment of glomerular fibrin deposition. False negatives are also likely to occur due to the size of the deposit. Davison and his colleagues (1973a), using material from Human renal biopsies, felt that, due to their lower sensitivities, the light microscope stains were unlikely to detect small subendothelial deposits that could be seen with both immunofluorescence and electron microscopy. On the other hand, false positives may also occur due to staining of globulins. Precipitates of macroglobulins formed in-vitro stain positively for fibrin with trichrome stains (Moe and Abildgaard 1969). In fact, trichrome stains can stain immune complex deposits as fibrin in cases of GN in Man (Davison et al. 1973a, Cohen 1975) and animals (Wright and

Spencer, unpublished observation). Thus, it is possible that the incidence of fibrin deposition in CGN was over-estimated. However, this is unlikely because, despite a careful search, immune complex deposits, which are much smaller on average than the large globules of fibrin, were never positively identified in histological sections in any of the cases of CGN. Finally, the change in staining reaction from fibrin to collagen with trichrome stains, noted here and described well by Lendrum et al. (1962), obscures the amount of fibrin deposited previously as it is now indistinguishable from mesangial matrix and GBM. Thus, light microscopic stains can only identify the amount of "recent" fibrin, and unfortunately the time span which "recent" covers has never been investigated. Moreover, it is not known if this change in staining reaction in the glomeruli indicates the replacement of fibrin by new mesangial matrix or GBM, or whether the identical staining reaction (with PAS and fibrin stains) obscures a different material. In this respect, Thoenes and Rumpelt (1977) described a process which they termed hyalinosis and which they judged to be separate from mesangial expansion or GBM changes. The hyalin material (an eosinophilic, homogeneous, refractile and glossly substance) formed in this process was distinguished from mesangial matrix and GBM by its negative reaction to silver stains but its positive one with orange G stain. Is this hyalin identical to the fibrin and/or the subsequent collagen staining material described in this study? Certainly, the H & E and PAS descriptions of the material are very similar but unfortunately neither

light nor immunofluorescence fibrin stains were used by Thoenes and Rumpelt. However, on the basis of previous studies on "focal and segmental sclerosing glomerulopathy" in Man, where much hyalin is formed but little or no fibrin is found, they concluded fibrin was not or only minimally involved. Obviously further studies are needed to fully elucidate whether the processes described here are the same as in Human nephropathies.

Immunofluorescence is theoretically the most accurate of the three methods used for identifying fibrin because of the specific nature of the immune reaction. There is evidence to support this theory. Moe and Abildgaard (1969) found it to be reliable, unlike light microscopic stains, in identifying fibrin clots formed in-vitro. Davison et al (1973a) found a close correlation between the distribution of fibrin in affected glomeruli using immunofluorescence and maximal urinary fibrin degradation product excretion. In this study, however, immunofluorescence appeared to give false negatives in some cases of CIN. This may have not been a true false negative but rather the result of the amount of tissue that can be routinely examined being too small to show the small focal deposition. On the other hand, immunofluorescence could theoretically give false positives. Because of antigenic similarity, anti-fibrinogen serum can identify: fibrinogen, fibrin, fibrin degredation products, and large circulating complexes composed of fibrin monomer, fibrinogen and fibrin degradation products (Canavese et al. 1978). Thus, glomerular "fibrin" deposits could in, some instances,

represent fibrinogen passing through a damaged GBM, or trapping in the glomerulus of circulating complexes, or fibrin degredation products formed by coagulation distant to the glomerulus, rather than fibrin actually formed as a result of local events in the glomerulus (Canavese et al. 1978). However; for reasons of simplicity fluorescing deposits were called fibrin although such terms as fibrinogen or fibrin related antigens or materials are more accurate.

Although it cannot be used for a quantitative assessment of fibrin deposition, electron microscopy is useful in locating the exact position of deposits. A major drawback is that fibrin deposits are often composed of a matrix of fine granules and rudimentary fibrils and not of fibrils with the "characteristic" periodicity of 230A^{o}; such deposits are probably composed of intermediate molecular stages produced in the formation and breakdown of fibrin (Stewart 1970, Davison et al. 1973a). Unfortunately other protein deposits can also appear as electron dense granular masses e.g. immune complexes, so the incidence and extent of fibrin deposition may be overestimated by using the electron microscope (Davison et al. 1973a). In this study only granular or rudimentary fibrillar material was seen, and therefore, it cannot be taken unequivocally to be fibrin. However, in case 23 subendothelial deposits of dense granular and fibrillar material surrounded by an electron translucent zone were present in the glomerular capillaries. Such areas have been reported in a wide variety of Human nephropathies in which the common factor appears to be the active deposition and lysis of fibrin within glomerular capillaries (Kincaid-Smith 1972). In

case 24 dense granular material was seen in peripheral capillaries associated with adhesions, and light and immunofluorescence microscopy showed this to be the commonest site of fibrin deposition. Thus, this material was possibly fibrin or derived from fibrin. It is interesting to note that the ultrastructural description and photographs of Thoenes and Rumpelt's (1977) "hyalin" were very similar to this.

A discussion of the identification of fibrin in tissue sections would not be complete without a discussion of the terms "fibrinoid" and "hyalin". These terms have been used in the past and are still used by some authors to denote fibrin and the subsequent collagen staining materials in light microscopic sections. In addition, "hyalinosis" along with "sclerosis" has been used to denote the process of glomerular scarring. Although it may be inaccurate to use the term fibrin when deposits almost certainly contain other proteins (see above), the use of the term "fibrinoid" which has no accurate definition only serves to obscure the fact that fibrin is the major component. Thus "fibrinoid" was not used in this study. In addition, "fibrinoid" has also been used to denote fibrin deposits which are not composed of fibres with the "characteristic" periodicity under the electron microscope. This granular material is now known to be intermediate stages in the formation and breakdown of fibrin although it may contain other proteins (see above). Thus, although calling this material fibrin is not strictly accurate, the term "fibrinoid" only obscures its dynamic relationship with fibrin. Hyalin, on the other hand, is a descriptive term applied to any eosinophilic,

homogeneous, refractile, glossly appearing cell or tissue component (Thoenes and Rumpelt 1977). It has been used in the past to denote several unrelated materials: old collagenous fibrous tissue, amyloid, alteration in blood vessel walls due to plasmatic vasculosis, and lesions of glomerular scarring (Nagle et al. 1969). The use of a word which can apply to materials of differing pathogenesis and chemical nature is to be discouraged if a more specific definition can be given. Its use in denoting glomerular scarring is to be particularly discouraged as the process consists of four components: mesangial expansion, GBM abnormalities, collagen formation and fibrin deposition; of these only fibrin and collagen staining material derived from fibrin have a hyalin appearance. Thus, it was judged that the word "scarring" conveyed more accurately to the reader the processes of glomerular obliteration with loss of function, than "hyalinosis" (or "sclerosis" which means hardening). It is interesting to note that Thoenes and Rumpelt (1977) independently have come to a similar conclusion. However, they retained "sclerosis" to specifically denote mesangial matrix expansion and "hyalinosis" to define the production of a hyalin material (see above). Similar hyalin material was seen in this study but the fibrin stains used here allowed the process of "hyalinosis" here to be identified as a sequel of fibrin deposition. Thus to use the term "hyalin" may obscure the fact that fibrin is or has been present.

Even when the limitations in identifying fibrin are taken into account, it is still true to say that fibrin

deposits were a prominent feature in CGN in particular, and in CIN, in the dog. But what is the significance of this? As already stated the process of glomerular scarring was very similar in these two nephropathies to that in various Human nephropathies (see above). This may be explained by the glomerulus reacting in the same way to a variety of insults or the presence of a final common pathway acting in a variety of nephropathies with differing aetiologies. Fibrin deposition is a candidate in this latter explanation. Not only was fibrin present in both nephropathies, but in histologic sections it appeared that obliteration of the tuft by mesangial matrix and GBM (the major features of glomerular scarring) could be intimately related to fibrin deposition. Moreover, the past 15 years has seen the accumulation of much evidence implicating fibrin deposition to be of major importance in the scarring of glomeruli, in a variety of Human and experimental animal nephropathies (Vassalli and McCluskey 1971, Kincaid-Smith 1972, 1975b, Kincaid-Smith et al. 1973).

A major piece of evidence is that experimental fibrin deposition alone may produce glomerular scarring (Hausman and Dreyfus 1953, Vassalli et al. 1963a, Humair et al. 1969a, Urizar et al. 1978). In these studies, the initiation of disseminated intravascular coagulation in rabbits, rats and mice produced widespread thrombosis of the glomerular capillaries. The initial reaction was swelling, proliferation and phagocytosis of the fibrin by the endothelial and mesangial cells. Healing involved localized thickening and duplication of GBMs, the expansion of the mesangium, and the formation (in rabbits) of crescents. In

severely damaged areas collagen fibres were also formed. Such lesions could obliterate a whole capillary, and very occasionally a whole glomerulus. The role of fibrin in producing glomerular scarring in this experimental model, is further shown by the amelioration of the lesions by anticoagulants or fibrinolytic agents (Evensen et al. 1967, Humair et al. 1969b, Urizar et al. 1976); and the reverse effect by fibrinolytic inhibitors (Vassalli et al. 1963a).

Similar lesions are seen in the Human nephropathies that follow intravascular coagulation (Vassalli and McCluskey 1971, Kincaid-Smith 1975b). A range is seen from mild reversible lesions to severe damage resulting in renal failure. In acute tubular necrosis (nephrosis) there is a short-lived episode of fibrin deposition in the glomerulus related to the cessation of blood flow, but the fibrin is rapidly removed as renal function returns and leads to no permanent damage (Clarkson et al. 1970, Kincaid-Smith 1975b). In pregnancy (pre-eclamptic) toxaemia there are much larger masses of fibrin, probably resulting from low grade intravascular coagulation over a period of time (Vassalli et al. 1963b, Kincaid-Smith 1973a,b, 1975b). In the acute stage much of this fibrin builds up in a subendothelial position and is accompanied by endothelial swelling. Once coagulation has stopped, repair is associated with thickening and duplication of the GBMs, and mesangial interposition around the capillary walls between the endothelium and GBM. Repeat biopsies, however, show that these lesions regress with time and glomeruli may return to normal. More severe permanent lesions,

however, are found in the various thrombotic micro-angiopathies (post partum renal failure and haemolytic-uraemic syndrome), sclerodoma and malignant hypertension (Kincaid-Smith 1973a,b, 1975b) where intravascular coagulation is more severe and may lead to renal failure. The build up of fibrin in the glomeruli leads to permanent thickening and duplication of the GBMs, and expansion of the mesangium including interposition of mesangial cells around the capillary circumference. Collagen fibres may be present in these areas indicating severe damage. Glomeruli are partially or completely obliterated by these lesions.

Stronger evidence for a pathogenetic role of fibrin in these nephropathies would be if anticoagulants reduced the severity of the renal lesions. However, evaluation of reports is complicated by differences in patient selection, control procedures and the combination of anticoagulants with other drugs. Heparin has been the most widely used, but although Proesmans and Eeckels (1974) judged it to be beneficial in treating the haemolytic-uraemic syndrome, this has been disputed (Kaplan et al. 1976); no improvement has been found in the treatment of pregnancy (pre-eclamptic) toxaemia (Studd 1977) or malignant hypertension (Zimmerman and Bergin 1974) with heparin.

Thus, in both experimental animals and in spontaneous disease in Man, GBM wrinkling, thickening and duplication, expansion of the mesangium, and formation of collagen in the tuft are produced as a result of fibrin deposition in the glomeruli. Such lesions are similar to those described here in both CIN and CGN in the dog. In addition, there is also evidence which implicates fibrin deposition as an

important secondary pathogenetic mechanism in GN in Man and animals.

Fibrin is prominent in the glomeruli in experimental GN mediated by anti-GBM antibodies (Vassalli and McCluskey 1964a,b, Briggs et al. 1969, Borrero et al. 1973, Watanabe and Tanaka 1976). Large amounts of fibrin are present both in the glomerular capillaries and urinary spaces, accompanied by cellular proliferation, infiltnation of circulating mononuclear cells, and the formation (in rabbits, hares and mice) of crescents. This results in 2 to 3 weeks in local and global obliteration of the glomeruli by excess mesangial matrix and basement membrane. This type of GN has been produced in dogs (Movat et al. 1961, Wright et al. 1973b) and similar terminal lesions (except for crescents) were seen. However, the role of fibrin was not specifically investigated.

The fact that glomerular scarring follows fibrin deposition does not automatically link the two. There would be evidence for this link if anticoagulants prevented fibrin deposition and subsequent scarring while not affecting the immunological process. Unfortunately the reports in the literature conflict on this point and none of this work has been carried out in dogs. Some authors report a reduction in glomerular damage and crescent formation following heparin (Kleinerman 1954, Halpern et al. 1965, Watanabe and Tanaka 1975) or warfarin treatment (Vassalli and McCluskey 1964a,b, Borrero et al 1973). However, Thomson et al. (1975a) found heparin only effective in very high doses while in three reports it gave no

reduction in the amount of fibrin present or the severity of glomerular damage (Briggs et al. 1969, Border et al. 1975, Bone et al. 1975). In addition, whilst Watanabe and Tanaka (1976) found that trans-aminomethylcyclohexane carboxylic acid (a fibrinolytic inhibitor) increased fibrin deposition and glomerular scarring, Briggs et al. (1969) found that urokinase (a fibrinolytic agent) did not affect the degree of glomerular scarring, although it reduced the amount of fibrin present. A close study of these papers suggests that anticoagulants were effective where the disease was not very severe. These inconsistent results do not disprove the link between fibrin deposition and glomerular scarring as coagulation may be primarily mediated by platelets so avoiding the intrinsic and extrinsic pathways on which heparin and warfarin act. In support of this theory, defibrination of serum by Ancrod (the coagulant fraction of Malayan pit viper venom) produced a marked reduction in both the amount of fibrin deposited and the degree of glomerular scarring. Moreover, this was true even when given after extensive fibrin deposition had occurred, and renal failure was developing (Naish et al. 1972, Thomson et al. 1975a, Thomson et al. 1976a). Thus, there is evidence, albeit inconclusive, that fibrin deposition leads to glomerular scarring in experimental GN mediated by anti-GBM antibodies.

As immune complex mediated GN only has been positively identified in the dog, the role of fibrin in this type would be more significant. It has been shown that the massive injection of preformed soluble immune complexes (Lee 1962, Humair et al. 1969b) or the formation of immune

complexes in-vivo by antigen injections into a strongly immunised animal, can result in glomerular fibrin deposition (Lee 1962, Vassalli and McCluskey 1964b, Evensen et al. 1972, Gabbiani et al. 1975). In addition, Humair et al. (1969b) reduced the amount of fibrin deposited by pretreatment with urokinase and with a combination of warfarin and heparin. However, only small numbers of animals were used in this study. Despite these experiments which relate fibrin deposition to immune complexes, coagulation appears to play at the most only a minor role in the two classical forms of experimental GN mediated by immune complexes, acute and chronic serum sickness (Vassalli and McCluskey 1971, Germuth and Rodriguez 1972). In acute serum sickness there is a short period of immune complex deposition in the glomeruli which leads to a proliferative GN with little scarring. Fibrin is present in only a few glomeruli, and neither the amount nor the other glomerular lesions are affected by anti-coagulant treatment (Baliah and Drummond 1972). Perhaps it could be argued that the minimal scarring was a result of this lack of fibrin (Vassalli and McCluskey 1971). In chronic serum sickness, the characteristic lesion is a membranous nephropathy, the only type of glomerular lesion not produced from experimental disseminated intravascular coagulation (Vassalli and McCluskey 1971). However, there may also be some glomerular fibrin deposition, proliferation, crescent formation and scarring. Border et al (1975) found heparin did not alter these events, but Thomson et al. (1975b) found defibrination with Ancrod gave some but not complete protection to the glomeruli from them.

On the other hand, fibrin deposition plays a major role in two types of spontaneous GN of animals mediated by immune complexes: Aleutian disease in mink (Henson et al. 1967) and the GN in the New Zealand Black (NZB) mouse and its NZB/NZW hybrid (Hicks and Burnet 1966, Howie and Helyer 1967). Both diseases are characterized by a proliferative GN with widespread severe fibrin deposition, crescent formation and glomerular scarring. Again, anticoagulant treatment has given conflicting results. Lambert in 1970 (quoted by Vassalli and McCluskey 1971) produced a striking decrease in the severity of the glomerular lesions and a prolongation of life, in NZB mice given heparin; the underlying immunological reaction, viz the deposition of immune complexes and complement, appeared unchanged. In contrast, McGiven (1967) failed to ameliorate the lesions in these mice with warfarin. Thus, under certain conditions, fibrin deposition does play a role in both spontaneous and experimental immune complex mediated GN in animals.

Further support for the role of fibrin in GN comes from this group of diseases in Humans. Fibrin deposits can be present in many of the morphological types, both those mediated by immune complex and those mediated by anti-GBM antibody (Paronetto and Koffler 1965, Koffler and Paronetto 1965, McCluskey et al. 1966, McIntosh et al. 1971, Min et al. 1974). Among the forms in which fibrin deposits are prominent are acute GN, rapidly progressive GN, Goodpasture's syndrome, GN associated with systemic lupus erythematosus (which resembles the disease in NZB mice described above), and in particular, in terms of this discussion, CGN. Moreover, if the GBM ruptures, fibrin is

liberated into the urinary space, and crescents then form (Churg et al. 1973, Min et al. 1974). This combination of glomerular fibrin deposition with subsequent crescent formation is well recognised as a poor prognostic sign (Chirawong et al. 1971). The evidence for the role of fibrin in these nephropathies would be further strengthened if anticoagulants had a beneficial effect. Although there are reports which indicate this to be so, none of these have proper controls and other drugs e.g. anti-platelet and anti-inflammatory drugs have often been used with the anticoagulants (Kincaid-Smith 1972, Cameron 1977).

In conclusion, there is much evidence implicating fibrin deposits in the process of glomerular scarring, in both experimental and spontaneous nephropathies, but none of this had been done in the dog or the results applied to the dog. However, the similarities of the lesions described here in CIN and CGN to those associated with fibrin deposition i.e. GBM wrinkling, thickening and duplication, mesangial matrix expansion, glomerular hypercellularity and capsular adhesions, suggests that fibrin deposition is important in these diseases. More conclusive evidence would be the experimental production of these lesions in dogs by fibrin deposition. The next section is, therefore, devoted to the investigation of the connection between fibrin deposition and glomerular scarring in the dog itself using an immunological method (anti-GBM antibody) and a non-immunological method (liquoid).

PART 3:

EXPERIMENTAL INVESTIGATIONS INTO THE ROLE OF FIBRIN IN THE PATHOGENESIS OF GLOMERULAR SCARRING

A. LIQUOID NEPHROPATHY

MATERIALS AND METHODS

Liquoid

Sodium polyanetholsulphonate - liquoid (Sigma Chemical Company, St. Louis, U.S.A.) was dissolved in sterile distilled water immediately before use, to obtain solutions of varying concentrations (2.5 to 50 $mg.ml^{-1}$). These were then injected intravenously into the experimental animals at doses ranging from 8 to 166 $mg.kg^{-1}$. All injections were given very slowly to try and avoid fatal pulmonary thrombosis and haemorrhage.

Experimental Animals

Male and female puppies 8 to 12 weeks old and weighing between 1.5 kg and 4 kg were used. 38 animals in all were given liquoid. 13 were given varying doses of liquoid to find a regime which gave extensive fibrin deposition in the glomeruli, and yet was compatible with survival. 25 animals were then given this dose (a 4 $mg.ml^{-1}$ solution given at a rate of 10 $mg.kg^{-1}$) and killed at a range of times up to 39 days after injection. 6 animals of similar age and weight received no treatment and formed a control group. The animals were sedated with "Immobilon" (Reckitt and Colman, Hull, Britain) before treatment, and revived immediately after with "Revivon" (Reckitt and Colman, Hull, Britain).

Light, Electron and Immunofluorescence Microscopy

Methods used in these studies were identical to those

described in Part 2 of this thesis except that a) anti-fibrinogen serum only was used in the immunofluorescence studies, and b) all 7 fibrin stains were used routinely in every case in the histological studies.

Biochemistry

Whenever possible samples were taken at the time of death for blood urea and urine protein measurements.

TABLE 21

ACUTE LIQUOID NEPHROPATHY

GENERAL INFORMATION AND POST MORTEM FINDINGS

CASE	LIQUOID CONCENTRATION $mg.ml^{-1}$	LIQUOID DOSE $mg.kg^{-1}$	SURVIVAL TIME	BLOOD UREA $mmol.l^{-1}$	URINE PROTEIN $mg.100ml^{-1}$	RENAL CONGESTION/ HAEMORRHAGE	PULMONARY OEDEMA/ HAEMORRHAGE	OTHER LESIONS
41	5	33	30 mins[d]	NR	NR	-	++	
42	50	116	80 mins[ke]	NR	NR	-	++	Pulmonary arterial thrombosis Intestinal haemorrhage
43	10	40	2 hours[d]	NR	NR	-	++	Intestinal haemorrhage
44	5	16	3 hours[d]	NR	NR	-	-	
45	2.5	10	3 hours[ke]	9.8	NR	++	+	
46	4	10	4 hours[ke]	NR	NR	++	-	
47	4	10	<24 hours[d]	NR	NR	++	++	
48	4	10	<24 hours[d]	NR	NR	++	++	Bladder petechiae
49	4	10	<24 hours[d]	NR	NR	++	++	
50	5	12.5	24 hours[ke]	NR	NR	++	+	Bladder petechiae
51	4	10	27 hours[ke]	39.0	128	+	+	
52	4	10	28 hours[ke]	49.3	NR	+	+	
53	4	10	28 hours[ke]	NR	168	+	+	
54	4	10	29 hours[ke]	NR	30	+	-	
55	4	8	2 days	3.8	24	-	-	
56	5	16	2 days[d]	NR	NR	++	++	
57	5	12.5	3 days	NR	NR	++	+	

d Died, ke Killed in extremis

TABLE 22

CHRONIC LIQUOID NEPHROPATHY
GENERAL INFORMATION AND POST MORTEM FINDINGS

CASE	LIQUOID CONCENTRATION $mg.ml^{-1}$	LIQUOID DOSE $mg.kg^{-1}$	SURVIVAL TIME Days	BLOOD UREA $mmol.l^{-1}$	URINE PROTEIN $mg.100\ mls^{-1}$	RENAL SCARRING/ CALCIFICATION	FOCAL PULMONARY CONGESTION
58	4	10	4	5.2	NR	-	-
59	4	10	5	NR	NR	++	+
60	4	10	6	30.3	171	++	-
61	4	10	7	2.8	274	-	-
62	4	10	8	3.1	144	+	-
63	5	25	8	NR	NR	+	-
64	4	10	9	10.6	153	++	-
65	4	10	10	2.8	78	-	-
66	4	12.5	10	5.8	55	+	-
67	4	10	13	4.5	NR	-	+
68	4	10	14	6.3	34	++	-
69	4	10	15	9.2	NR	++	-
70	2.5	10	17	NR	22	-	-
71	4	10	21	2.0	NR	-	+
72	4	10	23	7.1	61	-	+
73	4	10	25	3.1	NR	+	+
74	4	10	28	2.7	217	+	+
75	4	10	28	NR	25	-	-
76	4	10	34	6.0	25	-	+
77	4	10	35	4.9	39	-	-
78	4	10	39	7.3	NR	-	-

TABLE 23

ACUTE LIQUOID NEPHROPATHY

GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	<50% SCARRING	> 50% SCARRING	100% SCARRING CONTRACTED	100% SCARRING CYSTIC
41	207	97	1.0	0.5	1.0	0.5
42	226	94.8	2.2	0.4	2.2	0.4
43	222	96.7	1.4	0.5	1.4	0
44	174	97.2	1.1	0	1.7	0
45	221	97.3	0	0.9	1.8	0
46	285	98.0	1.0	0	1.0	0
47	156	96.2	2.6	0.6	0.6	0
48	184	98.4	0.5	1.1	0	0
49	335	97.6	0.9	0.6	0.9	0
50	245	96.3	2.5	0.8	0.4	0
51	210	80.9	17.1	1.0	1.0	0
52	218	86.7	9.6	3.2	0.5	0
53	253	80.2	16.2	3.2	0.4	0
54	173	91.8	6.4	1.2	0.6	0
55	344	97.9	0.6	0.6	0.9	0
56	207	91.7	6.8	0	1.5	0
57	251	80.1	15.9	1.6	2.4	0

TABLE 24

CHRONIC LIQUOID NEPHROPATHY

GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	< 50% SCARRING	> 50% SCARRING	100% SCARRING CONTRACTED	100% SCARRING CYSTIC
58	200	96.5	2.0	0.5	1.0	0
59	216	75.9	20.4	1.9	1.8	0
60	281	70.1	24.5	2.9	2.5	0
61	211	52.1	41.2	4.8	1.9	0
62	227	88.5	10.6	0	0.9	0
63	244	69.7	24.6	3.7	1.6	0.4
64	295	64.1	25.8	4.4	5.4	0.3
65	242	72.3	21.9	4.1	1.7	0
66	302	85.4	10.9	1.0	1.0	1.7
67	320	97.5	0.6	1.3	0.6	0
68	248	63.3	23.0	7.3	6.4	0
69	189	63.5	28.6	4.2	3.7	0
70	225	98.2	0	0.9	0	0.9
71	188	62.8	29.8	5.3	2.1	0
72	457	96.7	1.1	1.1	1.1	0
73	286	82.5	12.9	1.4	3.2	0
74	163	82.8	11.0	2.5	3.7	0
75	213	96.7	1.9	0	1.4	0
76	247	89.9	5.3	2.0	2.0	0.8
77	267	96.6	1.9	0	1.5	0
78	205	96.6	1.9	0	1.5	0

TABLE 25

CONTROLS

GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	$\leq 50\%$ SCARRING	$> 50\%$ SCARRING	100% SCARRING CONTRACTED	100% SCARRING CYSTIC
79	219	97.2	1.8	0.5	0.5	0
80	193	93.8	4.1	0	1.6	0.5
81	171	94.2	2.9	0.6	2.3	0
82	201	97.0	1.0	0	1.5	0.5
83	340	96.7	0.6	0.6	1.5	0.6
84	153	95.4	2.0	0	2.6	0

TABLE 26

ACUTE LIQUOID NEPHROPATHY
GLOMERULAR MORPHOLOGY

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED							
		FIBRIN DEPOSITS	CONGESTION AND HAEMORRHAGE	NECROSIS	POLYMORPHO-NUCLEAR LEUCOCYTE INFILTRATION	GBM THICKENING			CAPSULAR ADHESIONS
						TOTAL[A]	LOCAL	GLOBAL	
41	194	51.5	0	0	2.1	7.7	7.7	0	0
42	212	32.1	0	0	1.9	4.7	4.7	0	0
43	224	60.7	11.2	0	16.1	16.5	16.5	0	0
44	170	100	88.8	0	10.0	21.8	21.8	0	0
45	215	54.4	94.9	15.8	42,3	59.0	53.9	5.1	0
46	279	73.1	95.7	13.6	58.8	78.5	66.7	11.8	0
47	155	58.1	5.8	11.0	13.5	63.9	51.0	12.9	0
48	183	89.6	98.9	32.8	45.4	84.7	67.8	16.9	0
49	322	42.5	99.1	23.6	38.8	39.4	35.4	4.0	0
50	236	57.6	39.8	40.7	74.6	32.2	22.9	9.3	0
51	200	11.0	10.0	7.5	10.5	9.5	9.5	0	0
52	216	45.4	41.2	31.0	39.8	41.2	35.2	6.0	0
53	245	30.2	42.0	24.1	20.4	27.3	22.4	4.9	0
54	175	6.3	2.3	0	8.0	17.1	17.1	0	0
55	340	0	0	0	3.2	2.9	2.9	0	0
56	201	66.5	85.5	92.0	29.5	42.0	36.0	6.0	8.0
57	251	88.8	84.9	84.1	29.1	54.6	43.4	11.2	5.2

GBM Glomerular basement membrane
A Total percentage of glomeruli affected

TABLE 27

CHRONIC LIQUOID NEPHROPATHY

GLOMERULAR MORPHOLOGY

CASES	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED									
		FIBRIN DEPOSITS	NECROSIS	HYPERCELLULARITY TOTAL[A]	LOCAL	GLOBAL	GBM THICKENING TOTAL[A]	LOCAL	GLOBAL	CAPSULAR ADHESIONS	CAPSULAR THICKENING
58	193	0	0	5.2	5.2	0	3.6	3.6	0	0	0.5
59	213	0	0.5	13.2	12.7	0.5	6.6	6.1	0.5	0.9	0
60	266	0	0	15.8	13.5	2.3	18.1	18.1	0	1.1	4.1
61	207	0	0	12.1[M]	11.6	0.5	15.0	15.0	0	0.5	0.5
62	219	0	0	8.7	8.2	0.5	5.9	5.9	0	0	0
63	247	0.8	1.2	7.3	6.9	0.4	13.4	10.5	2.9	2.4	1.2
64	296	0	0	17.9	13.5	4.4	22.0	19.6	2.4	0.3	1.4
65	240	0	0	9.2[M]	8.8	0.4	6.6	5.8	0.8	0.8	1.2
66	303	0	0	7.6	6.9	0.7	6.9	6.9	0	0.7	0
67	317	0	0	4.4[M]	4.4	0	2.8	2.8	0	0	0
68	223	0	0	15.3	12.6	2.7	24.2	22.4	1.8	3.1	5.8
69	184	0	0	15.2[M]	10.9	4.3	7.6	6.5	1.1	1.6	3.8
70	217	0	0	3.7[M]	3.7	0	3.7	3.7	0	0	0
71	191	0	0	9.9	9.4	0.5	6.3	6.3	0	0.5	0.5
72	450	0	0	4.4	2.0	2.4	2.7	2.7	0	0	0
73	279	0	0	9.3[M]	7.9	1.4	7.2	7.2	0	0	1.4
74	160	0	0	3.1	2.5	0.6	10.6	10.0	0.6	0.6	1.3
75	202	0	0	4.0	4.0	0	4.0	4.0	0	0	0
76	243	0	0	7.9	5.8	2.1	7.8	7.0	0.8	0	2.0
77	268	0	0	5.2	5.2	0	3.7	3.7	0	0	0.4
78	199	0	0	4.0	4.0	0	2.5	2.5	0	0	0.5

A Total percentage of glomeruli affected.
GBM Glomerular basement membrane
M Mitoses present

TABLE 28

CONTROLS

GLOMERULAR MORPHOLOGY[A]

CASES	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED							
		POLYMORPHO-NUCLEAR LEUCOCYTE INFILTRATION	HYPERCELLULARITY			GBM THICKENING			CAPSULAR THICKENING
			TOTAL[B]	LOCAL	GLOBAL	TOTAL[B]	LOCAL	GLOBAL	
79	222	6.3	2.7	2.7	0	1.4	1.4	0	0
80	180	5.0	4.4	3.3	1.1	2.8	2.8	0	1.1
81	171	5.8	4.7	4.1	0.6	2.9	2.9	0	0
82	198	6.6	7.6	7.1	0.5	1.0	1.0	0	1.0
83	317	5.0	5.0	4.1	0.9	0.9	0.9	0	0
84	147	6.8	6.1	6.1	0	3.4	3.4	0	0.7

A Fibrin, congestion and haemorrhage, necrosis and capsular adhesions never seen.

B Total percentage of glomeruli affected.

GBM Glomerular basement membrane.

TABLE 29

ACUTE LIQUOID NEPHROPATHY

NON-GLOMERULAR HISTOPATHOLOGY

CASE	TUBULES				INTERSTITIUM		BLOOD VESSELS
	DEGENERATION AND NECROSIS	TBM RUPTURE	PROTEIN CASES	HAEMORRHAGE	CONGESTION AND HAEMORRHAGE	POLYMORPHO-NUCLEAR LEUCOCYTE INFILTRATION	FIBRIN DEPOSITS
41	-	-	-	-	-	-	1+
42	-	-	-	-	2+[c]	-	1+
43	-	-	-	-	1+[c]	-	-
44	1+	-	-	-	1+[c]	1+	1+
45	1+	1+	3+	-	2+[c]	1+	1+
46	4+	-	1+	-	4+	1+	2+
47	4+	2+	3+	2+	4+	3+	3+
48	4+	2+	2+	-	4+	2+	3+
49	4+	2+	1+	1+	2+	1+	2+
50	3+	1+	4+	1+	2+	1+	1+
51	2+	1+	3+	-	2+	1+	1+
52	4+	2+	4+	1+	4+	2+	2+
53	3+	2+	4+	1+	4+	2+	1+
54	1+	1+	4+	-	2+	2+	-
55	-	-	-	-	-	-	-
56	4+	3+	3+	2+	4+	1+	3+
57	4+	3+	2+	-	4+	2+	2+

c congestion only
TBM Tubular basement membrane

TABLE 30

CHRONIC LIQUOID NEPHROPATHY

NON-GLOMERULAR HISTOPATHOLOGY

CASE	TUBULES						INTERSTITIUM		
	DEGENERATION AND NECROSIS	REGENERATION	CALCIFICATION	TBM RUPTURE	TBM THICKENING	PROTEIN CASTS	CELLULAR INFILTRATION	FIBROSIS	HAEMORRHA
58	-	-	-	-	-	-	-	-	-
59	3+	2+	1+	1+	-	3+	2+	1+	1+
60	4+	2+	3+	1+	2+	4+	3+	2+	-
61	3+	1+	-	1+	2+	2+	1+	1+	-
62	1+	1+	-	-	1+	1+	1+	1+	-
63	2+	2+	-	-	2+	2+	1+	1+	-
64	4+	2+	3+	1+	3+	1+	2+	2+	-
65	1+	1+	-	-	1+	1+	1+	2+	-
66	1+	1+	-	1+	1+	2+	1+	1+	-
67	-	-	-	-	-	-	-	-	-
68	2+	3+	3+	1+	3+	2+	1+	3+	-
69	1+	2+	3+	1+	3+	3+	1+	2+	-
70	-	-	-	-	-	-	-	-	-
71	-	1+	-	-	1+	-	-	1+	-
72	-	-	-	-	-	-	-	-	-
73	1+	-	-	-	1+	-	-	1+	-
74	-	-	-	-	1+	1+	-	2+	-
75	-	-	-	-	-	-	-	-	-
76	-	-	-	-	1+	-	-	1+	-
77	-	-	-	-	-	-	-	-	-
78	-	-	-	-	-	-	-	-	-

TBM Tubular basement membrane

TABLE 31

LIQUOID NEPHROPATHY

COMPARISON OF HISTOLOGICAL STAINS AND IMMUNOFLUORESCENCE MICROSCOPY IN THE IDENTIFICATION OF FIBRIN[1]

CASE	SURVIVAL TIME	GRAM WEIGERT	MSB	MASSON 44/41	OBADIAH	PICRO MALLORY V	PTAH	YELLOWSOLVE	IMMUNOFLUORESCENCE
41	30 mins	-	S	S	S	S	W	S	S
42	80 mins	-	W	S	S	S	W	W	S
43	2 hours	-	W	S	S	S	S	S	S
44	3 hours	S	S	S	S	S	S	W	S
45	3 hours	W	S	S	S	W	S	S	S
46	4 hours	S	S	S	S	S	S	S	S
47	<24 hours	W	S	S	S	S	S	S	S
48	<24 hours	W	S	S	S	S	W	S	S
49	<24 hours	-	S	S	S	W	W	W	S
50	24 hours	S	S	S	S	S	S	S	S
51	27 hours	-	S	W	-	-	-	S	S
52	28 hours	S	S	S	S	S	S	S	S
53	28 hours	S	S	S	S	S	S	S	S
54	29 hours	-	S	S	S	S	-	W	S
56	2 days	W	S	S	S	S	S	W	S
57	3 days	W	S	W	S	S	S	S	S
63	8 days	-	W	-	W	W	-	-	S

W Weakly staining
S Strongly staining
1 Negative cases not included.

The effects of liquoid varied greatly from animal to animal, even when an identical dose was given. Such a dose could produce no effect whatsoever, a non fatal renal lesion, or fatal pulmonary and renal lesions. This and the consequent variation in severity and type of the glomerular lesions, precluded a detailed study of their relationship with the dose given or the time after injection. Instead, this section will be structured so as to describe the whole range of renal, and in particular, glomerular lesions that fibrin deposition produced. Animals could be divided into 2 groups: those living up to 3 days which showed predominantly acute lesions and those living 3 days or longer which showed predominantly chronic changes.

1. ACUTE PHASE (16 animals)

Gross Pathology and Biochemistry

Liquoid proved to be a highly lethal compound. 14 animals died or were destroyed *in extremis* within 2 days of treatment due to pulmonary thrombosis, haemorrhage and oedema, and/or acute renal failure (Table 21). Typically (Fig. 45), the kidneys were very congested and swollen, with many small petechiae in their cortices, and larger focal haemorrhages at the cortico-medullary junction and beneath the capsule. As a consequence both blood urea and urine protein levels were raised. Despite the raised blood urea none of the morphological signs of uraemia, listed in Part 2, were present.

Light Microscopy (Tables 23, 26, 29)

No abnormalities were found in case 55, presumably liquoid had not triggered intravascular coagulation and this will be excluded from further discussion.

The major renal lesions were massive thrombosis and congestion with subsequent cortical necrosis (Fig. 47, 48). There was a progressive build up of thrombi and red blood cells in the glomerular capillaries over the first 24 hours after treatment. As well as producing occlusive thrombi (Fig. 47), fibrin could also form as a layer on the GBMs, producing apparent thickening of these structures. Thrombosis and congestion were followed by cellular swelling, necrosis, and haemorrhage into the urinary space. This necrosis led to an influx of polymorphonuclear leucocytes into the kidney, so that both necrotic and non-necrotic glomeruli contained them. The severity of these lesions varied from glomerulus to glomerulus (Fig. 48). In any one kidney section, some glomeruli could be normal, some others were swollen with local or global capillary thrombosis and congestion, while the remainder showed local and global necrosis as well, with blood cells, fibrin and cellular debris liberated into the urinary spaces. In the most severely damaged glomeruli (most prominent in cases 56 and 57), Bowman's capsules were swollen with fibrin, blood cells and cellular debris, with little if any of the capillary tufts remaining (Fig. 49). In a few instances Bowman's capsule had ruptured liberating this material into the interstitium. In dogs dying later than 24 hours after

treatment, some glomeruli were scarred. This was characterized by the expansion of the mesangium, and thickening of capillary walls with globules of collagen-staining material, which in some instances completely occluded the glomerular capillaries. This lesion was typical of the chronic stage and is described in more detail below.

Thrombosis and congestion were also present in afferent and a few efferent arterioles (Fig. 47), occasional interlobular arteries and a few of the vasa recta and peritubular capillaries. Not only were fibrin and blood cells seen occluding the lumina of the arteries and arterioles but they could also be present in the walls of such vessels. As a consequence of this deranged blood supply there was interstitial haemorrhage and oedema. Haemorrhage was most marked in the outer medulla (corresponding to the gross picture) where it led to foci of liquefactive necrosis; this was most severe after 2 or 3 days. Polymorphonuclear leucocyte infiltration was also present in the interstitium, presumably as a result of this necrosis.

As a result of the ischaemia produced by the arterial and glomerular thrombosis, there was widespread tubular degeneration and necrosis, with the proximal convoluted tubules primarily and most severely affected. Distal convoluted tubules could also be affected, so producing almost total necrosis of the cortex (e.g. cases 56, 57). The medulla was relatively spared, apparently because there was little obstruction to its blood supply: part of the medullary blood supply (the vasa recta) is from the

arteriola recta vera which arise from the interlobular arteries and so bypass the glomeruli, and neither the vasa recta nor the interlobular arteries were commonly blocked by thrombi. Featureless eosinophilic cytoplasmic debris completely occluded the lumina of affected tubules, and any remaining nuclei showed either pyknosis, karyolysis or karyorrhexis. Often the tubular basement membrane remained intact, but rupture was seen, particularly after 2 or 3 days, indicating irreversible nephron damage. Surviving tubules often contained hyaline or fibrillar protein casts which commonly stained for fibrin, necrotic debris and occasional red blood cells.

Fibrin Deposits (Table 31)

Fibrin was found in all acute cases and one chronic case (63). Usually a weak staining reaction meant less fibrin was seen. Where a strong reaction was present there was no consistent difference in the amount seen with each stain. In addition, there was no clear differences in the stains in their ability to distinguish fibrin of different ages. It is obvious from the table, however, that PTAH and gram-Weigert stains were unreliable. Yellowsolve should be added to this as several attempts were often needed to obtain a satisfactory result. However, when these stains worked well they all gave good contrast between fibrin and the background allowing small deposits to be seen. On the other hand, Masson 44/41 and Obadiah although reliable, lacked good contrast and small deposits could be overlooked. Picro-Mallory V and MSB emerged the best stains: not only were they reliable with good

contrast, they also had the advantage of showing the change in staining reaction to that of collagen. This change in staining reaction was seen in all animals surviving a day or more after administration of Liquoid. Mixed staining reactions were only very occasionally seen, the deposits staining just for fibrin or collagen. The formation of collagen staining material is indicative of glomerular scarring and characterized the chronic phase (see below).

Electron Microscopy

Not only did the electron microscope confirm many of the light microscopic findings, it also enabled the reaction of the individual parts of the glomerulus to be seen more clearly.

In animals killed up to 2 days after treatment, every glomerulus examined contained fibrin (Figs.54,55,56), with many capillaries being blocked by thrombi (Fig. 55). Typically, emeshed in the fibrin were varying numbers of red blood cells, platelets and sometimes polymorphonuclear leucocytes (Fig. 54). Occasionally a capillary could be occluded by a mass of platelets and blood cells with little or no fibrin to be seen. Many of the red blood cells had an abnormal reduction in electron density particularly in their centres (Fig. 54), while polymorphonuclear leucocytes usually had increased numbers of vacuoles containing granular material of varying electron densities. Smaller deposits of fibrin were present elsewhere in the glomerulus (Fig. 56). It was often seen embedded in the mesangial matrix, lying under the endothelium on the GBM, and in

phagocytic vacuoles of glomerular cells and polymorphonuclear leucocytes. In addition, in the more severely affected glomeruli, fibrin was present in the urinary space (Fig. 57). Some would have reached here via rupture of the GBM (seen with light but not with the electron microscope), but, in addition, fibrin also possibly formed in the urinary space following filtration of fibrinogen and incompletely polymerized molecules of fibrin.

Most fibrin was in an electron dense fluffy, granular or fine non-banded fibrillar form; only in a minority of thrombi were fibres with the "characteristic" periodicity of fibrin seen (Figs. 55, 58). Very occasionally deposits of an electron translucent finely granular and fibrillar material were seen in animals surviving more than 24 hours (Fig. 56). Such material was very similar to that which characterized the chronic phase (see below) and probably corresponded to the collagen staining material seen with the light microscope in these cases.

All parts of the glomerular tuft were affected by the deposition of fibrin, but the reaction varied with the amount present. Where only a little fibrin was seen and occlusive thrombi were absent, the major change was one of endothelial and mesangial cell phagocytosis and swelling (Fig. 56). Material present in the vacuoles in these cells (and also in polymorphonuclear leucocytes and epithelial cells) appeared to undergo a progressive decrease in electron density. This was interpreted as phagocytosis and subsequent lysis of fibrin. Cellular swelling was most notable in the endothelial cells which often expanded to cover over a mass of fibrin (Fig. 56). In some

capillaries this could be repeated several times resulting in partial or complete occlusion of the lumen with alternate layers of fibrin and cytoplasm. Mesangial cells were also swollen, and this plus expansion of the matrix by fibrin deposits resulted in axial expansion of the mesangium (Fig. 56). The swelling of these cells was associated in some instances with an apparent increase in the number of organelles. Epithelial cells were little changed in these capillaries although foot processes could be fused (Fig. 56) and vacuoles increased in number.

In most glomeruli examined large amounts of fibrin were present resulting in capillary thrombosis (Fig. 55). As a result of this all parts of the glomerular tuft showed lesions of degeneration and necrosis. All 3 cell types showed varying degrees of cytoplasmic swelling associated with a loss of density and derangement of organelles. Loss of ribosomes, swelling and distortion of the mitochondria, formation of myelin bodies, increase in the number of vacuoles, and clumping of nuclear chromation were the most prominent changes seen. The foot processes of the epithelial cells were often "fused" indicating a glomerular protein leak (Fig. 57). In addition in such glomeruli, the GBM could be frayed and the mesangial matrix partially or completely destroyed. In the most severely damaged capillaries (Fig. 59) only a thin frayed denuded GBM was left surrounding a mass of thrombus and cellular debris, with no intact glomerular cells to be seen. The urinary spaces of such glomeruli were filled with fibrin, cellular debris and occasional blood cells.

Glomeruli from case 57 which survived for 3 days were different in that some showed extensive production of GBM and matrix-like material characteristic of the chronic phase, in addition to the acute lesions described above.

Only a few blood vessels (afferent arterioles and interlobular arteries) were examined. The light microscopic picture of thrombosis with extrusion of fibrin and red blood cells into the walls of these vessels was confirmed.

Many of the tubules (proximal and distal convoluted tubules) examined showed signs of degeneration and necrosis. Cytoplasmic swelling with loss of electron density, vacuolation, swelling and disruption of mitochondria, severe distortion of the brush border and infoldings of the basal plasma membrane, loss of ribosomes, and formation of myelin bodies, were the most prominent changes present. In the most severely affected tubules no intact cells were seen, the surviving tubular basement membranesenclosing masses of cytoplasmic debris. Fibrin and blood cells were present in some of the tubules examined. Rupture of tubular basement membranes was not seen.

Immunofluorescence Microscopy

Immunofluorescence findings correlated well with those from the light microscope. Fibrin was found to build up progressively in the 24 hours after treatment, within the glomerular capillaries and, to a lesser extent, the afferent arterioles and interlobular arteries. Occasional deposits were also found in the vasa recta. In the glomeruli most fibrin was present as occlusive thrombi

(Fig. 69), but smaller deposits were also found in a linear pattern along the capillary walls and as globules lodged in the mesangium. Immunofluorescence also confirmed that fibrin was present in the tubular lumina.

2. CHRONIC PHASE (21 animals)

Gross Pathology and Biochemistry

In animals surviving 4 days or more after administration of Liquoid a very different picture was seen at necropsy (Table 22). Renal swelling, congestion, and except in one case, haemorrhage were absent. Instead, the kidneys were either normal in appearance or had varying numbers of pale foci in their cortices. As will become apparent later, these foci reflected interstitial fibrosis and, in 4 cases, tubular calcification. This renal damage resulted in some animals having a raised proteinuria, and was severe enough in 3 cases to produce a raised blood urea. However, none of the morphological signs of uraemia were present.

Histological Studies (Tables 24,27,30)

No abnormalities were found in the kidneys of 7 animals (Case 58, 67, 70, 72, 75, 77, 78); presumably intravascular coagulation was not triggered in these dogs and they will be excluded from further discussion. The major renal lesions present in the remaining 14 dogs were focal glomerular scarring, tubular repair, and tubular obliteration by calcification and interstitial fibrosis.

Unlike the typical acute case where most of the glomeruli were affected, residual damage was present in

less than half and usually less than a third of the glomeruli in a chronic case, and lesions were usually local, as opposed to global in the acute cases. Moreover, the nature of the lesions was very different: congestion, haemorrhage and polymorphonuclear leucocyte infiltration were absent, while necrosis and fibrin were very rarely found.

Affected glomeruli were now characterized by lesions of scarring. The major feature was expansion of the mesangial matrix. The staining properties of the new material varied; in some glomeruli it stained like normal mesangium but in cases killed up to 21 days after treatment there were other areas of the mesangium which were expanded by globules of homogeneous, bright, eosinophilic, faintly PAS positive material. In particular, these globules stained very brightly for collagen allowing them to be distinguished from normal mesangial matrix. Occasionally such globules were also present in a peripheral capillary loop as well producing nodular thickening of the capillary wall. However, the major lesions of the capillary wall were thickening, wrinkling and duplication of the GBMs. A small percentage of glomeruli had local or rarely global hypercellularity (Fig. 51). The cells involved had the morphology and position of endothelial and mesangial cells and mitoses were seen in one or two glomeruli from several cases. However, this is of dubious significance as a glomerular mitosis was seen in two cases (67, 70) where no renal lesions were present.

Most damaged glomeruli ($<$ 50% scarring) had localized areas of one or more of these lesions. A few glomeruli

were more severely damaged (> 50% scarring), and not only were the above features more severe and widespread, but there could be other lesions viz: capsular adhesions, CBM thickening and duplication, and obliteration of necrotic lobules by collagen staining material (Fig. 52). It was in such areas of necrosis that fibrin was still present in case 63: in two glomeruli a small focus of fibrin was seen surrounded by an area of collagen staining material which in one case formed a capsular adhesion. A few glomeruli were left completely scarred as a result of the fibrin deposition. These remained as shrunken nodules with their capillaries obliterated by collagen-staining material; some appeared to be hypercellular while others were hypocellular. Finally, there were glomeruli where complete global necrosis had occurred (Fig. 50). All that remained were circular areas bordered by remnants of the CBM containing necrotic debris, calcium precipitates and occasional viable cells, including macrophages and fibroblasts. Eventually such glomeruli were probably completely replaced by fibrous tissue. Necrotic tubules could be very similar in appearance to these glomeruli, but the presence of the remains of an arteriole supplying the structure indicated a glomerular origin. Such arterioles showed signs of degeneration with a loss of cellular detail. Despite a careful search, however, no lesions were identified in viable afferent arterioles or interlobular arteries.

Tubules damaged in the acute phase now showed a variety of lesions. Some were regenerating, and these were composed of a disorganized, crowded mass of proliferating

basophilic cells, with no lumen (Fig. 50). Some still showed evidence of degeneration; these were lined by a low layer of atrophic basophilic epithelium surrounding an abnormally large lumen (Fig. 50). The basement membranes of both types often showed progressive thickening and wrinkling. Finally many tubules were now non viable; regeneration could not occur because either the basement membrane had ruptured or they were blocked by calcium precipitates (Fig. 53). The necrotic debris of these tubules were progressively replaced by fibrosis leaving collapsed remnants of basement membrane and calcium deposits embedded in scar tissue. In contrast, deranged but viable tubules eventually returned to normal. In animals killed 25 days or more after treatment thickened, wrinkled basement membranes around otherwise normal tubules indicated previous damage.

Electron Microscopy

The electron microscope proved invaluable in revealing the nature of the glomerular lesions in the chronic phase. Corresponding to the light microscope findings some glomeruli were normal but others showed localized areas of scarring. No severely scarred glomeruli (> 50% or 100% scarring) were present in the tissues examined. Electron microscopy confirmed the two major lesions to be mesangial expansion and GBM thickening, wrinkling, and duplication.

In affected segments of the tuft, the mesangium was often abnormally prominent due to the presence of increased amounts of matrix or matrix-like material and enlargement and axial expansion of mesangial cells (Fig. 60). Although mitoses were never seen, proliferation was judged to have

taken place as 2 or 3 mesangial cells were seen together more often than in the glomeruli from the control animals. Moreover, in a few instances 4 mesangial cells were seen in one area (Fig. 63). Very occasionally there was circumferential interposition by mesangial cells and matrix between the GBM and endothelium of a capillary.

In animals killed up to 21 days after treatment areas of mesangial enlargement were also caused by the presence of discrete foci of another material (Figs. 61-63). This was composed of granular, fluffy and fine non-banded fibrillar components and was less electron dense than normal mesangial matrix and GBM (Figs. 61,63). In a few foci, banded fibres closely resembling collagen were also present (Fig. 62). Presumably these foci were the ultrastructural equivalent of the brightly staining collagen globules seen with the light microscope. Such foci were usually lodged in the axial region of the capillary and were separated from the capillary lumina by a layer of endothelium, Less often foci, which were usually smaller in size, were lodged in the capillary walls between the endothelium and GBM.

The most common GBM abnormality was irregular subendothelial expansion, accompanied in many instances by splitting or vacuolation of the original basement membrane. As a result, a clear distinction into lamina densa and rara interna was often not possible (Fig. 64). Although similar areas may be present in normal glomeruli (see page 78) they were much more numerous and extensive in liquoid nephropathy. Less often there was irregular subepithelial thickening, alone or in conjunction with the lesions described above. A lesion that was only rarely found

was the formation of a new layer of basement membrane trapping segments of endothelium between it and the original GBM.

Like mesangial cells, endothelial cells in scarred areas were sometimes swollen and occasionally two nuclei could be seen very close together suggesting cell proliferation (Fig. 63), but mitoses were never found. As a result of the cellular swelling, mesangial enlargement and GBM thickening, affected capillaries had narrowed lumina but complete occlusion was not seen. "Fusion" of the epithelial cell foot processes was often present along stretches of deranged GBM indicating a protein leak across them (Fig. 64). In addition, there was an increase in the number of vacuoles in, and microvillous formation by, many of the epithelial cells.

Where interstitial fibrosis was seen around a glomerulus the CBM was often wrinkled. However, thickening of the CBM was never seen nor were adhesions found. In addition, there was no evidence of proliferation of either type of epithelial cell.

The electron microscope revealed that chronic lesions were, in fact, present in the interlobular arteries and afferent arterioles (Fig. 65). Small foci of basement membrane-like material were found in the intima and media of several different vessels. There was also a variety of tubular morphology conforming to the light microscopic findings. Where a tubule was regenerating a mass of haphazardly arranged cells was seen obliterating the lumen (Fig. 66). The cells lacked microvilli and often contained

excess numbers of vacuoles and myelin bodies. Similar cells but much reduced in height were present in tubules with atrophic flattened epithelium. Both types of tubules often had thickened,wrinkled basement membranes. Necrotic tubules were composed of a wrinkled,collapsed mass of basement membrane containing,if anything, an atrophic cell (Fig. 67), necrotic debris and/or a crystalline precipitate (Fig. 68). Interstitial fibrosis was often present around these abnormal types of tubule.

Immunofluorescence Microscopy

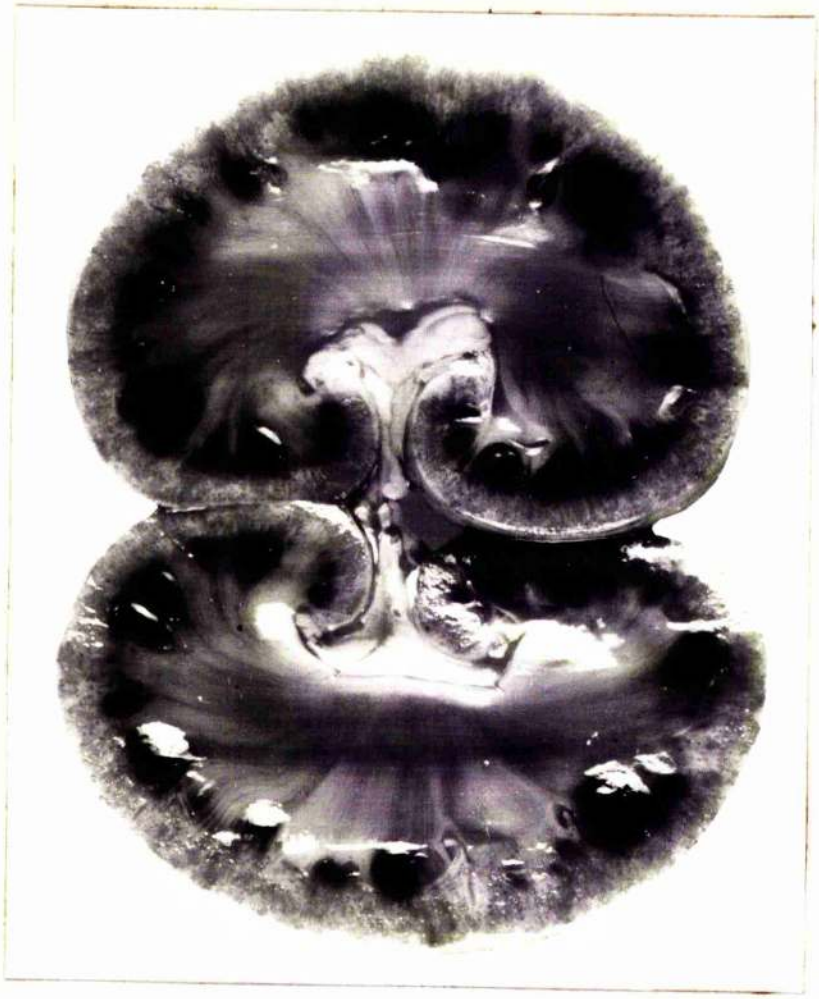
Fibrin was found only in case 63 confirming the light microscope findings. Only a few glomeruli in the section were affected. Unlike the acute stage, fibrin was present only in the mesangium and not in the capillary lumina and walls (Fig. 70).

Fig. 45 Acute Liquoid Nephropathy, 24 hours, Case 50

The kidney is swollen and there are large focal haemorrhages at the cortico-medullary junction. Petechial haemorrhages in the cortex cannot be appreciated in this photograph.

Fig. 46 Chronic Liquoid Nephropathy, 15 days. Case 69

The kidney is shrunken and has a very pale outer cortex as a result of interstitial fibrosis and intra-tubular calcium precipitates.



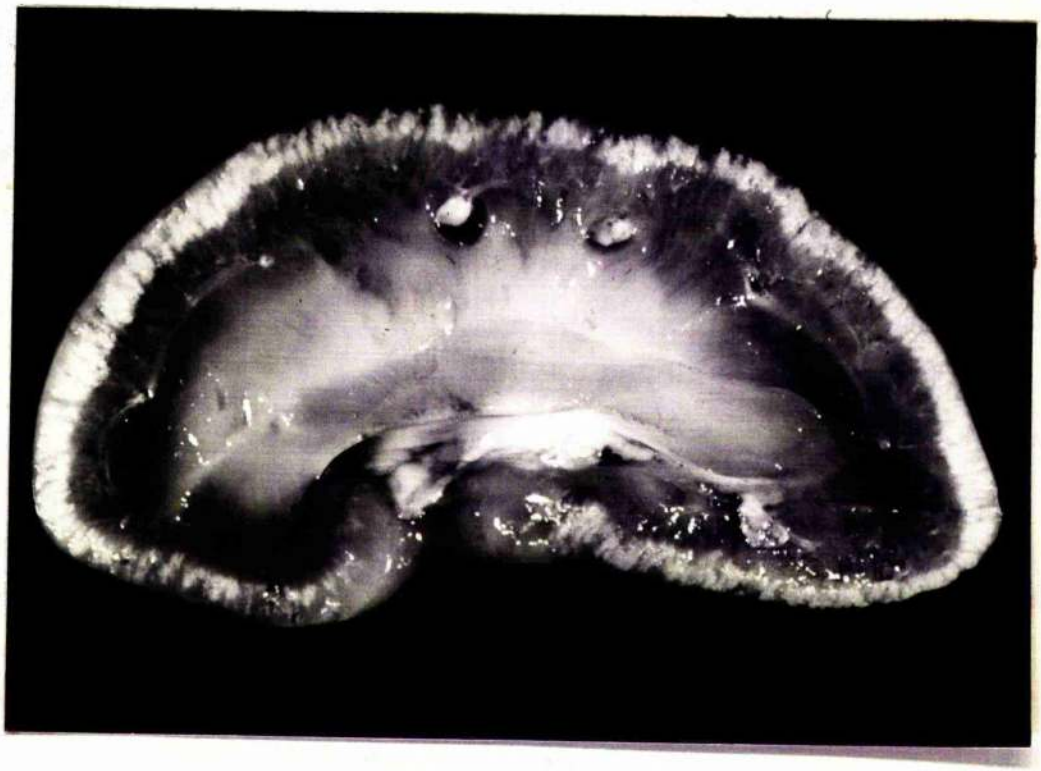


Fig. 47 Acute Liquoid Nephropathy, 3 hours. Case 45

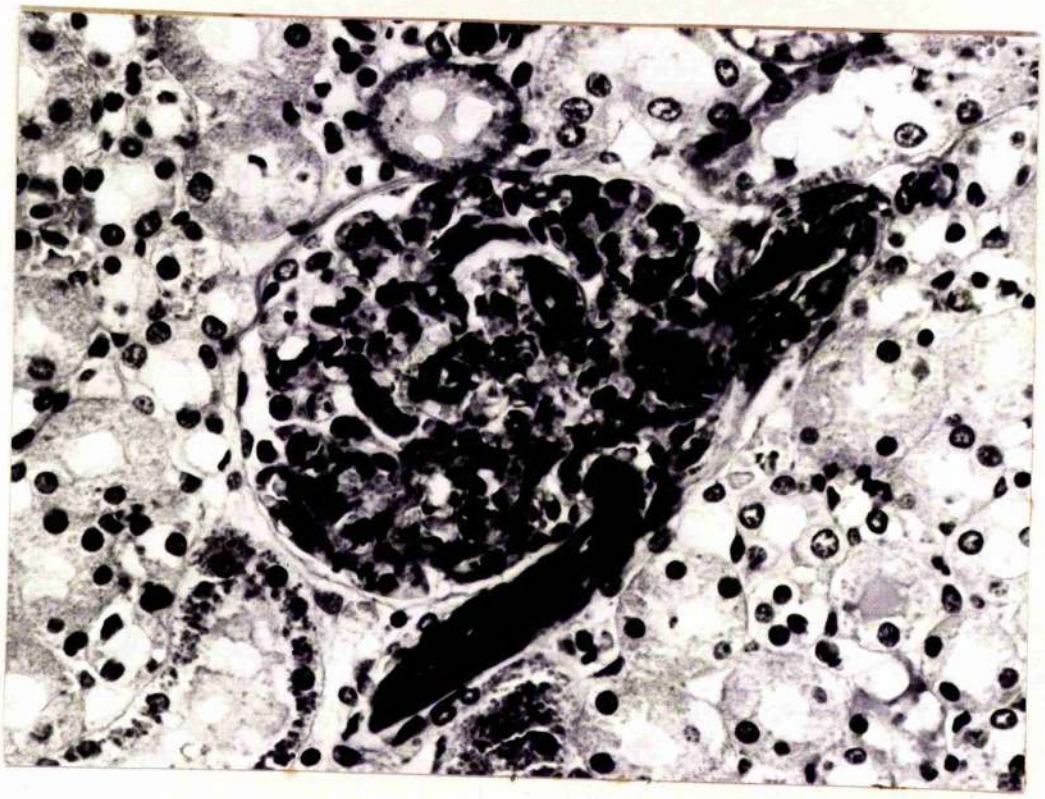
Thrombi (black) occlude both afferent and efferent arterioles as well as the glomerular capillaries.

(Obadiah x 400)

Fig. 48 Acute Liquoid Nephropathy, 24 hours, Case 50

Some glomeruli still appear normal (small arrow) but most are swollen due to thrombosis, congestion, haemorrhage and necrosis (large arrow). The capillary lumina and urinary space are difficult or impossible to distinguish in such glomeruli. Many tubules (T) are necrotic and reduced to featureless eosinophilic masses.

(H and E x 110)



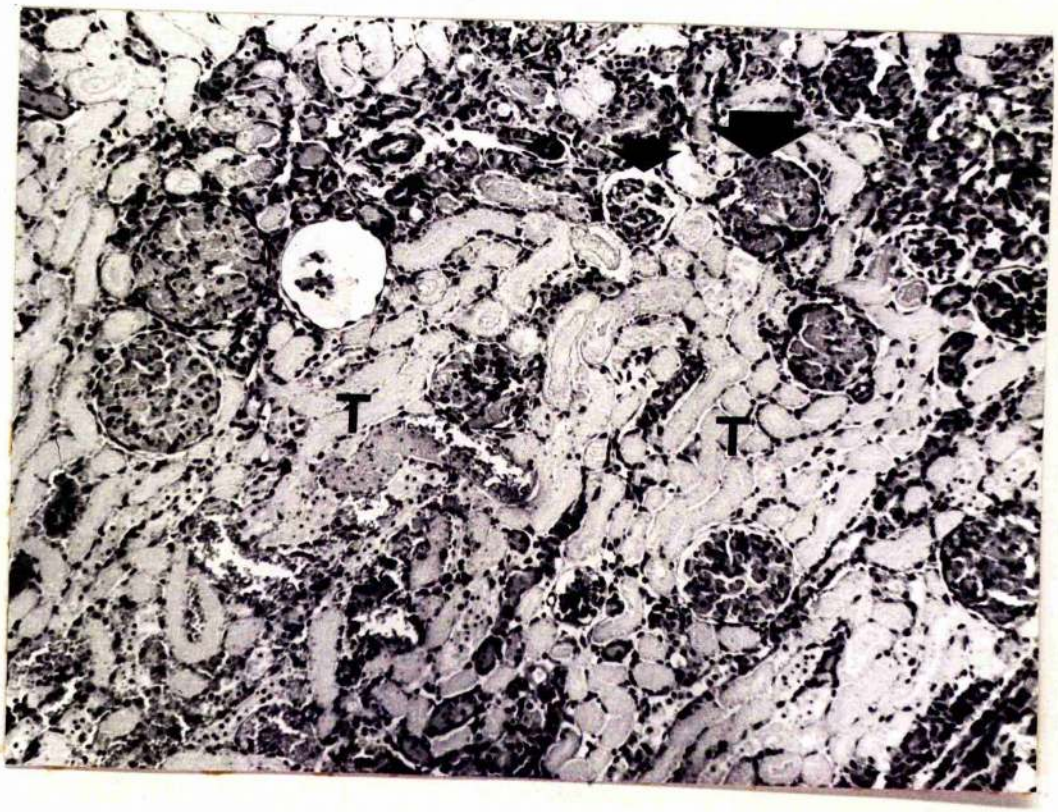

T
T

Fig. 49 Liquoid Nephropathy, 3 days, Case 57

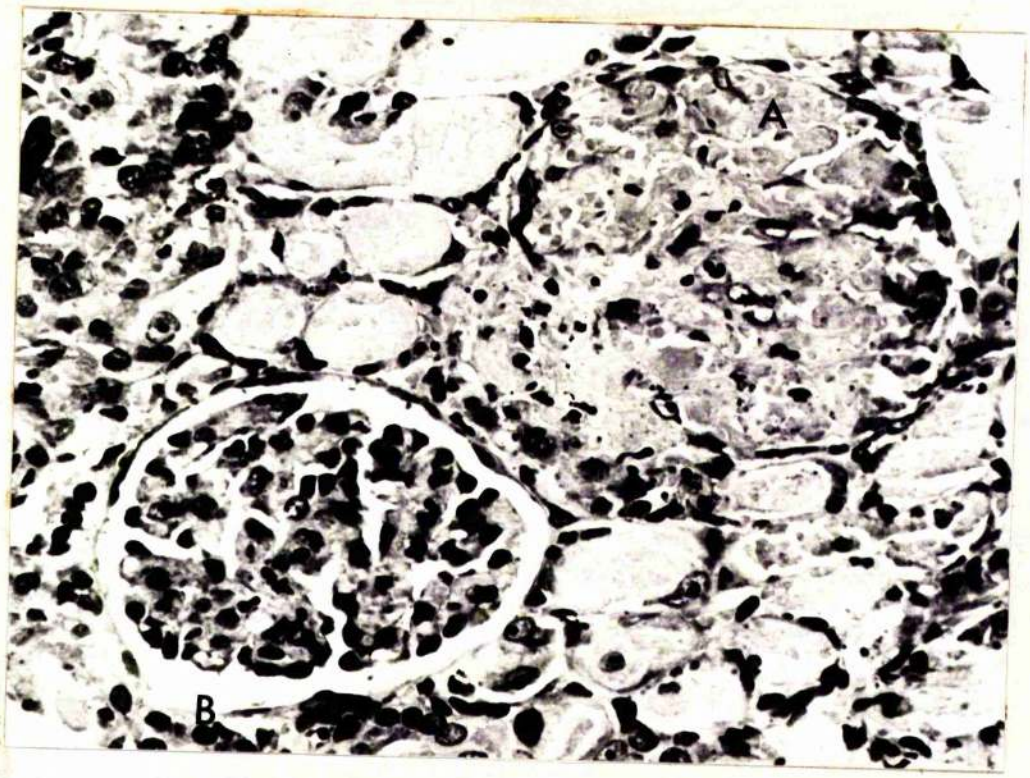
Two types of glomerulus are seen at this stage: those that have been destroyed (A) and those that are recovering and show evidence of scarring (B). Glomerulus A is reduced to a Bowman's capsule swollen with fibrin, necrotic debris, red blood cells and occasional polymorphonuclear leucocytes; no intact part of the tuft can be discerned. In contrast, glomerulus B is normal except that there is occlusion of the capillaries due to mesangial matrix expansion.

(H and E x 400)

Fig. 50 Chronic Liquoid Nephropathy, 5 days, Case 59

Fibroblasts and macrophages are invading the remains of a necrotic glomerulus (arrow). Eventually such a glomerulus will be completely obliterated. A variety of tubular morphology is present. Some contain hyaline casts and are lined by low atrophic epithelium (small *) whilst others are filled with regenerating epithelial cells (large *).

(H and E x 250)


A
B

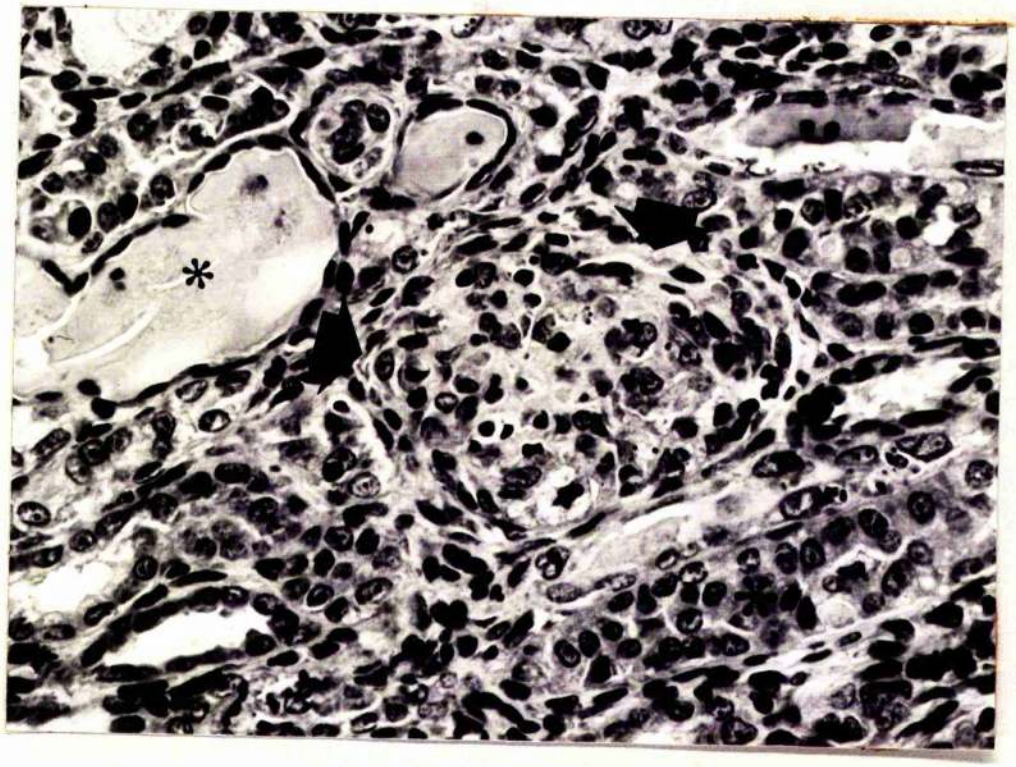

*

Fig. 51 Chronic Liquoid Nephropathy, 15 days, case 69

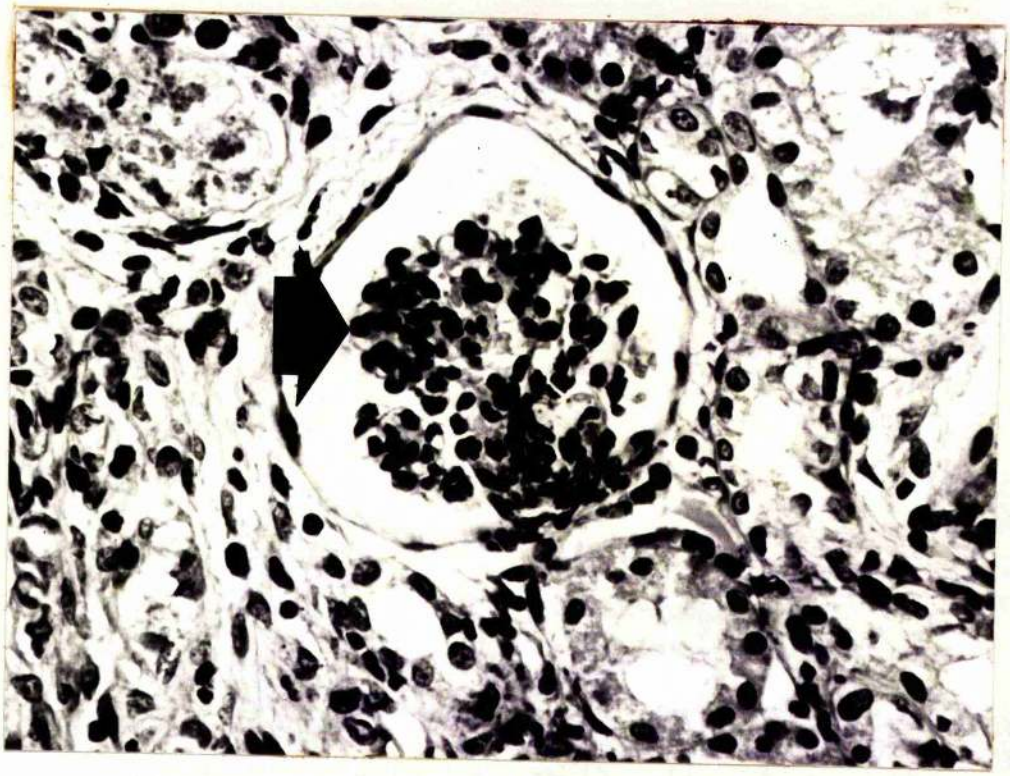
A glomerulus with < 50% of the tuft scarred is seen. The scarred area (arrow) is hypercellular due to an increase in the number of mesangial and endothelial cells.

(H and E x 250)

Fig. 52 Chronic Liquoid Nephropathy, 8 days, case 63

A glomerulus with > 50% of the tuft scarred is seen. The capillaries are obliterated by collagen staining material, and a capsular adhesion composed of similar material is also present.

(M.S.B. x 250)



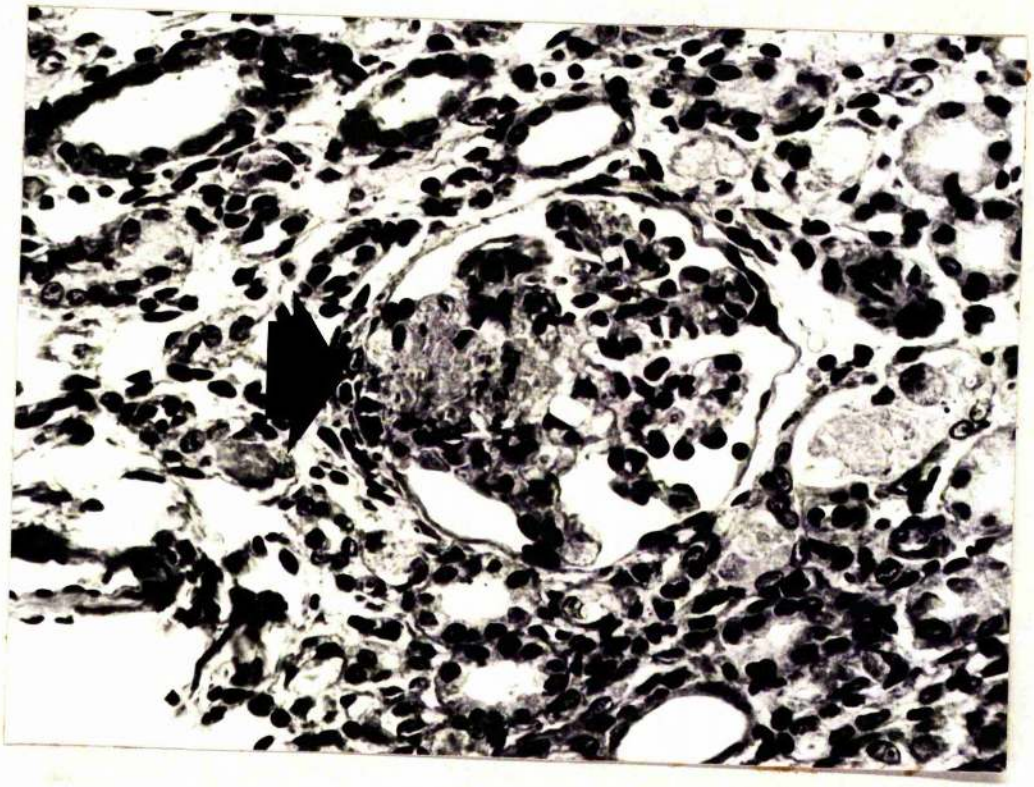


Fig. 53 Chronic Liquoid Nephropathy, 6 days, case 60

Intra-tubular precipitates of calcium (black deposits) are very prominent. These are a result of previous tubular necrosis.

(Von Kossa x 35)

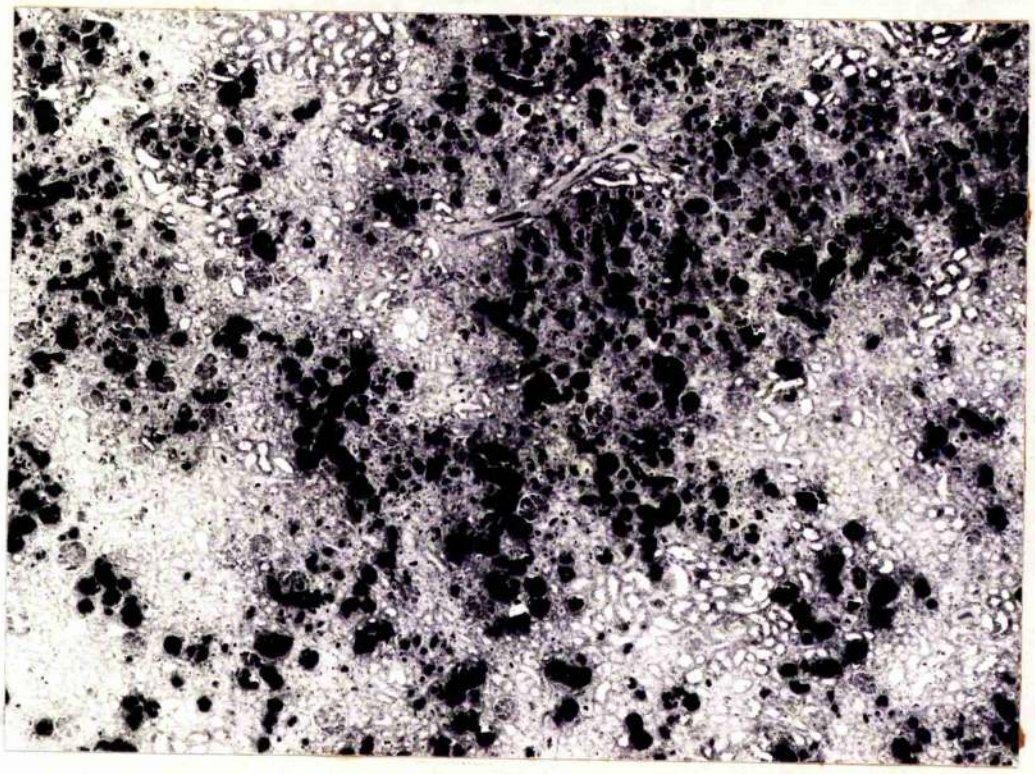


Fig. 54 Acute Liquoid Nephropathy, 4 hours, Case 46

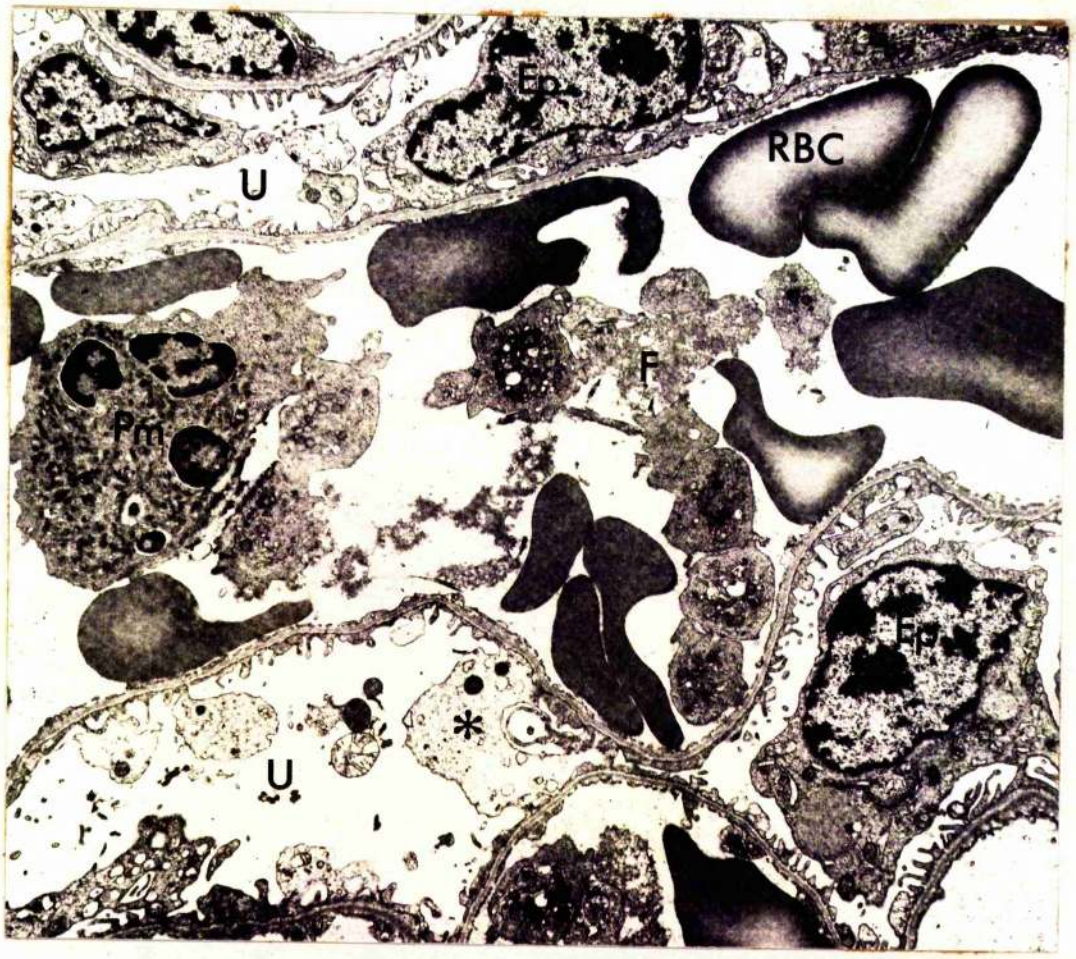
Varying quantities of fibrin (F), platelets(P), red blood cells (RBC) and polymorphonuclear leucocytes (Pm) are present in the glomerular capillaries in the acute phase. Note that the fibrin is in a granular form and that the red blood cells have abnormally pale staining centres. There is also degeneration of some epithelial cells (*); the cytoplasm is abnormally pale and contains a reduced number of organelles.
U. urinary space, Ep Epithelial cell.

(Electron microscopy x 6,000)

Fig. 55 Acute Liquoid Nephropathy, 3 hours, Case 45

Both capillaries are completely occluded by a mass of fibrillar fibrin (F) and pale staining red blood cells (RBC). Epithelial cell cytoplasm (Ep) is abnormally pale.

(Electron microscopy x 6,000)


Ep
RBC
U
F
Pm
Ep
*
U

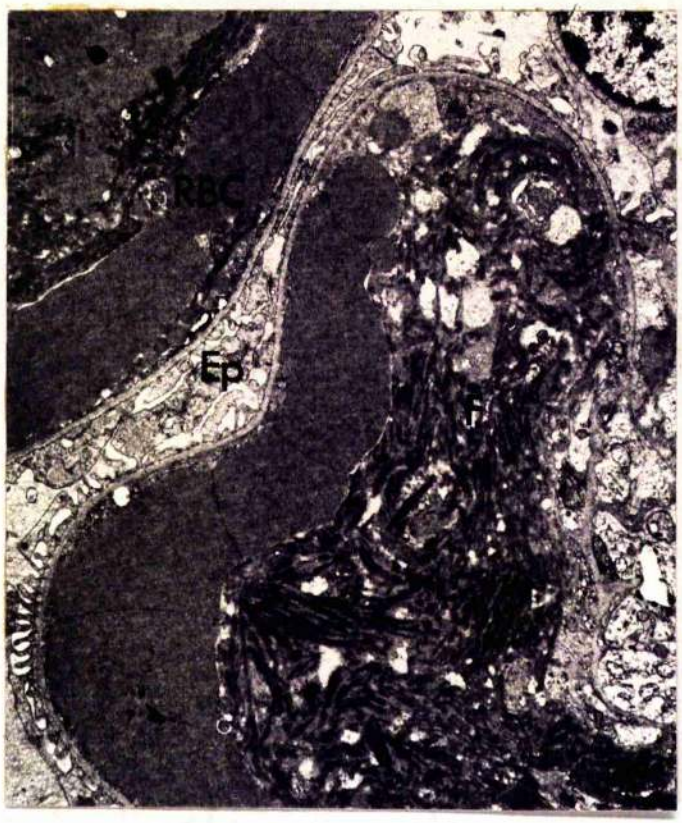

RBC
Ep
F

Fig. 56 Acute Liquoid Nephropathy, 27 hours, Case 54

Fibrin (F) is seen lodged in the mesangium, and between the endothelium and GBM. It is also present in vacuoles in the endothelial cells (arrow). Pale granular material (*) is also present, its position suggesting it is derived from fibrin. Similar material was prominent in the chronic phase (Figs. 61,63) C. capillary.

(Electron microscopy x 10,000)

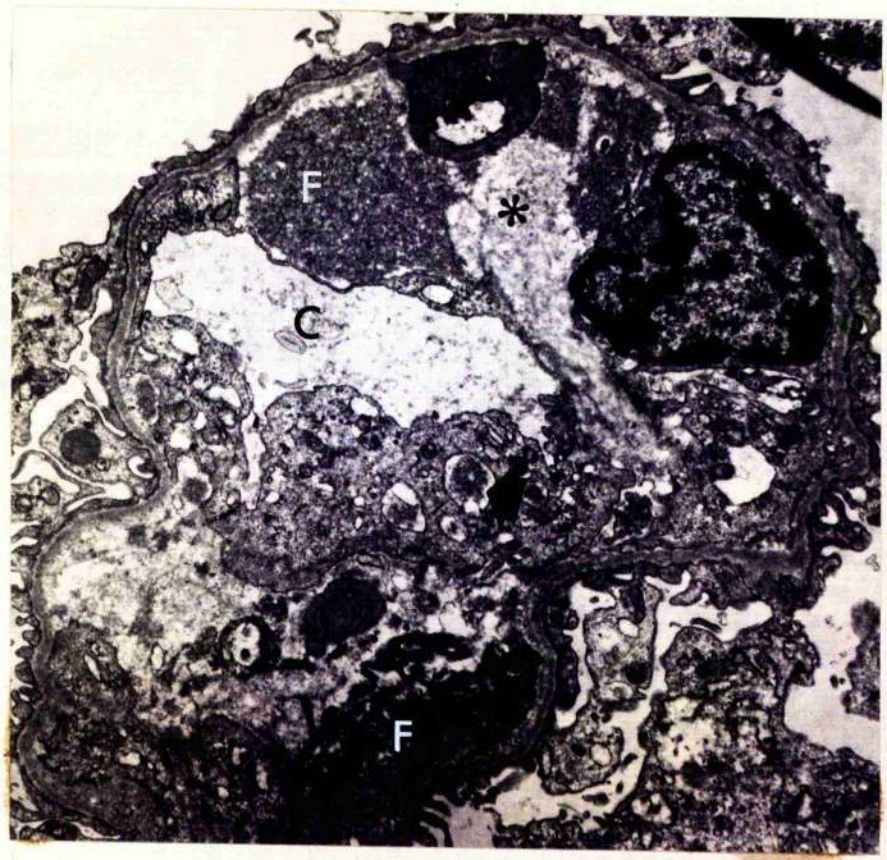

F
*
C
F

Fig. 57 Acute Liquoid Nephropathy, 4 hours, Case 46

Masses of fibrin (F) are seen in the urinary space (U). Any remaining epithelial cytoplasm is swollen and pale and many of the foot processes are "fused" and appear abnormally broad (arrows). Two capillaries are also seen occluded by red blood cells (RBC) and polymorphonuclear leucocytes (Pm).

(Electron microscopy x 15,000)

Fig. 58 High power of the fibrin deposits in Fig. 57. Note the periodic striations of the fibrin fibrils.

(Electron microscopy x 80,000)


U
F
Pm

Fig. 59 Acute Liquoid Nephropathy, 3 days, Case 57

Three necrotic glomerular capillaries (C) are seen. A thin frayed lamina densa (arrowed) remains enclosing a mass of fibrin (F) and occasional pale red blood cells (RBC). No intact glomerular cells remain. The urinary space (U) is filled with necrotic debris.

(Electron microscopy x 10,000)

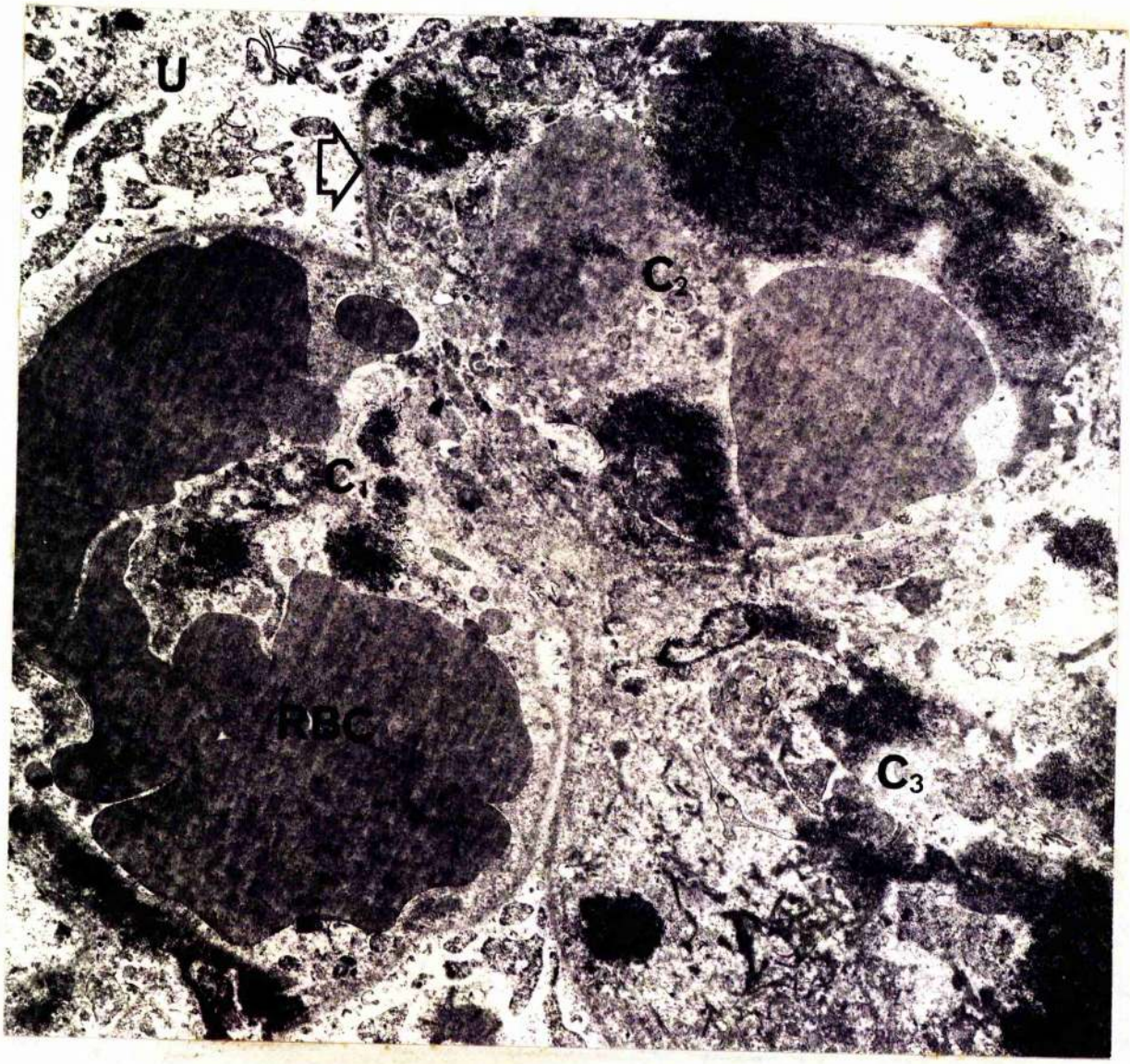

U
C2
C1
RBC
C3

Fig. 60 Chronic Liquoid Nephropathy, 8 days, Case 62

There is a marked reduction in the size of two capillaries (C) due to infiltration by a mesangial cell (M) surrounded by a fine fibrillar material (*). Ep. Epithelial cell, U. urinary space.

(Electron microscopy x 15,000)


Ep
C
*
U
V
*
C

Fig. 61 Chronic Liquoid Nephropathy, 7 days, Case 61

Distinct masses of pale finely granular and fibrillar material (*) are lodged in the mesangium and between the endothelium (E) and GBM (arrow), narrowing the capillary lumen (C). M. mesangial cell, Ep. Epithelial cell.

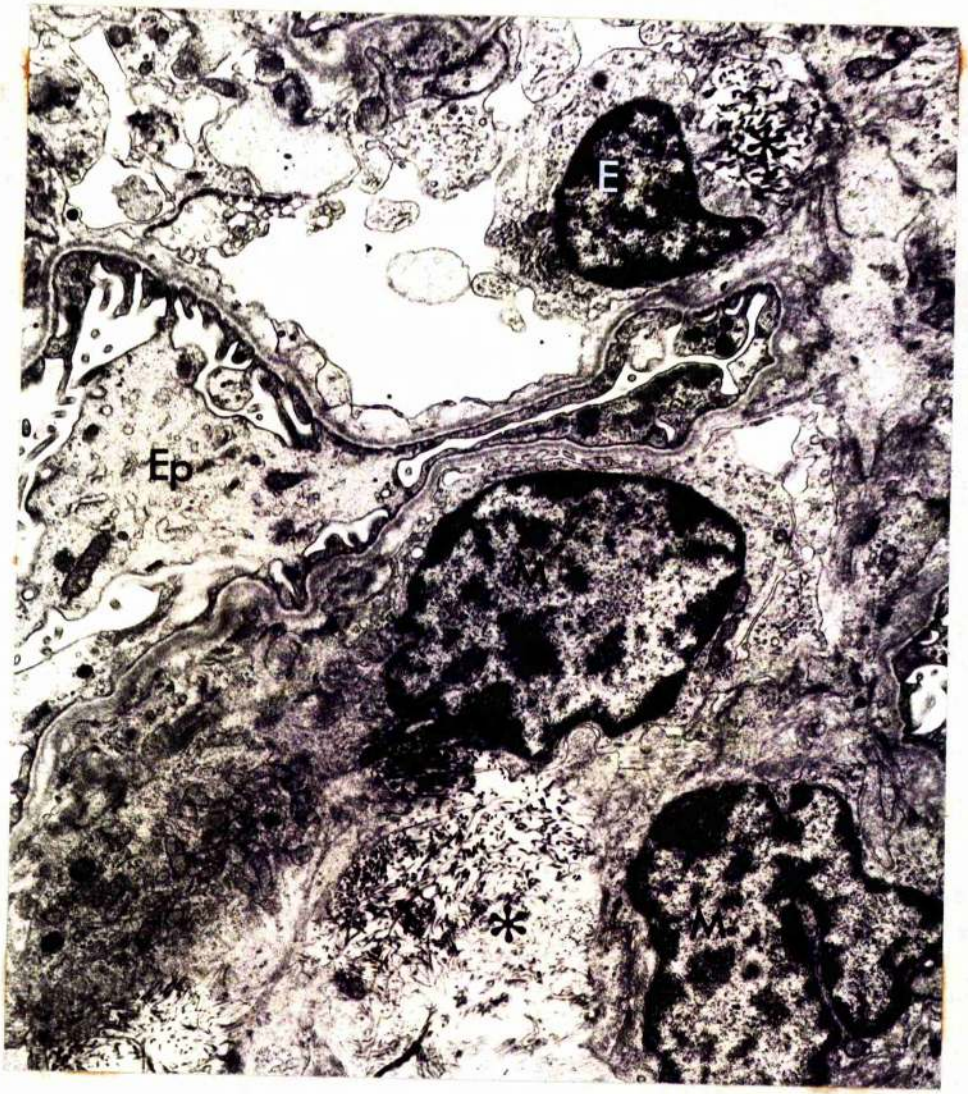
(Electron microscopy x 10,000)


E
C
*
Ep
*
*
C
M

Fig. 62(a,b) Chronic Liquoid Nephropathy, 21 days, Case 71

The mesangial regions in this field are expanded with a fibrillar material (*). Fig. 62(b) shows that some of these fibrils (arrow) have periodic banding and closely resemble collagen. M. Mesangial cell, E. Endothelial cells, Ep. Epithelial cell.

(Electron microscopy x 10,000 and 30,000)


E
Ep
M
M

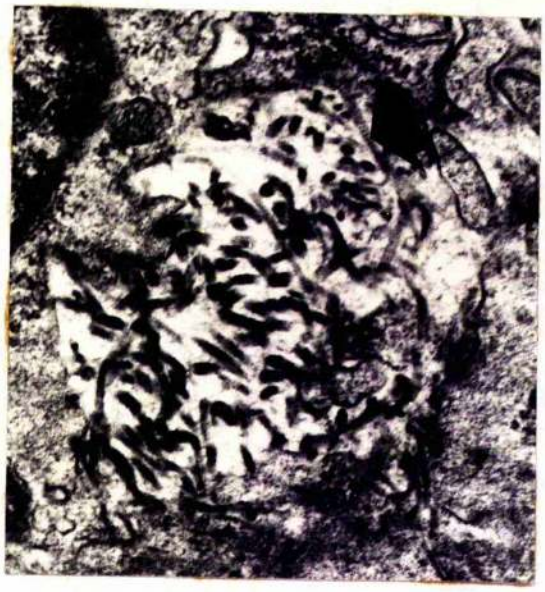


Fig. 63 Chronic Liquoid Nephropathy, 21 days, Case 71

4 mesangial cells (M) are present in this area of localized glomerular hypercellularity. In addition, one capillary contains 2 endothelial cells (E). Note also that foci of pale granular material (*) are present surrounded by endothelial or mesangial cytoplasm. Ep. Epithelial cells.

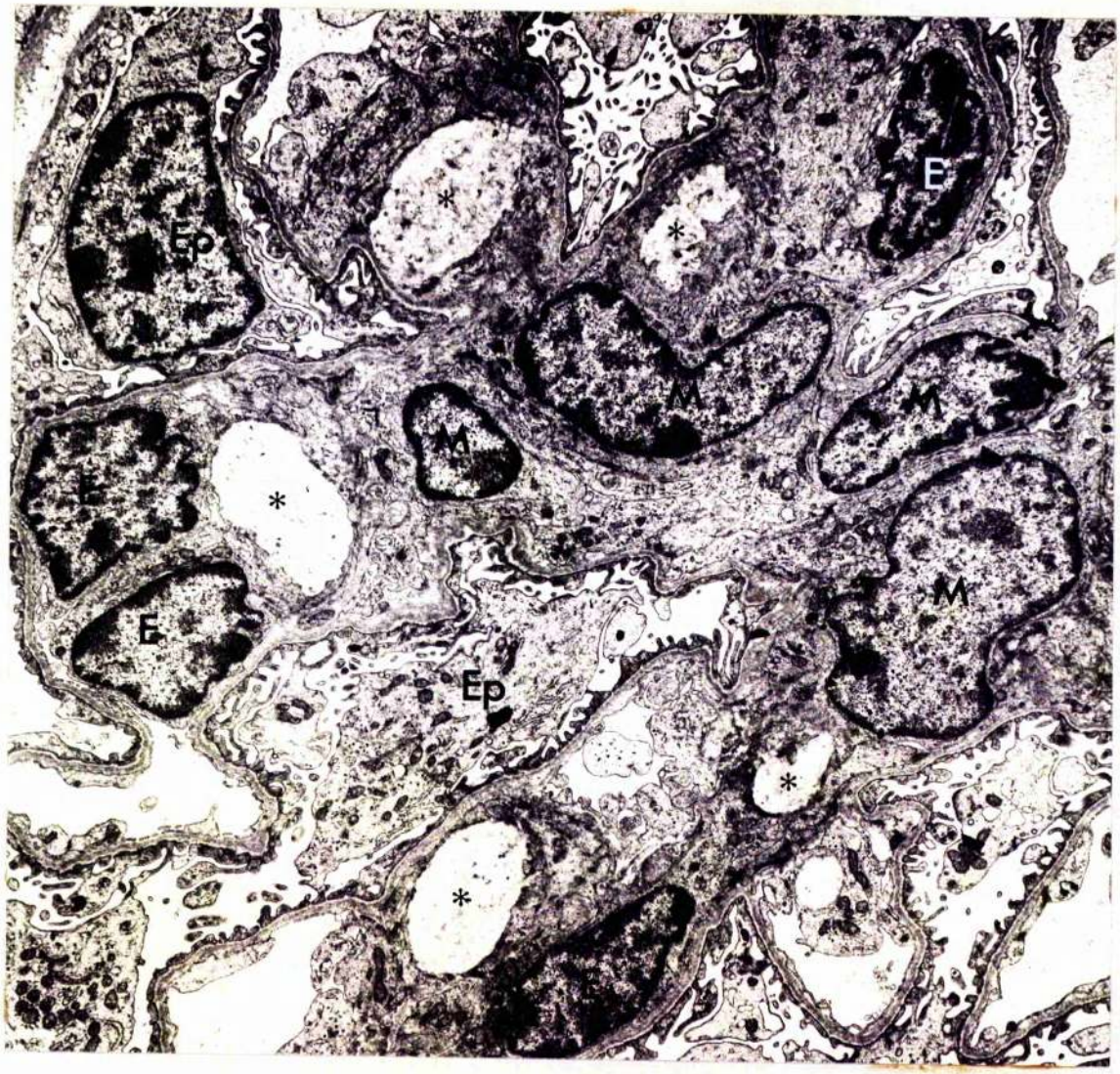

Ep
E
M
M
M
M
E
E
Ep
*
*
*
*
*

Fig. 64 Chronic Liquoid Nephropathy, 21 days, Case 71

A common form of glomerular basement membrane (GBM) abnormality is shown. It is thickened and split, and distinct lamina densa and rara interna cannot be seen. Epithelial foot processes (*) are often "fused" along such stretches of GBM indicating a glomerular protein leak. Note the similarity of this GBM to that in Fig. 25 from a case of CIN. C. capillary lumen containing 2 red blood cells.

(Electron microscopy x 30,000)

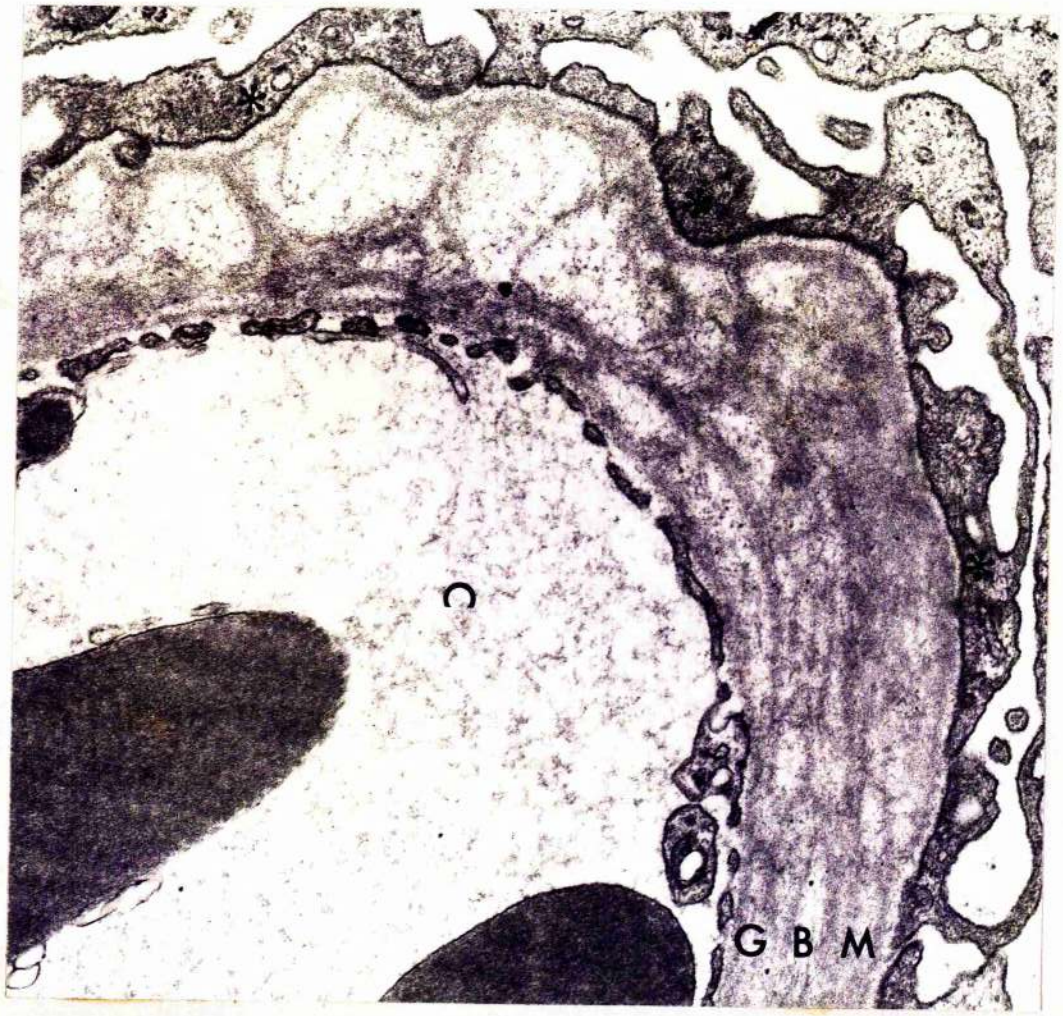

C
G B M

Fig. 65. Chronic Liquoid Nephropathy, 6 days, Case 60

A mass of pale granular and fibrillar basement membrane-like material (*) is lodged in the tunica intima of an afferent arteriole causing narrowing of the lumen (Lu). Such material is similar to that in the glomeruli in figs. 61 and 63. A necrotic nucleus (arrow) is also present. E. Endothelium.

(Electron microscopy x 10,000)

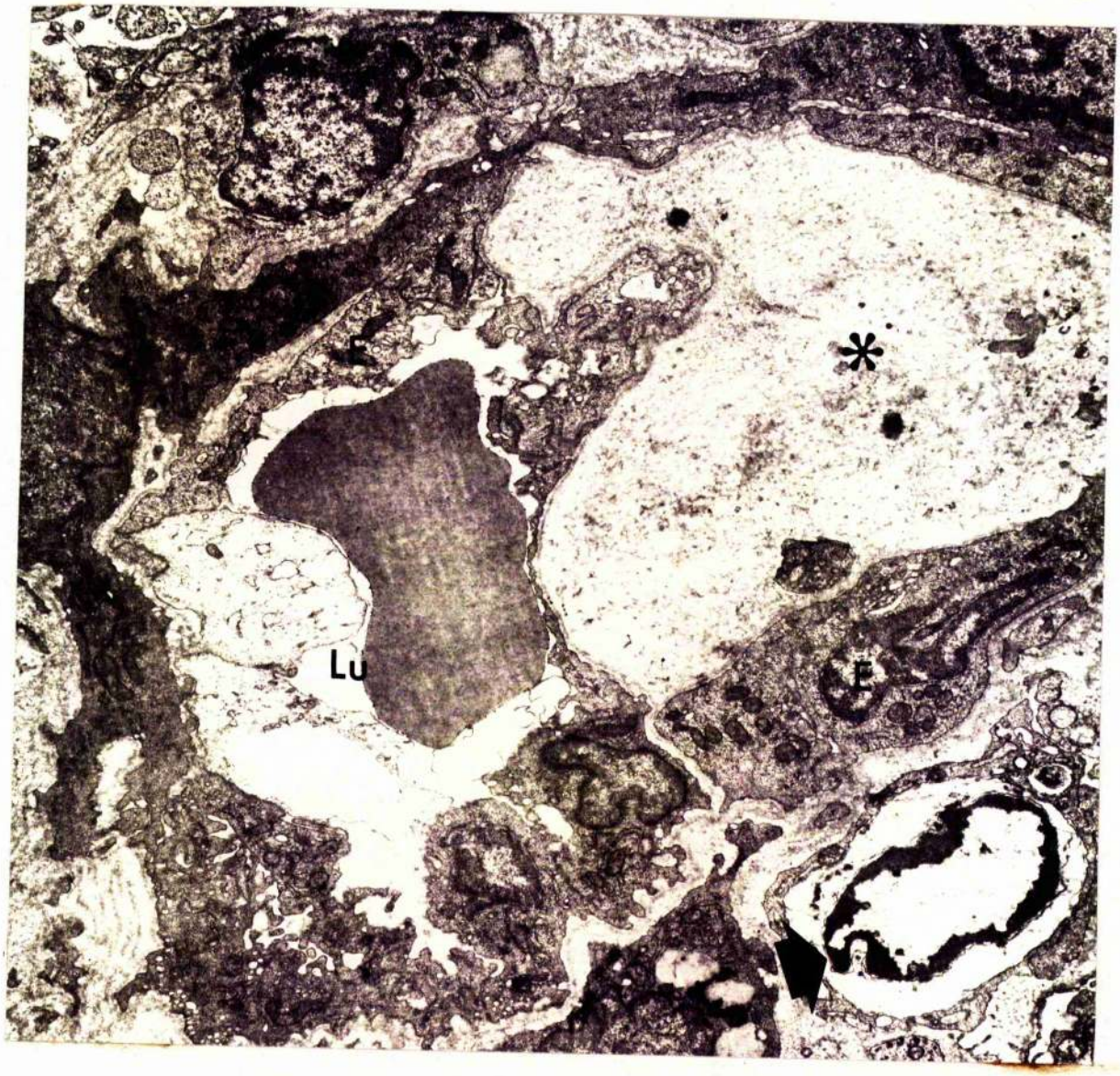

*
Lu
E

Fig. 66 Chronic Liquoid Nephropathy, 8 days, Case 63

Two tubules (T) are present. T_1 is regenerating; it is composed of a haphazard arrangement of epithelial cells which lack villi. No lumen is visible. In contrast cellular degeneration is seen in T_2 with the cells reduced to pale masses of cytoplasm containing few intact organelles.

(Electron microscopy x 6,000)

Fig. 67 Chronic Liquoid Nephropathy, 8 days, Case 63

A typical obsolescent tubule is shown. The tubular basement membrane has collapsed and only 1 atrophic epithelial cell remains.

(Electron microscopy x 10,000)


T1
T2

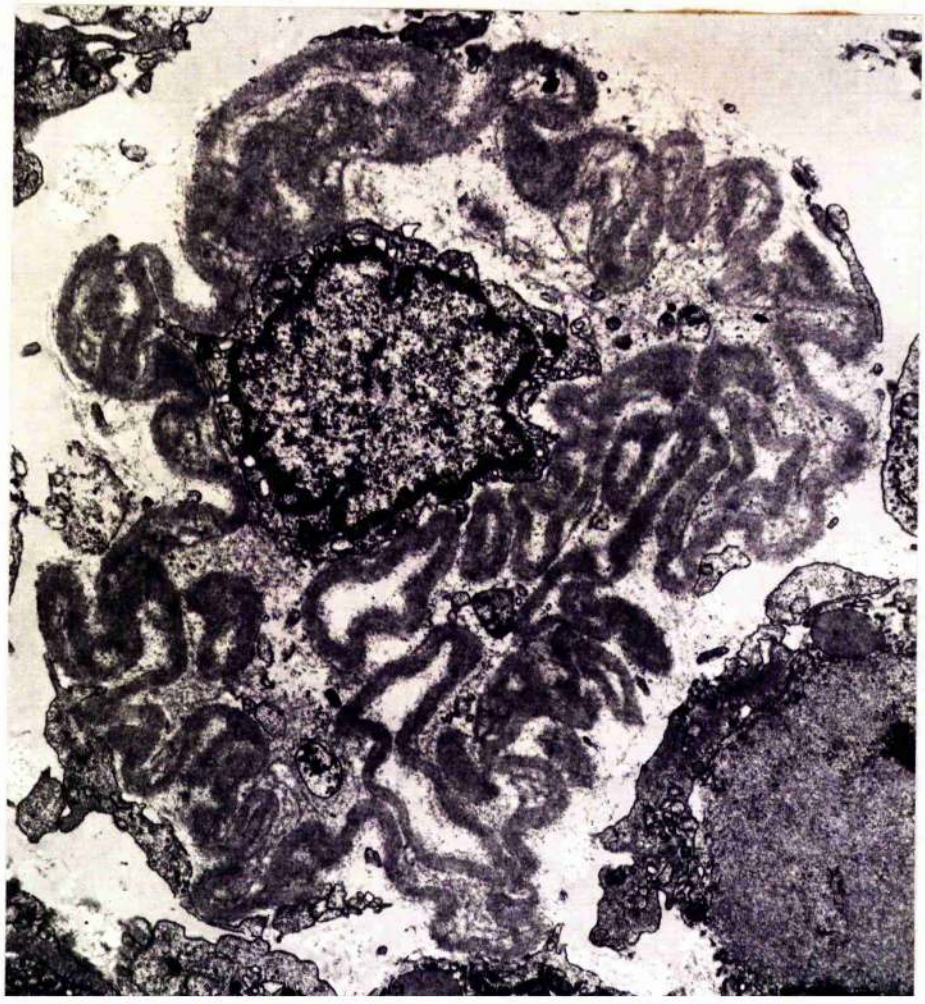


Fig. 68 Chronic Liquoid Nephropathy, 6 days, Case 60

Ultrastructural detail of Fig. 53 - Intra-tubular calcium precipitates (Ca) block the lumen and remaining epithelial cells show signs of degeneration.

(Electron microscopy x 6,000)

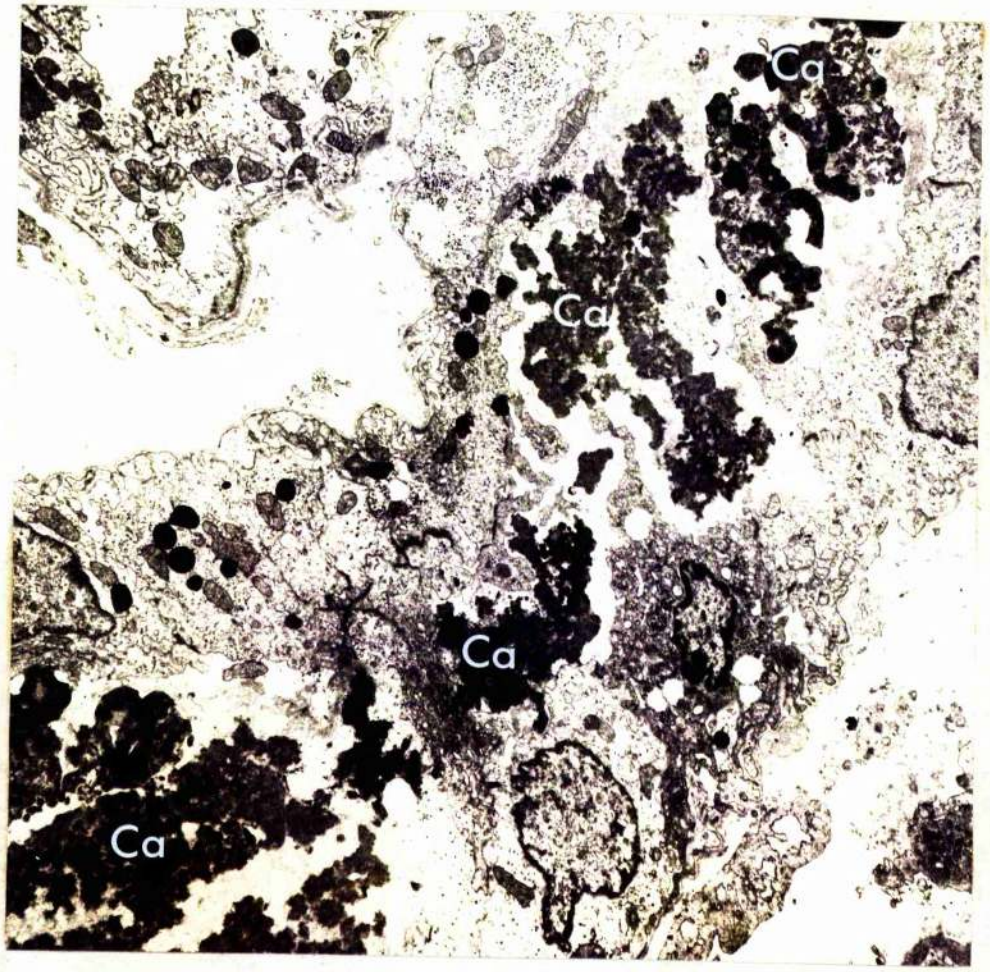

Ca
Ca
Ca
Ca

Fig. 69 Acute Liquoid Nephropathy, 24 hours, Case 50

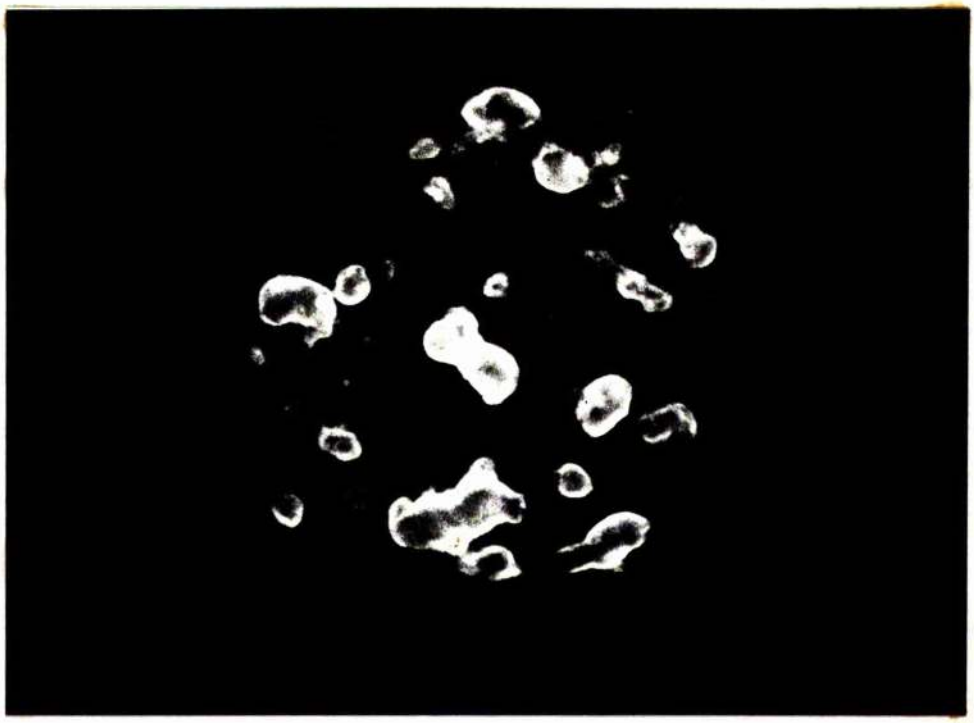
Brightly fluorescing fibrin thrombi are lodged in the glomerular capillaries.

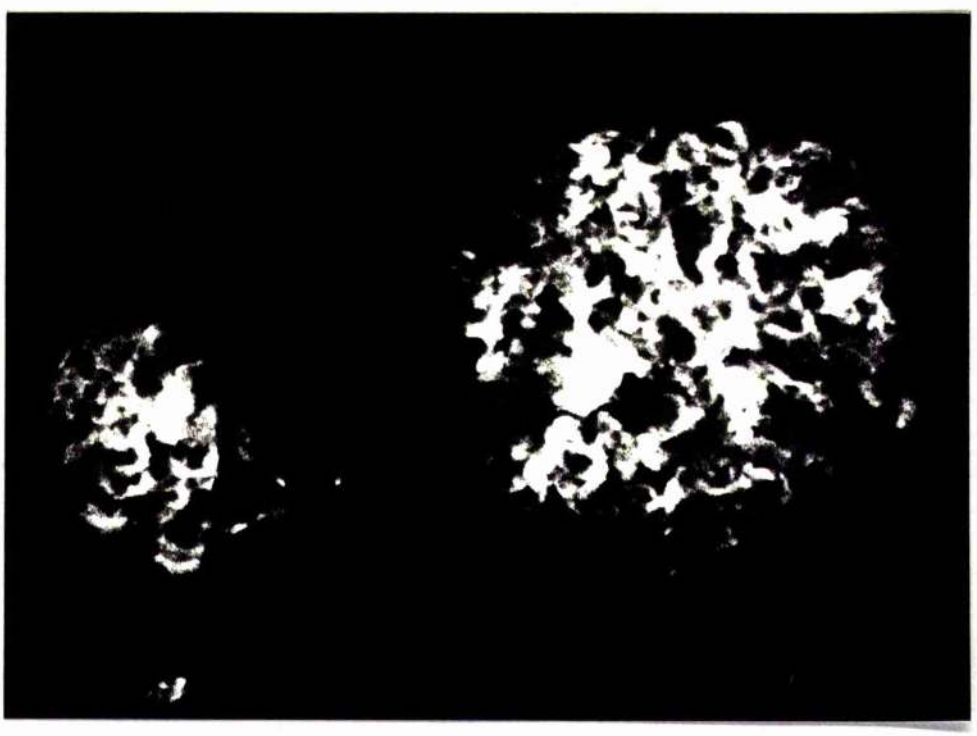
(Immunofluorescence x 300)

Fig. 70 Chronic Liquoid Nephropathy, 8 days, Case 63

Fibrin now remains only in the mesangial areas and the capillaries are free.

(Immunofluorescence x 300)





DISCUSSION

Liquoid is a synthetic acid polymer with anticomplementary, antiphagocytic and serum protein precipitating activity in-vitro (Traub and Lowrance 1970). It also acts as an anticoagulant with a heparin like action in-vitro and in-vivo (Evensen et al. 1967). Thus, the thrombosis produced in this study by the injection of liquoid appears to be paradoxical. It used to be thought this resulted from the precipitation of fibrinogen, a serum protein (Hausman and Dreyfus 1953). Although precipitation may contribute to the total effect, the major reaction has now been shown to be one of coagulation (Evensen et al. 1967). Liquoid triggers disseminated intravascular coagulation by activating Hageman's factor (Müller-Berghaus and Lasch 1970a,b, Urizar et al. 1975), probably via an injurious effect on endothelial cells releasing tissue thromboplastin (Evensen and Sherpo 1973). The clinical and pathological picture produced is indistinguishable from that seen in disseminated intravascular coagulation induced by thrombin or thromboplastin (Vassalli et al. 1963a, Donald et al. 1973), bacterial endotoxin - the generalized Schwartzman reaction (Hjort and Rapaport 1965); agar (Urizar et al. 1969) and, under certain specific conditions, immune complexes (see page 157), although the route by which the coagulation cascade is stimulated is different in each case.

Liquoid was chosen in this study as the glomerular lesions appear to be the result purely of fibrin deposition. However, Urizar et al. (1975) found complement (C3) and IgG in many of the glomerular thrombi formed in liquoid

nephropathy. In addition, Bergstein and Michael (1974) found IgG, IgM, C3 and albumin in glomerular thrombi formed in the generalized Schwartzman reaction in rabbits. Although the presence of albumin suggests non specific trapping of serum proteins, IgM and C3 were present in greater amounts than IgG and albumin, despite the higher serum concentrations of the latter. This, they suggested, could indicate that IgM and C3 play a role in the glomerular damage. However, this may not be relevant as liquoid and endotoxin act in different ways. Whether or not these findings reflect activation, precipitation or non-specific trapping of serum proteins in thrombi is not known. Certainly, liquoid precipitates complement and IgG in-vitro (Traub and Lowrance 1970), and Donald et al. (1973) showed that colloidal material can be trapped in or on glomerular thrombi (formed by thrombin injection). On the other hand, liquoid can activate complement in-vitro through the classical and/or alternate pathways (Loos et al. 1974). If the complement cascade is indeed activated, as it is by endotoxin and immune-complexes, it may be responsible for a part of the glomerular damage. However, two factors suggest that fibrin is the cause of most if not all of the glomerular damage in liquoid nephropathy. Firstly, there is the protection given by anticoagulant and fibrinolytic drugs, coupled with reverse effect of fibrinolytic inhibitors (see page 154). Secondly, identical lesions are produced by thrombin which acts specifically on the conversion of fibrinogen to fibrin (Vassalli et al. 1963a). However, the thrombin-induced lesions described by these authors were less severe than the liquoid ones suggesting other factors

could be involved. Thus, further work is needed to fully evaluate the role, if any, of complement in this model.

The acute lesions were identical to those described in other species, (rats, mice and rabbits) following liquoid injection (Hausman and Dreyfus 1953, Vassalli et al. 1963a, Humair et al. 1969a, Urizar et al. 1975). The basic reaction was one of glomerular and arterial thrombosis leading to ischaemic necrosis of the glomeruli and tubules. As a result of this necrosis there was an influx of polymorphonuclear leucocytes into the kidney. In a normal kidney (and in the chronic stage of liquoid nephropathy) a small proportion of glomeruli contain 1 or 2 circulating polymorphonuclear leucocytes (Table 25). In contrast, in the acute stage of liquoid nephropathy multiple numbers were present in many glomeruli, including ones where necrosis was not evident. In addition, these cells could be present sequestered in the mesangium or in the urinary space, as well as in the glomerular capillaries.

The chronic lesions have been described in detail in two reports. The lesions described in rabbits by Vassalli et al. (1963a) were very similar to those found in this study, viz: focal mesangial expansion, focal endothelial and mesangial hypercellularity, focal wrinkling, thickening and duplication of the GBMs, occasional capsular adhesions and very occasionally partial or complete obliteration of glomeruli. In addition, a few crescents were present in these rabbits. However Urizar et al. (1968) produced slightly different lesions in the rat. In addition to the above, there were also electron dense deposits in a sub-endothelial or mesangial location, infiltration by

circulating mononuclear cells and the presence of variable numbers of cytoplasmic vacuoles and bodies of variable sizes, densities and structure in these cells and in mesangial and endothelial cells. These differences may reflect a species variation, or were the result of a different treatment regime of twice weekly injections of a low dose of liquoid for up to 13 weeks; the lesions produced being most severe at this time. Neither group reported the presence of brightly staining collagen material or its ultrastructural equivalent, foci of electron translucent fluffy, granular and finely fibrillar material.

What is not clear from these studies is the mechanism(s) by which fibrin deposition leads to glomerular scarring. Initially fibrin will be removed from the glomeruli by 3 mechanisms. Firstly, the electron microscope showed that the endothelial and mesangial cells plus polymorphonuclear leucocytes all phagocytose intracapillary fibrin. This process leads to swelling of the endothelial and mesangial cells but its role in the subsequent proliferation and hypercellularity was not clear. The ability of the mesangial cells to remove foreign material (including fibrin) appears to be an important factor in protecting the glomerulus. In a study of human proliferative GN Davison et al. (1973b) found an apparent inverse relationship between mesangial activity and the severity of glomerular scarring. Secondly, the electron microscope also showed that fibrin can pass into the urinary space, and be removed by epithelial cell phagocytosis or excretion in the urine. Fibrin will enter the urinary space via breaks in the GBM but it is also highly possible that the smaller molecules formed during

the formation and breakdown of fibrin pass through an intact but abnormally permeable GBM. Thirdly, where deposits are exposed to the circulation there is lysis by the fibrinolytic systems. The importance of this mechanism cannot be judged from our studies. However, Vassalli et al. (1963a) and Humair et al. (1969b) both produced evidence for its existance. The former authors found that epsilon-amino-caproic acid (a fibrinolytic inhibitor) accentuated the fibrin deposition and subsequent scarring. The latter authors showed fibrinolytic activity to be present in the glomeruli in acute liquoid nephropathy using a fibrin slide test. In addition, they reported that urokinase (a fibrinolytic agent) protected the glomeruli. Once the rate of deposition exceeded the rate of clearance by these mechanisms, fibrin built up and persisted in the glomeruli. This persistance appeared to lead to scarring via two pathways. Firstly, thrombosis led to glomerular necrosis and damaged areas appeared to be replaced by excess matrix and GBM produced by the remaining, viable glomerular cells; a process akin to scarring elsewhere in the body. Secondly, persisting fibrin deposits appeared to be transformed into an electron translucent finely granular fluffy or fibrillar substance which could contain collagen fibres. Large masses of this were easily distinguished from mesangial matrix and GBM with the electron microscope. However, there were many areas of localized GBM thickening and mesangial matrix expansion where it was not possible to tell which process, or indeed if a combination of both, was involved.

Are these lesions permanent? At first glance the

milder, less widespread scarring coupled with the lack of brightly staining collagen material or its ultrastructural equivalent, in cases killed 23 days or later suggests this. However, the lack of tubular damage and interstitial fibrosis indicate the original glomerular fibrin deposition in these animals was less severe than most of the cases killed before this time.

PART 3B: NEPHROTOXIC SERUM NEPHRITIS

MATERIALS AND METHODS

Preparation of Glomerular Basement Membrane (GBM) Antigen

GBM was isolated from normal dog kidneys by a modification of the method used by Wright et al. (1973b). After removal of the capsule and medulla, the cortex was minced with scissors and washed repeatedly with cold (4°C) PBS (pH 7.2). The minced tissue was then gradually forced through a copper sieve with a mesh size of 212 mμ. The resultant filtrate was collected in an ice bath and then poured through a sieve with a mesh size of 63 mμ. The predominantly glomerular residue left was washed off the sieve with cold PBS and the suspension obtained centrifuged at 500g for 5 minutes. After two further washings with cold PBS, a smear was made of the residue and stained with methylene blue to assess the purity of the sample; only fractions containing a high percentage of glomeruli were used (Fig. 72). The glomeruli were then resuspended in an equal volume of cold PBS and ultrasonicated for 5 minutes to break Bowman's capsule and separate cells from the GBM. This suspension was stored at -20°C until use.

Preparation of Nephrotoxic serum (NTS)

Young adult white rabbits were inoculated subcutaneously with 5 ml of a mixture of 2 parts GBM suspension to 1 part complete Freund's adjuvant, weekly for 1 month. Beginning 14 days after the final injection, the animals were bled at weekly intervals and the sera

individually tested for antibody by a gel diffusion test, using the GBM extract as the antigen. 150 ml of sera thus obtained were pooled and tested for specifity of anti-GBM activity on normal dog kidney sections by an indirect immunofluorescence test (see part 2: Materials and Methods), using sheep anti-rabbit immunoglobulin serum conjugated with FITC (Grand Island Biological Company, New York, USA). A sharp linear band of fluorescence was present only on the GBM; this showed the serum did not contain antibodies to other renal antigens.

Experimental Animals

15 puppies aged between 8 and 12 weeks and weighing between 2 and 4½ kgs were used. As a preliminary experiment to find the smallest dose of antiserum that gave a severe degree of renal damage, 2 pups were given an intravenous injection of 5 ml and 10 ml of anti-GBM antiserum and killed 6 and 7 days later respectively. 10 ml was subsequently given to 10 dogs which were killed at a range of times from 3 to 29 days later. 3 control dogs were given 10 ml of normal rabbit serum and killed at 3, 11 and 14 days respectively after injection. All dogs were anaesthetised with "Immobilon" before treatment and revived immediately afterwards with "Revivon" (both products; Reckitt and Colman Hull, Britain).

Light, Electron and Immunofluorescence Microscopy

Methods used in light and electron microscopy were identical to those described in Part 2. In the immuno-fluorescence studies, the following FITC conjugated antisera

were used: rabbit anti-dog IgG, IgM, C3 and fibrinogen (Cappel Laboratories, Downingtown, USA) and sheep anti-rabbit immunoglobulin (Grand Island Biological Company, New York, USA).

Biochemistry

Whenever possible samples were taken at the time of death for blood urea and urine protein measurements.

TABLE 32

NEPHROTOXIC SERUM (NTS) NEPHRITIS

GENERAL INFORMATION AND POST MORTEM FINDINGS

CASE	TREATMENT	DOSE ml	DAY KILLED	BLOOD UREA mmol.L^{-1}	URINE PROTEIN mg.100ml^{-1}	RENAL PATHOLOGY		
						SWELLING	HAEMORRHAGE	SCARRING
85	NTS	10	3	4.2	> 550	+	+	-
86	NTS	10	4	20.1	330	++	++	-
87	NTS	10	5	25.0	240	+	+	-
88	NTS	5	6	4.2	NR	-	-	-
89	NTS	10	7	NR	NR	+	+	-
90	NTS	10	7	13.2	320	-	+	-
91	NTS	10	9	5.4	300	+	+	-
92	NTS	10	11	5.7	180	+	+	-
93	NTS	10	14	11.1	160	+	+	-
94	NTS	10	16	3.8	530	+	+	+
95	NTS	10	21	9.4	105	-	+	+
96	NTS	10	29	3.1	160	-	+	+
97	NRS	10	3	14.8	100	-	-	-
98	NRS	10	11	4.2	50	-	-	-
99	NRS	10	16	4.9	160	-	+	-

NTS Nephrotoxic serum
NRS Normal rabbit serum

TABLE 33

NTS NEPHRITIS: GLOMERULAR SCARRING

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED				
		NO SCARRING	<50% SCARRING	>50% SCARRING	100% SCARRING CONTRACTED	100% SCARRING CYSTIC
85	206	39.8	45.6	12.6	2.0	0
86	219	15.1	58.4	24.2	2.3	0
87	311	11.2	64.0	21.9	1.9	1.0
88	227	30.4	52.9	14.1	2.6	0
89	267	26.2	55.8	16.9	1.1	0
90	355	6.8	56.4	31.8	4.2	0.8
91	201	4.0	51.7	39.8	4.5	0
92	292	7.5	40.7	39.4	11.0	1.4
93	309	2.3	23.3	32.7	38.2	3.5
94	251	10.0	62.5	21.5	6.0	0
95	183	4.9	56.9	33.9	3.8	0.5
96	217	29.0	51.1	11.1	6.0	2.8
97	264	97.0	1.5	0	1.5	0
98	229	93.9	5.2	0	0.9	0
99	239	97.9	0.8	0.5	0.8	0

TABLE 34a

NTS NEPHRITIS: GLOMERULAR MORPHOLOGY

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED						
		TOTAL[B]	FIBRIN DEPOSITS[A]				NECROSIS	POLYMORPHONUCLEAR LEUCOCYTE INFILTRATION
			EPITHELIAL CELLS	URINARY SPACE	CAPILLARY	ADHESIONS		
85	200	66.0	1.0	32.0	33.0	0	61.0	11.0
86	222	89.7	3.2	66.2	17.1	3.2	83.3	19.8
87	296	30.1	13.9	5.4	8.4	2.4	59.8	12.2
88	216	19.9	7.9	1.8	5.6	4.6	8.8	3.7
89	251	31.1	8.0	10.8	2.8	9.5	17.5	5.2
90	356	37.1	21.1	5.9	6.7	3.4	18.5	6.2
91	189	59.3	10.1	17.5	13.7	18.0	35.5	10.6
92	271	52.4	23.2	5.5	3.0	20.7	18.5	7.0
93	169	36.7	30.8	0	4.1	1.8	20.7	4.7
94	238	39.9	29.9	2.0	2.5	5.5	2.5	6.7
95	198	26.8	20.7	0	1.0	5.1	6.6	6.2
96	203	4.4	4.4	0	0	0	0	3.4
97	257	0	0	0	0	0	0	6.2
98	225	0	0	0	0	0	0	5.3
99	243	0.8	0.8	0	0	0	0.4	7.0

A Position of largest deposit
B Total percentage of glomeruli affected.

TABLE 34b

NTS NEPHRITIS: GLOMERULAR MORPHOLOGY (CONTINUED)

CASE	NUMBER OF GLOMERULI COUNTED	PERCENTAGE OF GLOMERULI AFFECTED								
		TUFT HYPERCELLULARITY			GBM THICKENING			CAPSULAR ADHESIONS	CAPSULAR THICKENING	"CRESCENTS"
		TOTAL	LOCAL	GLOBAL	TOTAL	LOCAL	GLOBAL			
85	200	82.0[M]	23.0	59.0	72.0	55.0	17.0	20.0	0	1.0
86	222	98.2[M]	9.9	88.3	79.7	58.1	21.6	22.1	0	1.8
87	296	79.7[M]	27.3	52.4	68.3	52.4	15.9	29.4	0	2.0
88	216	82.9[M]	13.0	69.9	55.6	41.7	13.9	18.5	0	1.4
89	251	82.1[M]	23.1	59.0	59.3	41.4	17.9	19.9	0	4.0
90	356	90.2[M]	25.6	64.6	75.5	40.4	35.1	29.2	0	8.4
91	189	95.8[M]	8.5	87.3	83.5	46.5	37.0	54.0	15.9	19.0
92	271	94.8[M]	15.5	79.3	91.9	31.4	60.5	50.9	36.9	15.5
93	169	97.7	16.6	81.1	95.3	21.3	74.0	62.7	68.6	30.2
94	238	78.6[M]	21.0	57.6	66.4	49.6	16.8	49.2	34.0	13.9
95	198	68.6[M]	34.3	34.3	93.4	41.9	51.5	43.9	40.4	6.1
96	203	64.0	25.6	38.4	76.3	50.2	26.1	35.5	37.9	6.4
97	257	2.7	2.7	0	5.1	5.1	0	0	0	0
98	225	4.9[M]	4.9	0	7.1	6.7	0.4	0	0	0
99	243	7.0	7.0	0	7.8	7.4	0.4	0	0	0

GBM Glomerular basement membrane
M Mitoses present
"crescents" - crescent shaped mass of cells in or around a glomerulus.

TABLE 35

NTS NEPHRITIS: NON-GLOMERULAR HISTOPATHOLOGY

CASE	TUBULES					INTERSTITIUM		
	DEGENERATION AND NECROSIS	REGENERATION	BASEMENT MEMBRANE THICKENING	PROTEIN CASTS	HAEMORRHAGE	OEDEMA	FIBROSIS	CELLULAR INFILTRATE
85	1+	-	-	2+	1+	1+	-	-
86	3+	-	-	4+	2+	4+	-	-
87	2+	-	-	4+	1+	2+	-	--
88	1+	-	-	1+	1+	-	-	-
89	1+	-	-	1+	1+	1+	-	-
90	2+	1+	1+	3+	1+	1+	1+	1+
91	2+	1+	1+	1+	1+	1+	1+	1+
92	2+	1+	1+	4+	2+	1+	1+	1+
93	3+	1+	2+	4+	1+	1+	2+	1+
94	2+	1+	1+	2+	1+	1+	2+	1+
95	1+	1+	1+	1+	1+	1+	1+	2+
96	1+	-	1+	2+	1+	-	1+	2+
97	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-
99	-	-	-	1+	1+	-	-	1+

RESULTS

Gross Pathology and Biochemistry (Table 32)

In animals killed up to 16 days after being given NTS, the kidneys were swollen and had varying numbers of petechial haemorrhages in their cortices. After 16 days the post mortem picture altered; the kidneys were normal in size and had very few haemorrhages, but they were streaked and pitted with fine pale scars running through both cortex and medulla. As a reflection of the renal lesions, the urine protein level was raised in all 11 cases tested, and the damage was severe enough in 5 cases to cause retention of urea. However, none of the morphological signs of uraemia (described in Part 2) were ever present. Extra-renal lesions were present in only one case (86) where a purulent pneumonia was present in both anterior and middle lobes of the lung. Bordetella bronchiseptica and a coliform bacillus were isolated from the pulmonary lesions.

In the control animals (given normal rabbit serum) swelling and scarring of the kidneys were absent, but in case 99 a very few petechial haemorrhages were present in the renal cortices. Low levels of proteinurea were present in all 3 animals and case 97 had a slightly raised blood urea level.

Light Microscopy (Tables 33, 34a, b, 35)

Animals killed up to 7 days after treatment (cases 85-90) were characterized by glomerular fibrin deposition, necrosis and hypercellularity. Fibrin deposits were present focally in the glomeruli (Fig. 73). In the tuft itself

intracapillary deposits often lay against the GBM causing apparent thickening of this structure, while the larger deposits formed occlusive thrombi. Fibrin was often associated with areas of tuft necrosis, and the destruction of the capillary walls in such areas allowed large quantities of fibrin (and some red blood cells) to be liberated into the urinary space; in the majority of affected glomeruli this was where the largest masses of fibrin were present. In some glomeruli this liberated fibrin penetrated between the parietal epithelial cells and the CBM, and very rarely, into the periglomerular interstitium as well. In many instances the presence of fibrin in the urinary space led to the formation of capsular adhesions composed of a combination of collagen staining material and fibrin. Not all fibrin in the urinary space had this fate; the presence of globules staining for fibrin in both visceral and parietal epithelial cell indicated these cells were active in the phagocytosis and removal of fibrin. Most areas of glomerular necrosis ranged in size from a few capillaries to a whole lobule. Very occasionally a whole tuft was necrotic (Figs. 73, 74, 76). In areas of necrosis the tuft was reduced to an eosinophilic mass of cytoplasmic debris, often containing fibrin, scattered nuclear fragments and occasional polymorphonuclear leucocytes. Elsewhere, tuft structure was well maintained but many capillary loops were occluded by a combination of cellular swelling, mesangial matrix expansion, GBM thickening and duplication, and a local or global increase in cellularity (Fig. 74). Mitoses were identified in a proportion of the glomeruli in all cases indicating active cell proliferation.

Many of the excess cells resembled endothelial and mesangial cells but the extensive infiltration of monocytes clearly seen under the electron microscope could not be appreciated with the light microscope. Polymorphonuclear leucocyte infiltration was limited to small numbers in areas of necrosis and was not a major cause of tuft hypercellularity. Very occasionally an increase in cellularity resulted from the presence of a crescent shaped mass of cells in the urinary space (Fig. 76). The exact nature of these cells could not be determined with the light microscope.

As a result of this glomerular damage, protein casts and occasional blood cells appeared in the tubules. Where glomerular damage was particularly severe, restriction in blood supply to the tubular capillaries resulted in tubular degeneration and necrosis (Fig. 76). Some tubules were lined by irregularly arranged swollen cells with pyknotic nuclei, while others were lined by flattened atrophic epithelium. However, areas composed of many completely necrotic tubules, typical of acute liquoid nephropathy, were never seen.

In the period between 9 and 16 days after treatment (cases 91-94) the destructive changes in the glomerulus subsided, and scarring and hypercellularity became more prominent. Areas of glomerular necrosis, although still fairly widespread, were smaller in size whilst recent fibrin deposition appeared to be minimal. Little fibrin was now present in the capillaries or free in the urinary spaces. However, in many glomeruli there were small deposits associated with capsular adhesions and/or globules staining for fibrin in visceral and parietal epithelial cells. All

the lesions described before as comprising glomerular tuft scarring were now prominent, viz.: thickening, wrinkling and duplication of the GBMs, expansion of the mesangium and, in about half the glomeruli, the formation of capsular adhesions (Figs. 75, 77, 73). These areas were composed of collagen staining, PAS positive material. Although in many glomeruli this new material had a fairly bright staining reaction for collagen, the discrete shiny foci that characterized chronic liquoid nephropathy were never seen. Accompanying these tuft lesions were often changes in Bowman's capsule involving thickening, wrinkling and duplication of the CBM (Fig. 78). In some glomeruli the CBM had ruptured, a feature often associated with cellular infiltration around the glomeruli (see below). Some glomeruli were completely obliterated by this scarring process and were reduced to shrunken, hypercellular or occasionally hypocellular nodules of collagen staining material with the remains of the capsule collapsed around the tuft (Fig. 77). Only very occasionally did Bowman's capsule remain dilated as the tuft collapsed so as to form a cyst. The increase in glomerular cellularity was even greater during this period than before. Tuft hypercellularity was as prominent but this was augmented by the presence of crescent shaped collections of cells around some 20% of the glomeruli, particularly the more severely scarred. Not only were they more common than in cases 85-90 but they were much larger in size. A few were again formed from small numbers of cells solely in the urinary space, but the majority were formed by masses of macrophages, lymphocytes and plasma cells in both urinary space

and periglomerular interstitium with breakup of the intervening CBM (Fig. 77). Occasionally such cells appeared to be present only in the interstitium (Fig. 78). Fibrin was only very rarely identified in these cellular masses.

Large numbers of protein casts and red blood cells were still present in the tubules indicating the continuing severity of the glomerular damage (Fig. 75). Focal cellular degeneration was also still present in the tubules (Fig. 75), while those tubules previously damaged were either being replaced by fibrosis or, in a few instances, appeared to be regenerating. A small degree of interstitial oedema was still present and foci of lymphocytes, plasma cells and macrophages were scattered through the areas of interstitial scarring in both cortex and medulla, in addition to the periglomerular infiltration.

In cases 95 and 96 (killed 21 and 29 days after treatment respectively) the glomeruli showed signs of recovery (Fig. 79). Only rarely were small areas of necrosis or fibrin found, and both tuft hypercellularity and cellular infiltration around the glomeruli were reduced. In addition, GBM thickening and mesangial expansion were less prominent. As a result of these changes, capillary lumina became more obvious again. As a reflection of this recovery tubular protein casts and haemorrhage were less common, and only occasional degenerate tubules lined by atrophic epithelium were seen. Fine strands of interstitial fibrosis were obvious where tubules had been destroyed.

In the control animals lesions were present only in case 99. In one glomerulus there was a small localized area of fibrin exudation and tuft necrosis, while in another

there were globules of fibrin-staining material in several visceral epithelial cells. As a result of these lesions a few tubules contained protein casts and red blood cells. Finally, a small infiltration of lymphocytes, plasma cells and macrophages was found around many of the interlobular arteries, but no lesions of degeneration were seen in the vessels themselves.

Electron Microscopy

The electron microscope proved invaluable, not only in confirming many of light microscopic features, but also in the identification of changes in cellularity and the study of the processes of scarring.

In animals killed between days 3 and 7 (cases 85-90) fibrin deposition (Figs. 80, 82, 83), tuft hypercellularity (Figs. 80-82), and localized areas of necrosis (Fig. 83) were the dominant features. Fibrin was present in all parts of the tuft: in the capillary lumina where deposits were accompanied by occasional platelets, lodged in the mesangial matrix, in phagocytic vacuoles and, in particular, lying between the endothelium and GBM. In addition, it was seen in the urinary spaces of many of the glomeruli. Fibrin was present in the majority of glomerular capillaries but the amount varied considerably. A range was seen from occasional small floccules to massive subendothelial deposits or occlusive intracapillary thrombi. Similarly the amount in the urinary spaces varied from small foci to large lakes of fibrin. This variation in size of deposits was accompanied by differences in structure. Most deposits were composed of electron dense finely granular and

fibrillar material (Fig. 80); fibres with the "characteristic" periodicity of fibrin were seen only in some of the larger deposits (Fig. 82, 83). Occasionally in the capillaries dense material was surrounded by a translucent zone (Fig. 86).

Differences in the amount of fibrin present resulted in variations in subsequent changes in glomerular structure. Capillaries where only a little fibrin was present were often hypercellular. This was the result of two processes: infiltration by circulating mononuclear cells (Figs. 80-82) and the proliferation of endothelial and mesangial cells (Fig. 82). The mononuclear cells closely resembled monocytes. Characteristically they possessed indented nuclei surrounded by pale cytoplasm which contained a well developed Golgi apparatus, varying numbers of lysosomal granules, vacuoles containing material of varying densities, and some mitochondria, endoplasmic reticulum and free ribosomes. Additional distinguishing features were the lack of filamentous cytoplasm (typical of mesangial cells), and lack of junctional complexes where two cells adjoined (unlike neighbouring endothelial cells). These monocyte-like cells could be present in the capillary lumina and lodged in the mesangium, but most were found lying between the endothelium and GBM.

Where smaller amounts of fibrin were present the endothelial and mesangial cells were swollen and possessed increased amounts of rough endoplasmic reticulum, Golgi apparatus and vacuoles coutaining material of varying densities (Fig. 82). This swelling resulted in the

obliteration of many endothelial cell fenestrae, and axial and/or circumferential expansion of the mesangial cells. The latter was often accompanied by expansion of the mesangial matrix. This was due to both the presence of fibrin embedded in it and the formation of new matrix. In addition, greater numbers of mesangial and endothelial cells were often present. This indicated proliferation of these cells was taking place, although they were never seen in mitosis. As a result of this swelling and proliferation, and the infiltration of mononuclear cells, the capillary lumina were partially or completely obliterated (Figs.80-82).

The GBMs could be normal in these hypercellular capillaries (Figs. 80-82). However, in many capillaries there was irregular distortion and thickening of the GBM (Figs. 85-87). The commonest lesion was wrinkling and splitting of the original GBM coupled with the build up of granular material on subendothelial, and to a lesser extent, subepithelial surfaces. This thickening appeared to result from fibrin deposition. Not only was fibrin commonly found between the endothelium and GBM, but in many areas granular fibrin appeared to merge into thickened segments of GBM. In some capillaries the wall was thickened by the circumferential interposition of a mesangial cell between the endothelium and GBM.

Virtually all the GBMs of these hypercellular capillaries,whether abnormal or not, were covered with broad segments of epithelial cytoplasm which were often abnormally dense (Figs. 80-82, 85-87). As stated before this "fusion" of the foot processes indicates a glomerular protein leak.

Some epithelial cells were further distorted and the normal arrangement of trabeculae lost, so that long distorted segments of cytoplasm were present in the urinary space (Fig. 86). Like endothelial and mesangial cells, both visceral and parietal epithelial cells could contain increased amounts of endoplasmic reticulum and Golgi apparatus, and vacuoles were often prominent. Increased numbers of villi were also formed by these cells. Both types of epithelial cell were active in the phagocytosis of fibrin, and like the other cells in the glomerulus, material in the vacuoles appeared to undergo a progressive decrease in electron density (Fig. 84). This was judged to indicate the progressive destruction of fibrin. In none of the glomeruli examined was there any evidence for proliferation of either visceral or parietal epithelial cells. Excess numbers of either cell type were not present and they were never seen in mitosis.

As the amount of fibrin in the capillaries increased, changes of degeneration and necrosis, identical to those seen in acute liquoid nephropathy, were superimposed on the above lesions. Thus, in the most severely affected capillaries the lumina were filled with large masses of fibrin (Figs. 82, 83) and the GBM was reduced to a thin, frayed or wrinkled lamina densa (Figs. 83, 85). Fibrin was seen traversing these severely damaged GBMs (Fig. 83) and in a few places the GBM had ruptured. This allowed large lakes of fibrin to form in the urinary space. Like the GBM, the influx of large amounts of fibrin had led to the derangement and destruction of the mesangial matrix (Fig. 82). Portions of swollen degenerate endothelial

cytoplasm often remained interspersed with fibrin, but in some capillaries only an occlusive thrombus could be seen (Fig. 82). Occasional monocyte-like cells were present in these severely affected capillaries but because of endothelial and mesangial cell necrosis, hypercellularity was not a feature. Epithelial cells were better preserved. Most severely damaged GBMs were still covered by "fused" foot processes but some stretches were completely denuded of an epithelial covering (Fig. 83).

Both proximal and distal convoluted tubules often contained casts of fibrin, necrotic debris and/or red blood cells. Changes indicative of cellular degeneration, identical to those described in acute liquoid nephropathy, were also present in some tubules although complete necrosis of a whole tubule was not seen.

Between days 9 and 16 after treatment (cases 91-94) the ultrastructural picture changed to one of scarring, confirming the light microscopic findings. Areas of severe capillary thrombosis, with subsequent necrosis and exudation of fibrin into the urinary space, were only occasionally seen. Fibrin was rarely present in the tuft distant from necrotic areas, but collections of vacuoles containing material of variable electron density were prominent in some visceral and parietal epithelial cells. Presumably, these were equivalent to the fibrin staining globules seen in these cells under the light microscope.

All capillaries were still partially or completely occluded, but mesangial matrix expansion and GBM thickening, wrinkling and duplication now played a much greater part in this process than before. In fact, whole lobules were seen that were completely obliterated by excess matrix and GBM,

containing only atrophic mesangial and endothelial cells (Fig. 88). Where a capillary lumen was still patent, it could be seen that mesangial expansion could take several forms. In most instances there was narrowing of the capillary lumina due to axial expansion of a hypercellular mesangium surrounded by increased amounts of matrix (Fig. 90, 91). A less common although still prominent change was the circumferential extension of mesangial cells around the capillary wall (Fig. 90). Thirdly, in a few capillaries it appeared that strands of matrix had formed across a capillary lumen dividing it into several compartments. Lesions of the GBM were similar to those present in the earlier cases but were more severe (Figs. 85-87, 89, 90, 94). The build up of new GBM was now greater on both subepithelial and subendothelial surfaces producing very distorted outlines. Occasionally a new basement membrane appeared to be forming trapping portions of endothelial or monocyte cells between it and the original GBM. In many of the affected capillaries the original GBM appeared to be breaking up with only a very thin, wrinkled and frayed lamina densa remaining (Figs. 86, 87, 89). In several capillaries there were points where the GBM appeared to have previously ruptured; these were now filled with an amorphous mass of new basement membrane material lacking any differentiation into lamina densa, and lamina rara interna and externa. Small electron dense granular deposits were common both in the mesangial matrix and GBMs (Fig. 90). Although fibrin often has this appearance, it is highly possible that some of the deposits may have been immune complexes formed in an acute serum sickness reaction (see below).

Although only a few monocyte-like cells remained in the tuft, hypercellularity was still prominent. In the less severely scarred areas there were increased numbers of swollen endothelial and mesangial cells crowding the capillary lumina. Both often showed evidence of increased synthetic activity with enlarged Golgi apparatus and endoplasmic reticulum and increased numbers of free ribosomes present. In contrast, in the severely scarred areas both endothelial and mesangial cells were often atrophic (Fig. 88). Such cells were composed of a small amount of cytoplasm containing only a few organelles. Sometimes their cytoplasm was abnormally dense or contained collections of myelin bodies and vacuoles.

A variety of changes were seen in the visceral epithelial cells. Most capillaries continued to be covered by "fused" foot processes composed of abnormally dense cytoplasm (Fig. 89, 91). However, along the more severely scarred capillaries the cytoplasm could be reduced to a thin, pale, atrophic layer (Fig. 90). In some instances the epithelium lay separated from the GBM, the space left being filled with a pale finely fibrillar material (Fig. 94). Where scarring was particularly severe the epithelial covering could be absent altogether. The main cytoplasmic mass of intact visceral epithelial cells often possessed increased amounts of endoplasmic reticulum, free ribosomes, Golgi apparatus and vacuoles. In contrast, some epithelial cells showed evidence of degeneration, and these contained many vacuoles, dense bodies and myelin figures (Fig. 91, 98).

In 3 of the more severely scarred glomeruli the urinary space was obliterated by a mass of cells of varying

types, interspersed with a scanty amorphous deposit of pale, granular and fibrillar material (Figs. 92-94). A variety of non-banded and banded fibrils were present including some of collagen. Fibrin was not prominent in these areas being limited to a few small masses of dense granular material (Fig. 92). These cells were surrounded by an intact CBM but in all cases a small accumulation of similar cells was present in the periglomerular interstitium. These then were the ultrastructural equivalent of the crescent shaped masses of cells seen around the glomeruli under the light microscope. The majority of cells in the urinary space and interstitium most closely resembled macrophages (Figs. 92-95). They could be distinguished from epithelial cells by their lighter cytoplasm which contained variable numbers of lysosomes, less prominent endoplasmic reticulum, more prominent mitochondria, and Golgi apparatus, and their very irregular borders which did not form junctional complexes with neighbouring cells. Vacuoles were usually prominent in these cells as well. In addition, occasional elongate cells with very prominent endoplasmic reticulum resembling fibroblasts, and lymphocytes were scattered amongst these cells, both in the urinary space and interstitium (Figs. 92, 95). Occasional plasma cells were present in the interstitium as well (Fig. 95), while in the urinary space this collection of cells was bordered by a ring of epithelial cells (Fig. 93). Usually these were elongate with very irregular cell borders often apparently unassociated with either GBM or CBM. A distinguishing feature of these cells was the formation of junctional complexes with adjoining epithelial cells. Some of these

epithelial cells had very filamentous cytoplasm suggesting they were of parietal origin.

In several of the glomeruli examined, including those with cellular infiltrates in the urinary space, there was uneven thickening of the CBM (Fig. 96). In all instances masses of granular and fibrillar material, including collagen (Fig. 97), had built up between the CBM and the parietal epithelium. In one instance, where a large amount of this material was present, a fibroblast and segments of macrophage-like cytoplasm were embedded in it (Fig. 96 In places it appeared that a new CBM was starting to be formed under the parietal epithelium. The original CBM was usually wrinkled but rupture was never seen. Capsular adhesions were found in 2 glomeruli (Fig. 98). In both instances a mass of granular and fibrillar elements linked the CBM to a collapsed, wrinkled GBM. Segments of fibrillar cytoplasm were present in the adhesions, but their cell of origin was not clear.

Most tubules were lined by normal cells but they could contain cellular debris or red blood cells. Other tubules were identical to those described in chronic liquoid nephropathy either being lined by low atrophic epithelium, or occluded by a mass of distorted cells surrounded by a wrinkled, thickened basement membrane. Interstitial fibrosis was usually present around these tubules.

Cases 95 and 96 (killed 21 and 29 days after treatment respectively) were characterized by glomerular repair. A few capillaries appeared to be permanently obliterated by matrix and GBM but the remainder showed a return to normality due to a reduction in cellularity, mesangial expansion and

GBM thickening. Mononuclear cells were now absent but there were still excess numbers of endothelial and mesangial cells. Cellular swelling was reduced so that fenestrated endothelium again lined some capillaries. Excess mesangial matrix was still prominent, but circumferential interposition by mesangial cells was only occasionally present. Thus, there was a reduction in the width of the capillary walls, although all GBMs were still irregularly thickened. Epithelial cell morphology also showed a return to normality with foot processes reappearing along some segments of GBM. No cellular infiltrates were present in the urinary spaces, nor were capsular adhesions or CBM thickening seen in the glomeruli examined. No tubular abnormalities were seen.

In the control animals glomerular lesions were present in cases 98 and 99. A few small electron dense granular deposits were present embedded in the mesangial matrix, and in the GBM in a subendothelial position, at or near its junction with the mesangium. These were almost certainly the immune complexes identified with immunofluorescence. No other glomerular lesions were present. In case 99 an interlobular artery was also examined and a small perivascular cellular infiltrate was present. Most cells were of the plasma cell and lymphoid series but several macrophages were also present.

Immunofluorescence Microscopy (Table 36)

The most striking deposits found were rabbit immunoglobulin, and host fibrin and complement. In the first 6 days after injection (cases 85-88) there was a diffuse deposition of rabbit globulin in a sharp, bright, continuous,

TABLE 36

NTS NEPHRITIS: IMMUNOFLUORESCENCE FINDINGS

CASE	GLOMERULI				
	RABBIT GLOBULIN	IgG	IgM	C3	FIBRIN
85	+	+^{F}	+^{F}	+	+
86	+	+^{F}	+^{F}	+	+
87	+	+^{F}	+^{F}	+	+
88	+	+^{F}	+^{F}	+	+
89	+	+^{F}	+^{F}	+	+
90	+	+	+	+	+
91	+	+	+^{F}	+	+
92	+	-	+	+	+
93	+	-	+	+	+
94	+	+	+^{F}	+	+
95	+	+	+^{F}	+	+
96	+	+	+	+	+
97	-	-	-	-	-
98	-	+	-	+	-
99	-	+	+	+	-

F = present in fibrin deposits only

linear band along the glomerular capillary walls (Fig. 99). In later cases (89-96) the staining became duller, distorted and discontinuous as a result of tuft scarring.

Fibrin was very prominent in the earlier cases, but with time the deposits became smaller and less widespread, so matching the light microscopic findings. In general, more fibrin was identified with immunofluorescence than with light microscopy; this was particularly true of the later cases (91-96), killed later than 9 days after injection. In the earlier cases there was a segmental (cases 85, 87-90) or diffuse (case 86) distribution. In the later cases, smaller deposits were present focally in the glomeruli. The most prominent deposits formed lakes in the urinary spaces, but large amounts were also present in the capillaries forming occlusive thrombi or lying in a linear band along the capillary wall (Figs. 101, 102).

Complement (C3) was present in every case, either bound to immunoglobulin or apparently trapped in thrombi (Fig. 100). In the early cases (85-88) there was a diffuse deposition of C3 along the capillary walls in a granular or broken linear pattern. With time (after 7 days, cases 89-96) only granules were seen. In addition to these deposits bound either by rabbit or host immunoglobulin, large globules of C3 were present in all cases in identical positions to fibrin deposits. In general in these areas, the staining for C3 was only slightly less bright and less extensive than that of fibrin.

IgG and IgM were present in most cases. Before day 7 (cases 85-89) focal deposits of IgM and IgG were present trapped like complement in some of the fibrin deposits. The

staining particularly of IgG was much duller than that of complement and fewer deposits were seen. After 7 days (cases 90-96) these deposits were augmented by the diffuse but rather sparse deposition of granules of IgG and/or IgM granules along the capillary walls and occasionally in the mesangium. It is interesting to note that the type of response varied from dog to dog; in most it was solely or predominantly an IgG response, but in two dogs (cases 92, 93) only IgM was present.

In the control animals a totally different picture was seen. A sparse diffuse deposition of fine granules of IgG, alone or with IgM, plus bound complement, was present in the mesangial regions in cases 98 and 99.

No rabbit globulin or fibrin was present in any control animal.

Fig. 71 Residue from glomerular extraction process

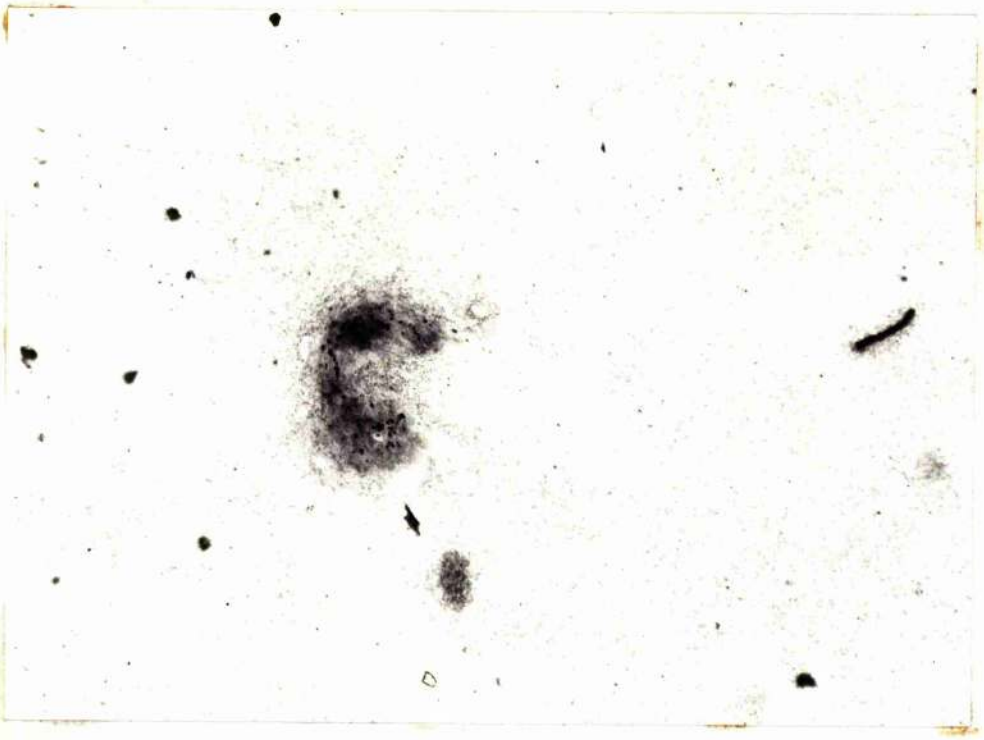
Cellular fragments only are seen, glomeruli are absent.

(Methylene blue x 35)

Fig. 72 Glomerular extract

Note the high purity of the glomerular extract obtained for the preparation of GBM antigen suspension.

(Methylene blue x 110)



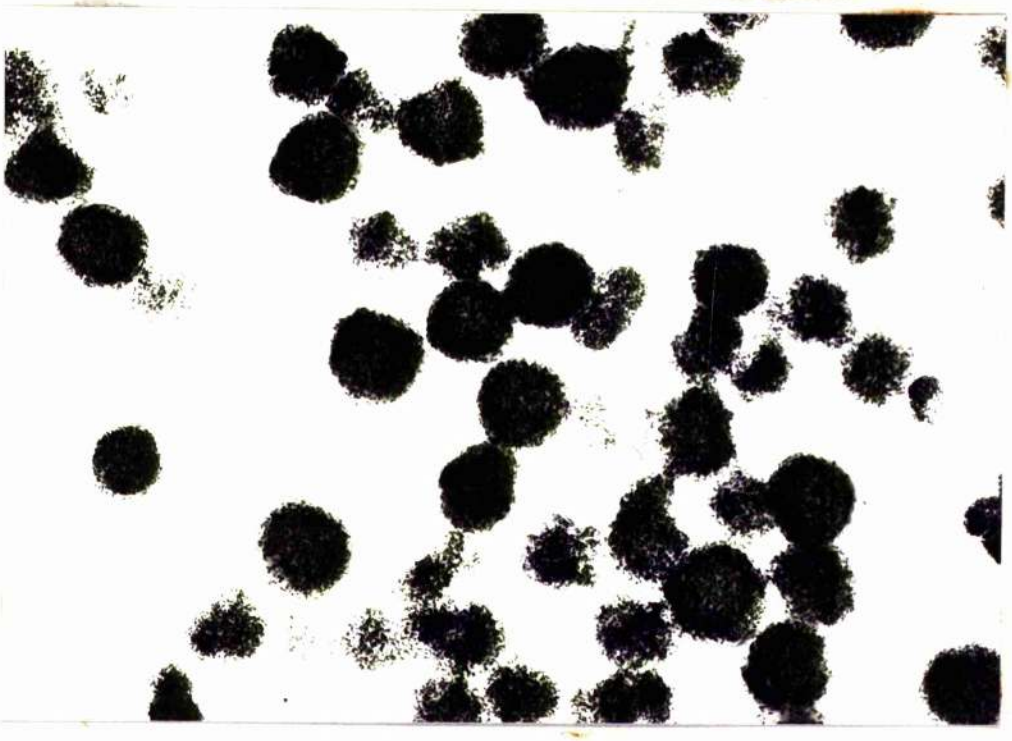


Fig. 73 Nephrotoxic Serum (NTS) Nephritis, 7 days

Case 89

Fibrin deposits (red) are seen in both glomeruli. They are present both in the capillaries, and as a result of tuft necrosis, in the urinary spaces (arrow).

(MSB x 250)

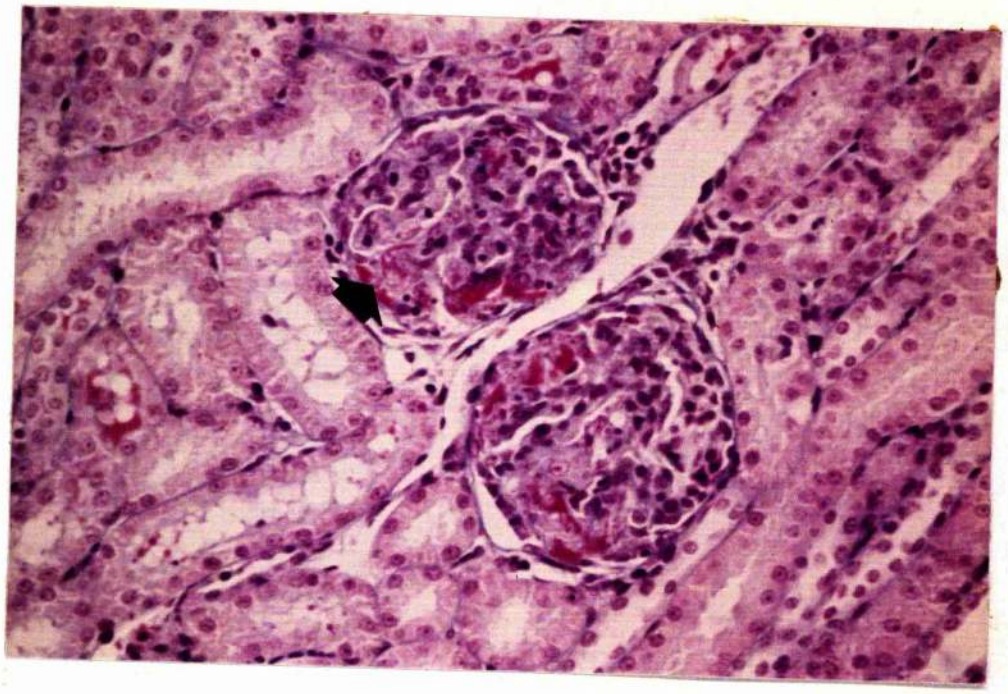


Fig. 74 NTS Nephritis, 3 days, Case 85

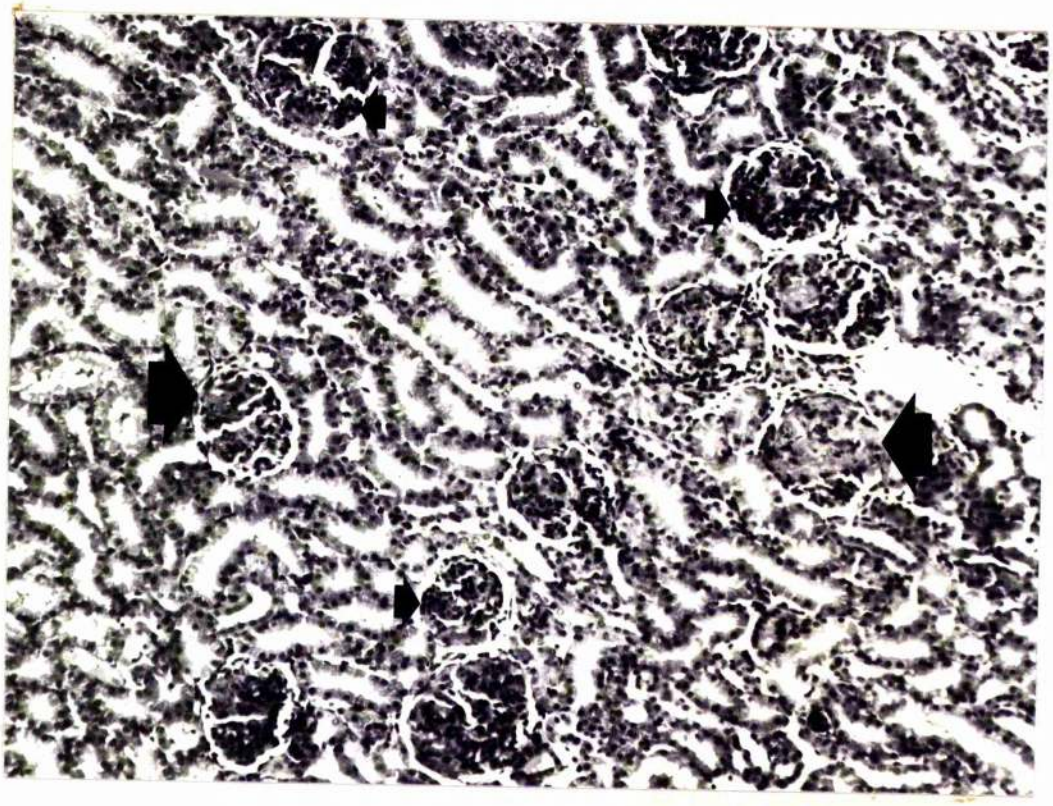
All glomerular tufts are swollen and show localized areas of hypercellularity (small arrow) and necrosis (large arrow). Consequently few patent capillaries can be seen and the urinary spaces are partially occluded. The tubules in this field are normal.

(H & E x 110)

Fig. 75 NTS Nephritis, 14 days, Case 93

In contrast to the last photomicrograph, glomerular scarring is prominent with the tufts reduced to hypercellular masses of collagen staining material. Varying degrees of mononuclear cell infiltration are present around the glomeruli (arrow). Some tubules are normal but others are lined by low atrophic epithelium and protein casts are common (*).

(H & E x 110)



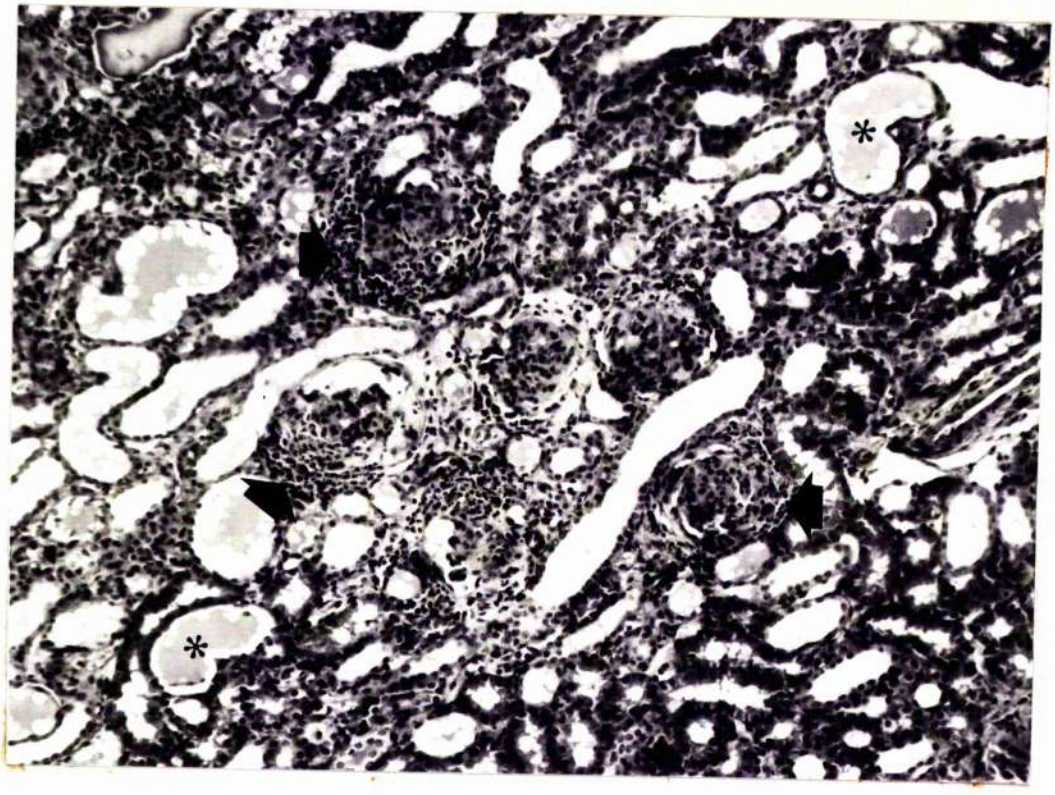


Fig. 76 NTS Nephritis, 11 days, Case 92

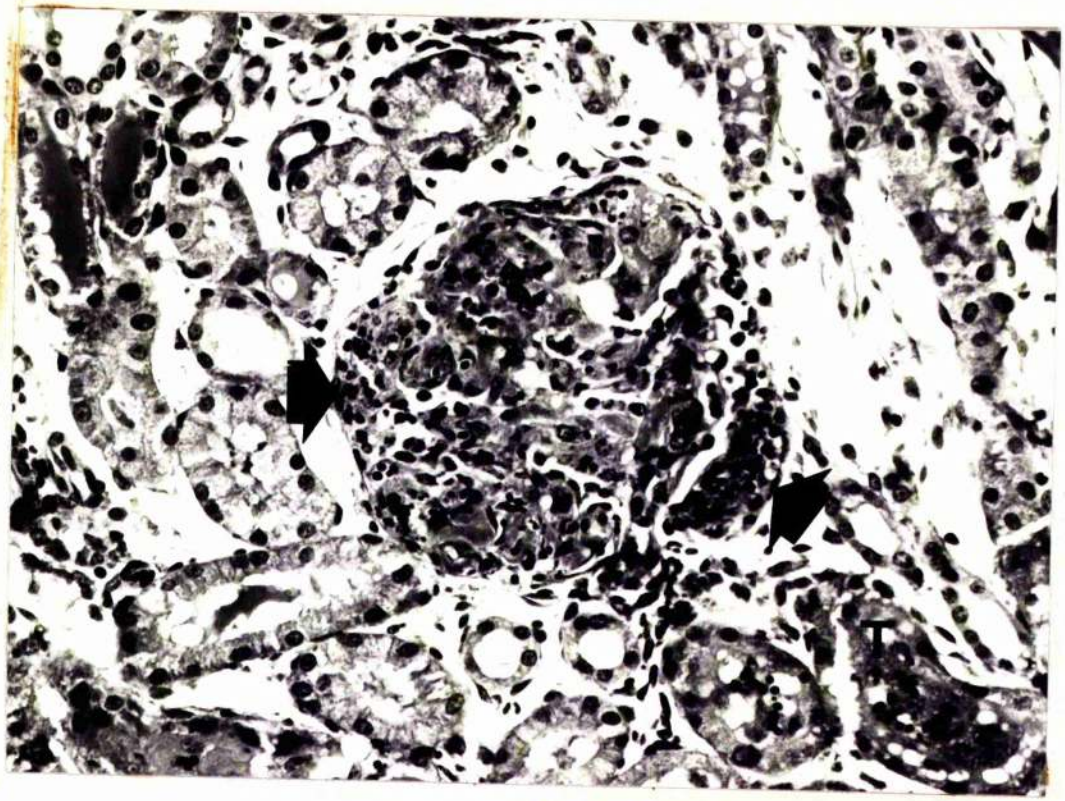
A mass of cells has accumulated at 2 points in the urinary space of this glomerulus. The nature of these cells cannot be accurately identified with the light microscope. Cellular infiltration is absent from the interstitium. Note also the widespread glomerular necrosis, tubular degeneration (T) and the abnormal separation of the tubules due to interstitial oedema.

(MSB x 250).

Fig. 77 NTS Nephritis, 14 days, Case 93

The vast majority of crescent shaped cellular infiltrates around the glomeruli resembled those shown here. Plasma cells, lymphocytes and macrophages are present both in the periglomerular interstitium and the urinary spaces of these 2 severely scarred glomeruli.

(MSB x 250)



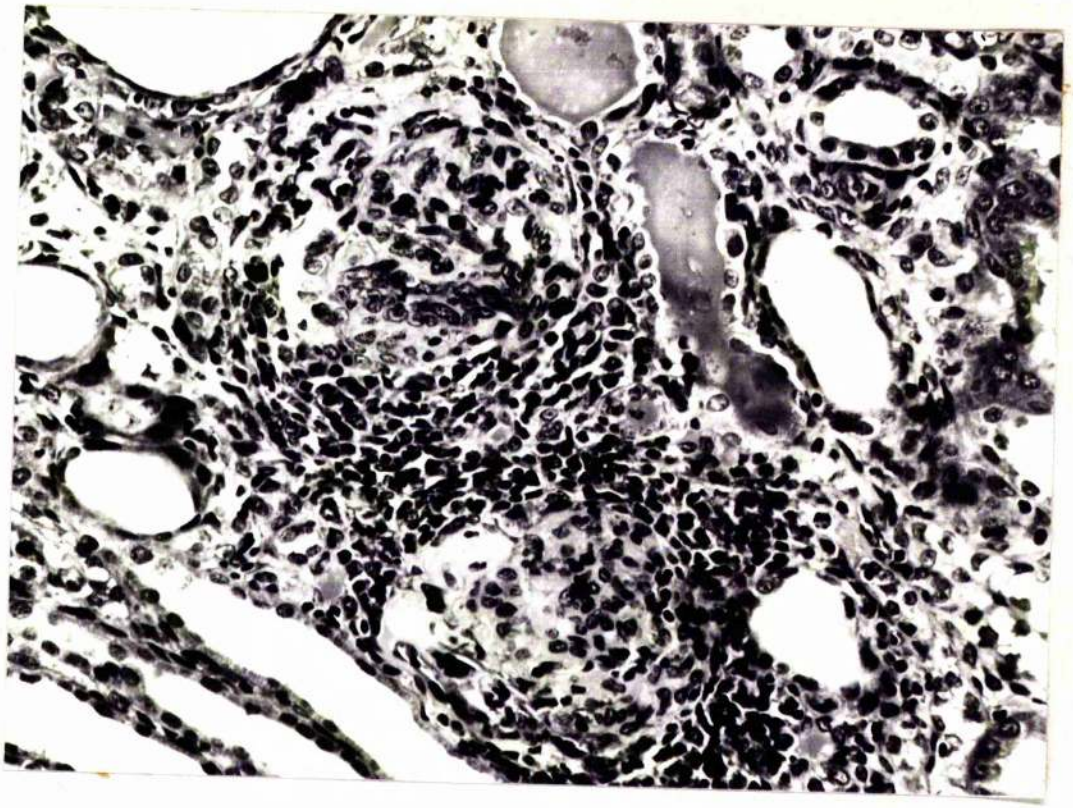


Fig. 78 NTS Nephritis, 16 days, Case 94

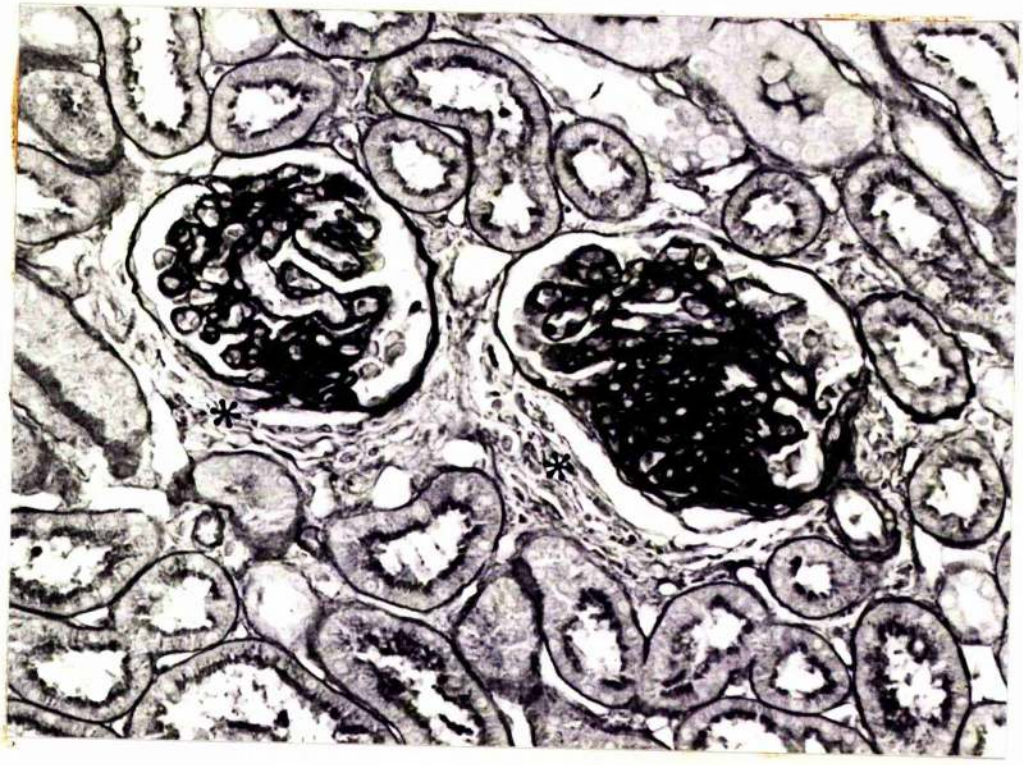
Global GBM thickening and mesangial matrix expansion are present and parts of both tufts have been completely obliterated. Capsular adhesions and periglomerular cellular infiltration (*) are also present. Note that, at this plane at least, the CBMs are intact and cellular infiltration is absent from the urinary spaces.

(PAS x 250)

Fig. 79 NTS Nephritis, 21 days, Case 95

Glomeruli at this stage show signs of recovery. Although mesangial expansion and tuft hypercellularity are still prominent, some capillaries are now patent. Note also that the tubules are all normal and only a small periglomerular infiltrate is present (arrow).

(MSB x 250)



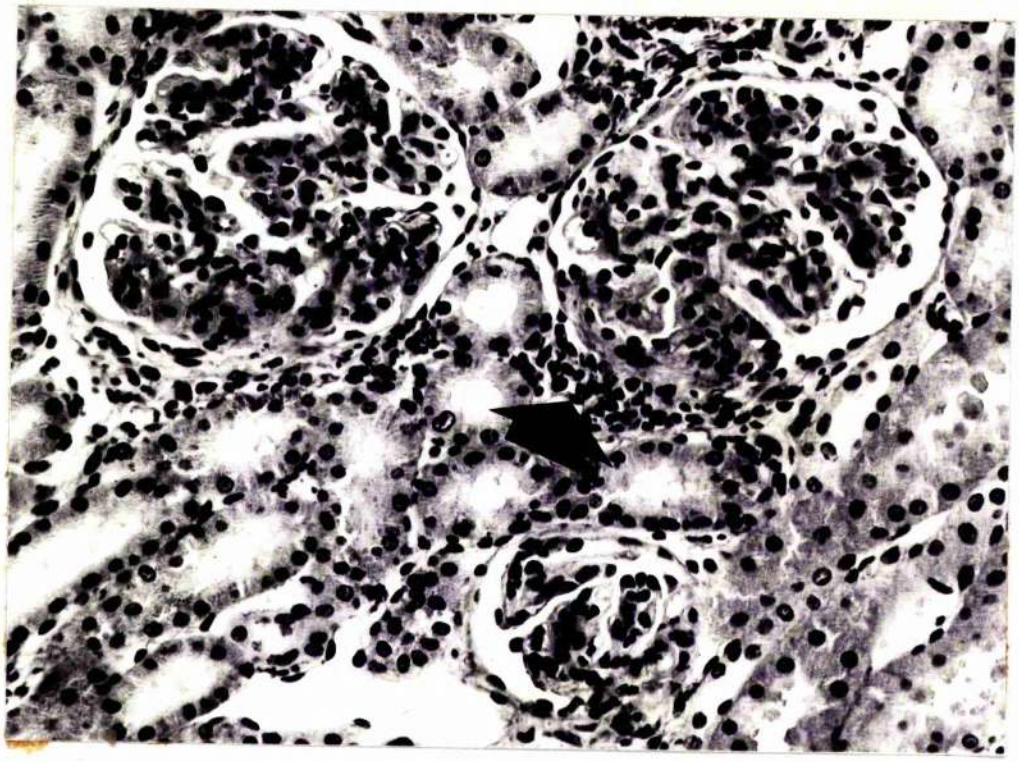


Fig. 80 NTS Nephritis, 3 days, Case 85

A hypercellular capillary loop is seen. The pale cytoplasm and collections of lysosomes clearly distinguish these 5 monocyte-like cells (Mo) from the mesangial (M) and endothelial cells (E). These cells are lodged either in the mesangium (3 and 4), in the capillary lumina (2 and 5) or under the endothelium (1). Note the fusion of the epithelial cell foot processes (*) and fibrin (F) in the capillary and urinary space (U).

(Electron microscopy x 15,000)

Fig. 81 NTS Nephritis, 9 days, Case 91

Many of the monocyte-like cells (Mo) were present between the endothelium (E) and GBM. Note the prominent golgi apparatus of this cell which helps distinguish it from endothelial (E) and mesangial cells (M). m. Mesangial matrix, U. urinary space, I. interstitium.

(Electron microscopy x10,000)


E
*
Mo1
U
Mo2
E
Mo3
Mo4
M
M
M

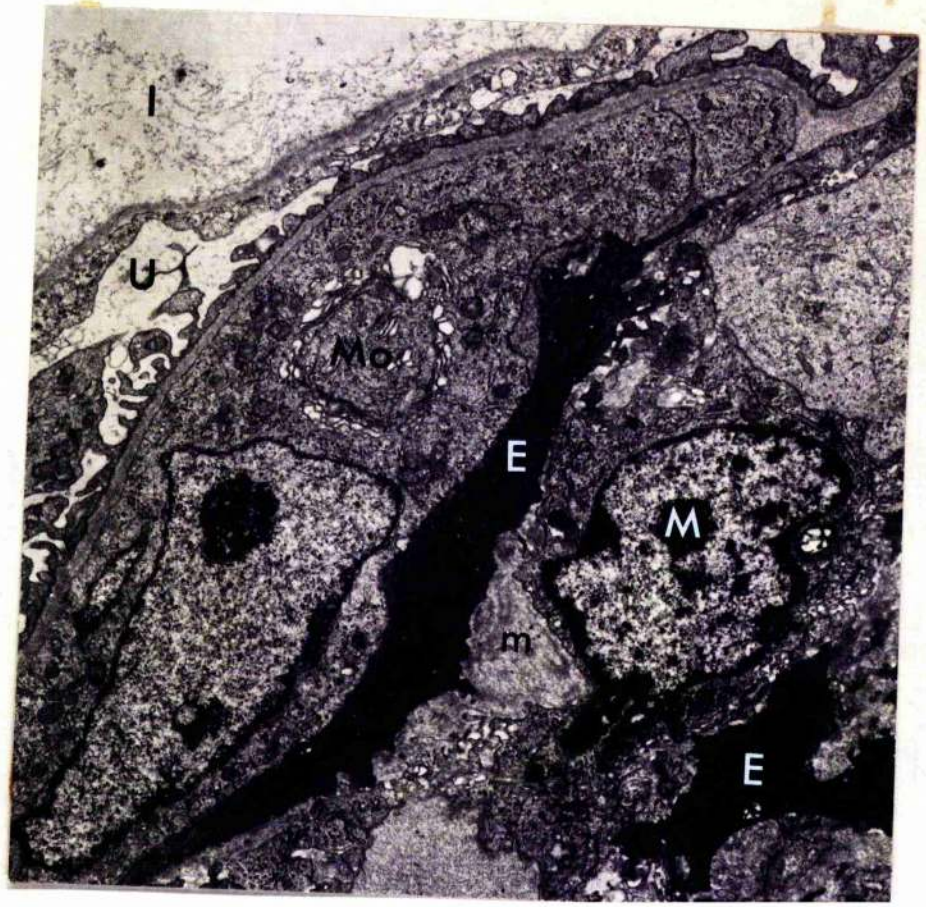

I
U
Mo
E
M
m
E

Fig. 82 NTS Nephritis, 3 days, Case 85

Hypercellularity and capillary occlusion have been produced by the combination of infiltration of monocyte-like cells (Mo) and a lymphocyte (L), and proliferation of endothelial cells (E). Granular and fibrillar deposits of fibrin are present and the capillary in the upper right quadrant is blocked by a thrombus. Note also that the fibrin and cellular infiltration have displaced or destroyed both mesangial cell and matrix between the 4 capillaries. P. Platelet.

(Electron microscopy x 10,000)

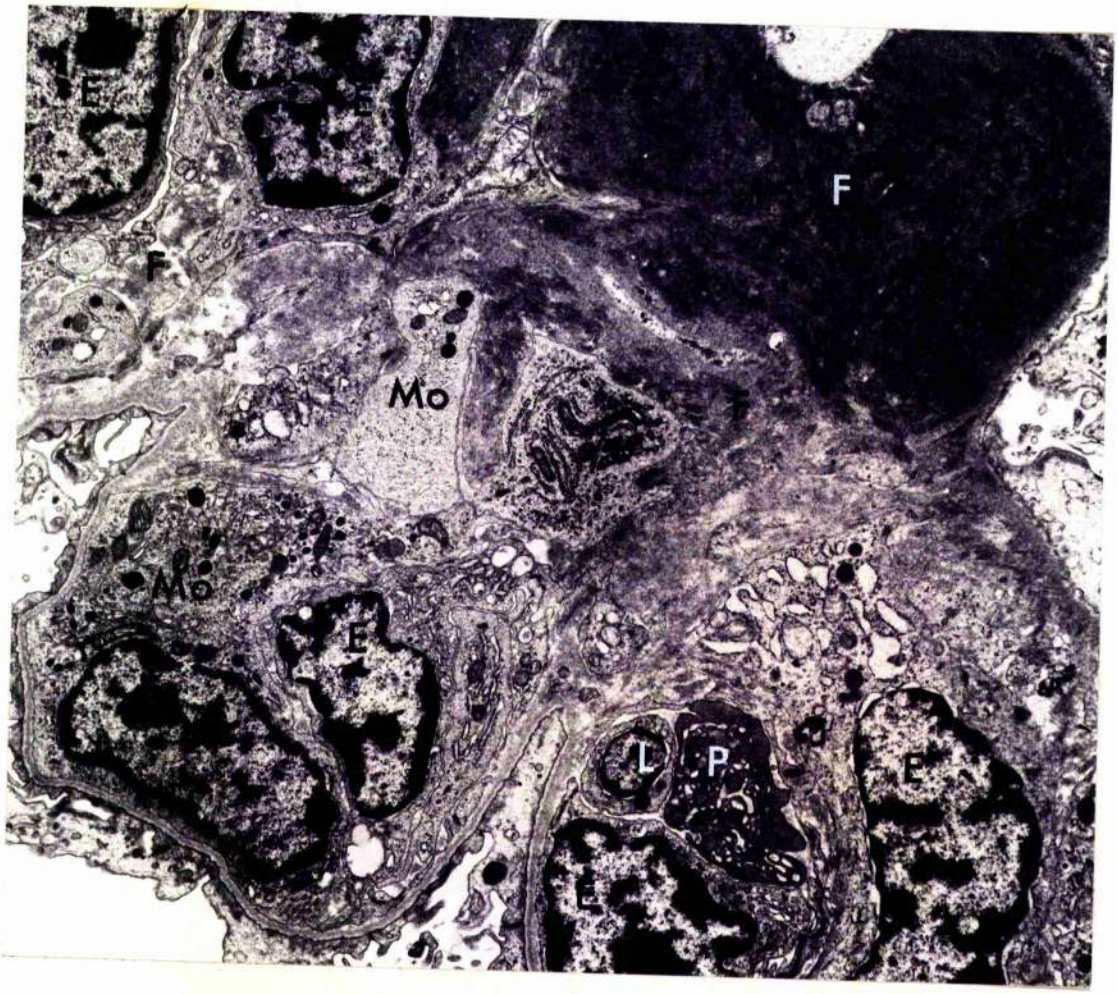

E
F
F
Mo
Mo
E
L
P
E
E

Fig. 83 NTS Nephritis, 3 days, Case 85

Severe fibrin (F) deposition leads to glomerular necrosis. Both endothelial (E) and epithelial (Ep) cells show signs of degeneration. Endothelium no longer lies on the GBM but segments of swollen, vacuolated endothelial cytoplasm are still evident. Epithelial cell foot processes are either "fused" or completely absent (arrow). The epithelial cytoplasm is pale and vacuolated with few intact organelles. The GBM is reduced to a thin frayed lamina densa. As a result of this degeneration and necrosis large amounts of fibrin have been liberated into the urinary space (U).

(Electron Microscopy x 10,000)


Ep
F
F
F

Fig. 84 NTS Nephritis, 9 days, Case 91

Epithelial cells (Ep) were active in the removal of fibrin (F) from the urinary spaces. Note vacuoles containing dense material with a pale halo suggesting its lysis (*).

(Electron microscopy x 15,000)

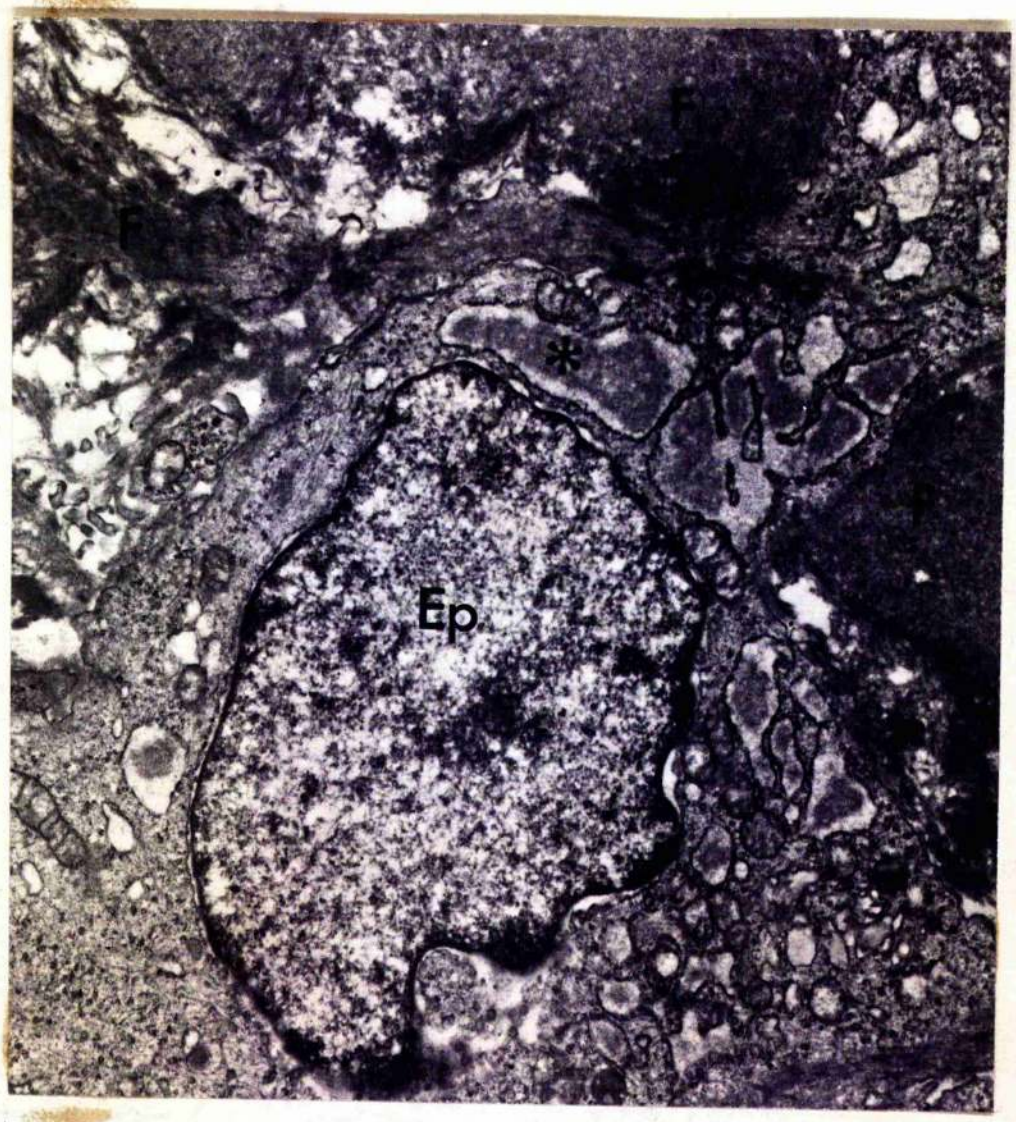

F
F
*
Ep

Fig. 85 NTS Nephritis, 6 days, Case 83

The original glomerular basement membrane (GBM) is reduced to a frayed, wrinkled, collapsed lamina densa, but there is irregular build up of new basement membrane on both subendothelial and subepithelial surfaces. Note the "fusion" of the epithelial cell (Ep) foot processes along the GBM. E. Endothelium, Mo. Monocyte-like cell.

(Electron microscopy x 20,000)


Ep
Ep
GBM
E
Mo

Fig. 86 NTS Nephritis, 9 days, Case 91

The capillary lumen has been obliterated by translucent matter containing dense fibrillar elements (F). A mesangial cell (M) is infiltrating this material. This lesion closely resembles that seen in CIN case 23 (Fig. 32). Such areas are a feature of nephropathies where active deposition and lysis of fibrin is occurring (Kincaid-Smith 1972). Note also the vacuolation and distortion of the epithelial cells (Ep) including the fusion of foot processes (small *). In one area the epithelium is necrotic (large *). There is irregular thickening of the GBMs but the original, frayed lamina densa is still visible (arrow). E. Endothelial cells.

(Electron microscopy x 15,000)

Fig. 87 NTS Nephritis, 6 days, Case 88

Granular fibrin deposits (F) have obliterated a capillary and produced marked thickening of the GBM. Only a few atrophic segments of endothelial cytoplasm (E) remain from the capillary, while the remains of the original GBM are arrowed. Note also there is build up of new GBM material in a subepithelial position (*) and the epithelial cell (Ep) foot processes are fused. M. Mesangial cell, C. capillary.

(Electron microscopy x 20,000)


Ep
F
M
E

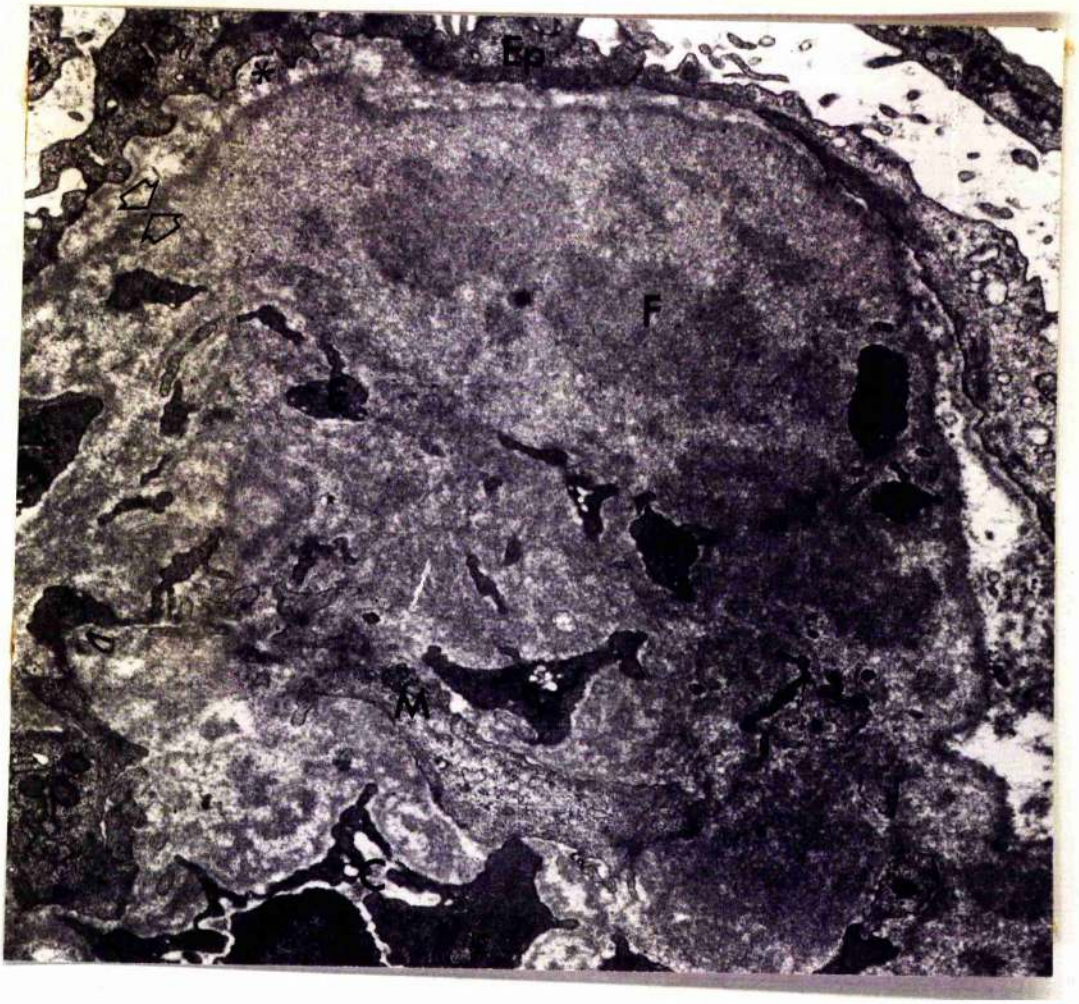

Ep
F
M

Fig. 88 NTS Nephritis, 14 days, Case 93

A severely scarred glomerulus is shown. The production of excess mesangial matrix and GBM have virtually obliterated the normal architecture. Remaining capillaries (C) are very narrow and the urinary space cannot be distinguished. The area is also hypercellular due to an increase in the number of mesangial cells (M). E. endothelial cells, Ep. epithelial cells.

(Electron microscopy x 10,000)


M
M
M
E
C
E
C
M
M
C
E

Fig. 89 NTS Nephritis, 16 days, Case 94

The original GBM can still be seen (arrowed) but it is irregularly thickened due to the formation of new GBM on both subepithelial and subendothelial surfaces. Note also that an endothelial cell (E) lies under a new layer of GBM. Epithelial cell (Ep) foot processes are fused. GBM glomerular basement membrane, L. lymphocyte.

(Electron microscopy x 20,000)

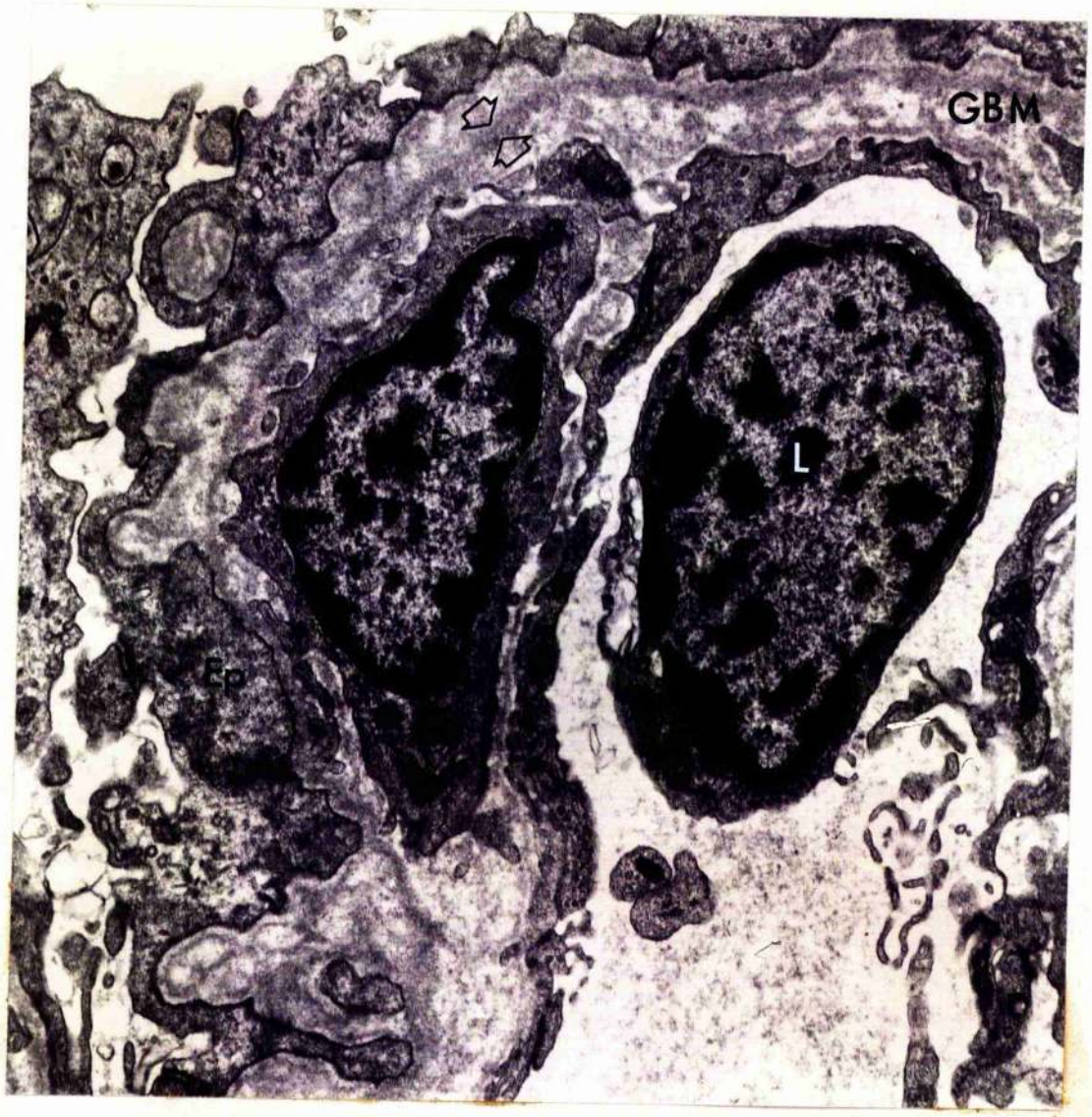

GBM
L
Ep

Fig. 90 NTS Nephritis, 14 days, Case 93

Dense granular deposits (d) are seen in the mesangium and in the capillary wall. This material could be fibrin, but similar small deposits were seen after the same time interval in animals given normal rabbit serum, where the deposits were immune complexes. Note also the enlarged mesangial area and circumferential interposition of the mesangial cell (M) around the capillary wall.
C. capillary, U. urinary space.

(Electron microscopy x 10,000)


U
C
M

Fig. 91 NTS Nephritis, 16 days, Case 94

Degeneration of an epithelial cell (Ep) is seen with dense bodies prominent. Previous fibrin phagocytosis is also evident; several vacuoles are present which contain granular material of varying densities (arrow). Note also the "fusion" of the epithelial cell foot processes (*) and the thickening of the capillary wall due to circumferential interposition of the mesangial cell (M).

(Electron microscopy x 10,000)

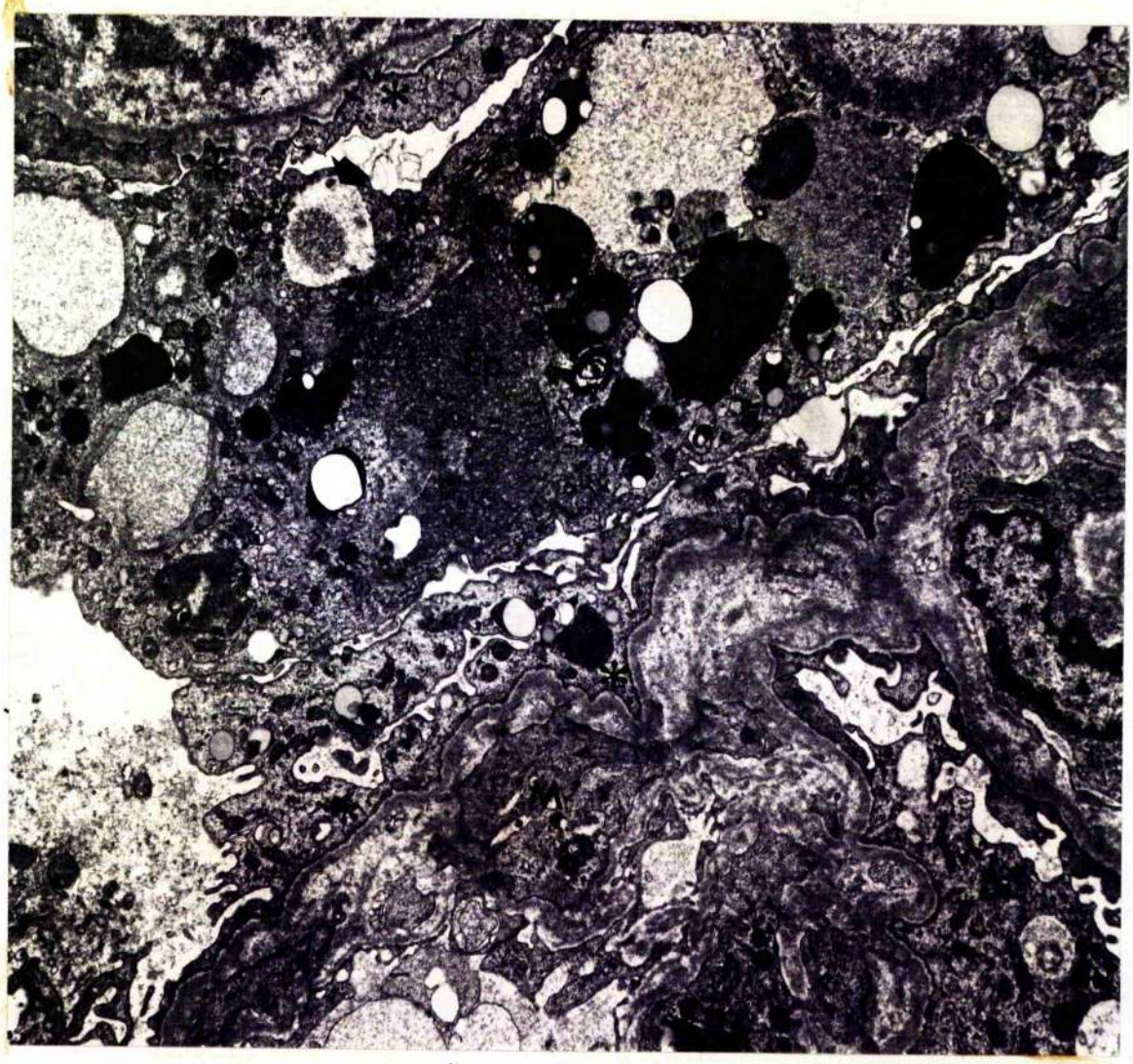


Fig. 92 NTS Nephritis, 9 days, Case 91

The urinary space of this glomerulus is filled with a mass of fibrin (F), and infiltrating cells. A lymphocyte (L), a fibroblast-like cell, and a cell resembling a macrophage (Ma) are present in addition to the epithelial cells (Ep). Similar cells are also seen in the interstitium (I). Note that the parietal epithelium is absent leaving a denuded CBM (arrowed). C. capillary.

(Electron microscopy x 6,000)


I
F
L
Ep
F

Fig. 93 NTS Nephritis, 14 days, Case 93

Ultrastructural appearance of a glomerulus similar to those shown in Fig. 77. The urinary space is filled with a mass of cells, most of which resemble macrophages (Ma). Occasional lymphocytes (L), Epithelial cells (Ep) and a fibroblast-like cell (Fi) are also present. Epithelial cells are identifiable by the formation of junctional complexes (arrow) where they adjoin each other. A pale, finely granular and fibrillar material is present between the cells. Fibrin is not seen in this field. CBM capsular basement membrane, I. Interstitium.

(Electron microscopy x 6,000)

Fig. 94 NTS Nephritis, 11 days, Case 92

Three macrophage-like cells (Ma) are easily distinguished by their very irregular cell borders and prominent lysosomes. The urinary space around these cells is being filled with a fine granular material (*). Note also the atrophic layer of epithelium (Ep) lacking foot processes and the thickened GBM. The original lamina densa can still be seen (arrows). GBM. Glomerular Basement Membrane.

(Electron microscopy x 10,000)


I
CBM
Ma
Ma
Ma
Ma
Ma
Ma
Fi
Ep
L
Ep

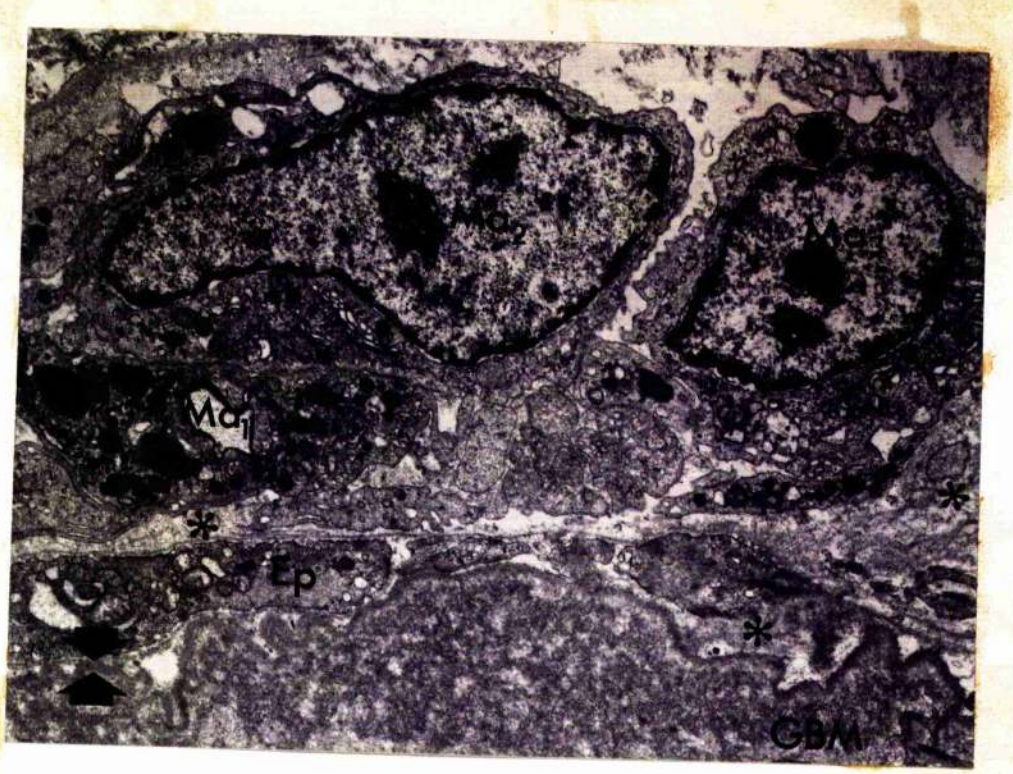

Ma
Ma1
Ep
GBM

Fig. 95 NTS Nephritis, 16 days, Case 94

Part of a cellular infiltrate in the periglomerular interstitium is shown. These were composed of macrophages (Ma), Fibroblasts (Fi), lymphoid cells (L) and occasional plasma cells (Pc).

(Electron microscopy x 10,000)

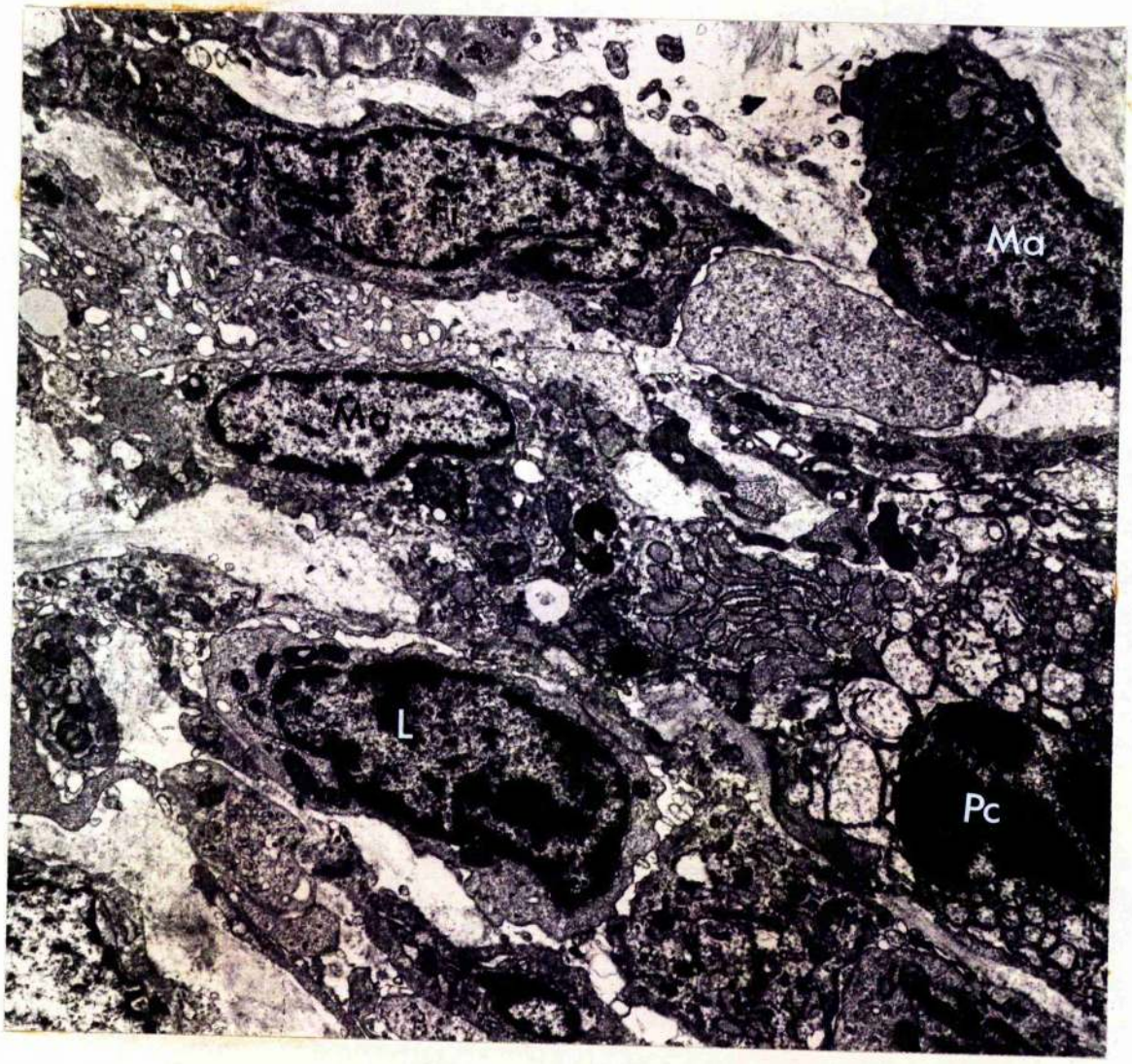

Ma
Ma
L
Pc

Fig. 96 NTS Nephritis, 14 days, Case 93

The original capsular basement membrane (CBM) is wrinkled and thickened by the haphazard formation of new basement membrane and other fibrillar elements. A fibroblast (Fi) and macrophage-like cell (Ma) are present in this new material. Distorted, swollen parietal epithelial cells (PEp) are also seen, distinguishable by their fibrillar cytoplasm (*) and cell junctions (arrow). I. Interstitium.

(Electron microscopy x 6,000)

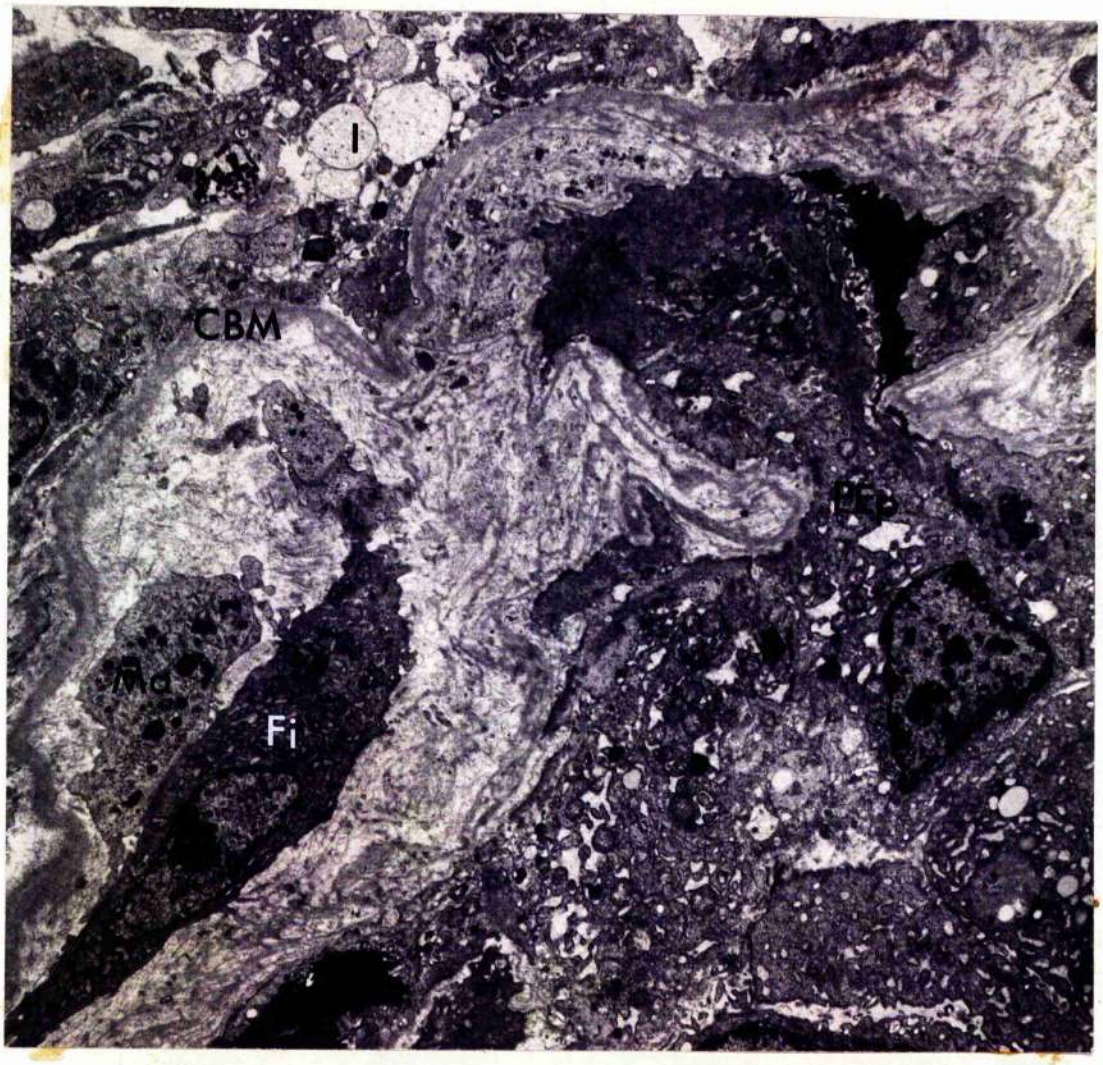

I
CBM
PEP
Ma
Fi

Fig. 97 High power electron micrograph of Fig. 96 to show that collagen fibres were amongst the elements present in the thickened CBM.

(Electron Microscopy x 80,000)

Fig. 98 NTS Nephritis, 16 days, case 94

A capsular adhesion (A) is formed by a mass of fibrillar material containing segments of fibrillar cytoplasm. The original GBM can be seen as a collapsed, wrinkled strand (arrow). The epithelial cell (Ep) associated with the adhesion is necrotic. C. capillaries, U. urinary space, CBM. capsular basement membrane.

(Electron microscopy x 10,000)

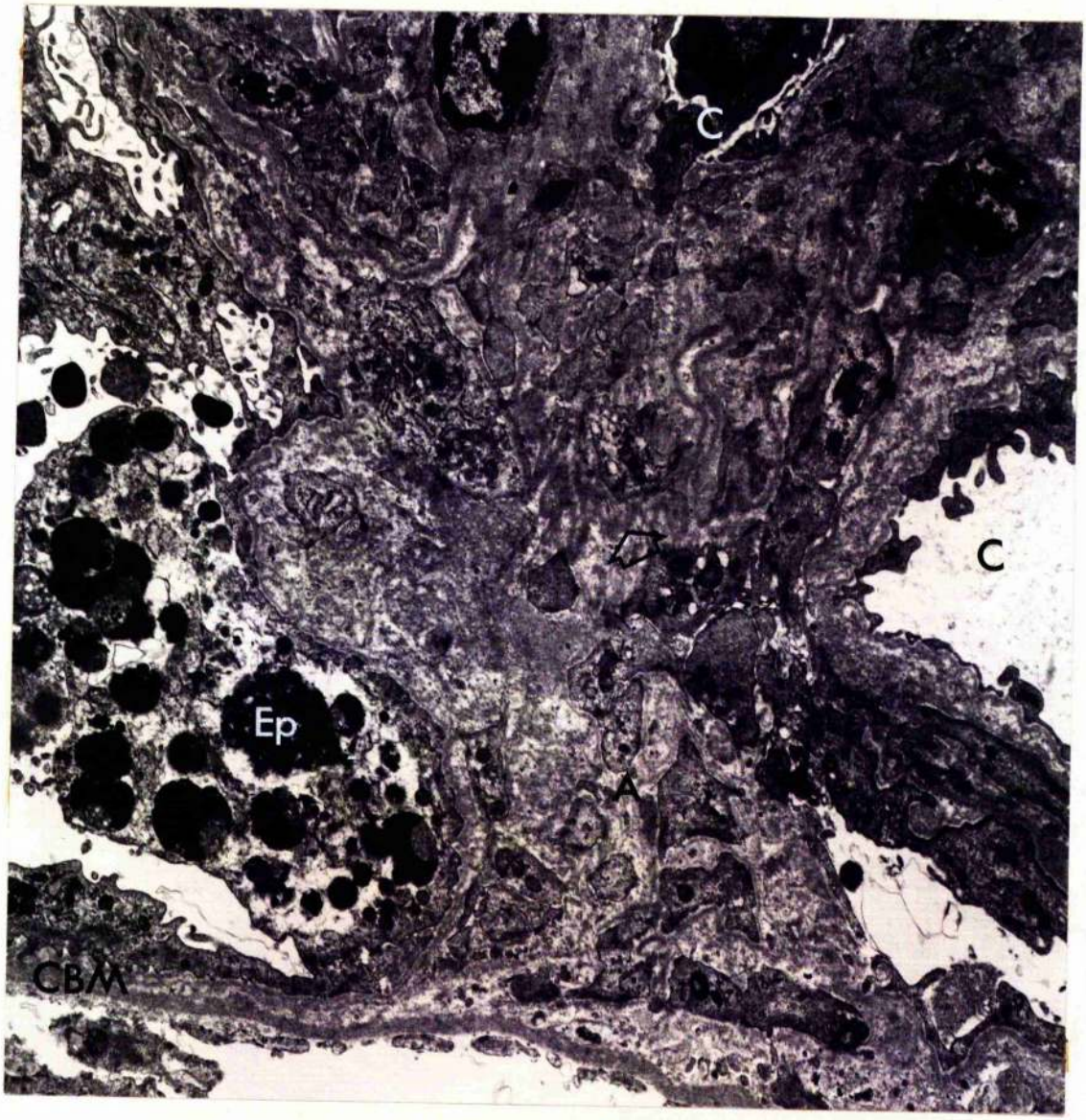

C
C
Ep
CBM

Fig. 99 NTS Nephritis, 4 days, Case 86

A linear deposit of rabbit immunoglobulin is present along the glomerular capillary walls.

(Immunofluorescence x 450)

Fig. 100 NTS Nephritis, 4 days, Case 86

Irregular linear deposits of complement are present along the glomerular capillary walls, while larger masses are present in the urinary space. Fibrin was identified in similar positions to these larger masses.

(Immunofluorescence x 300)

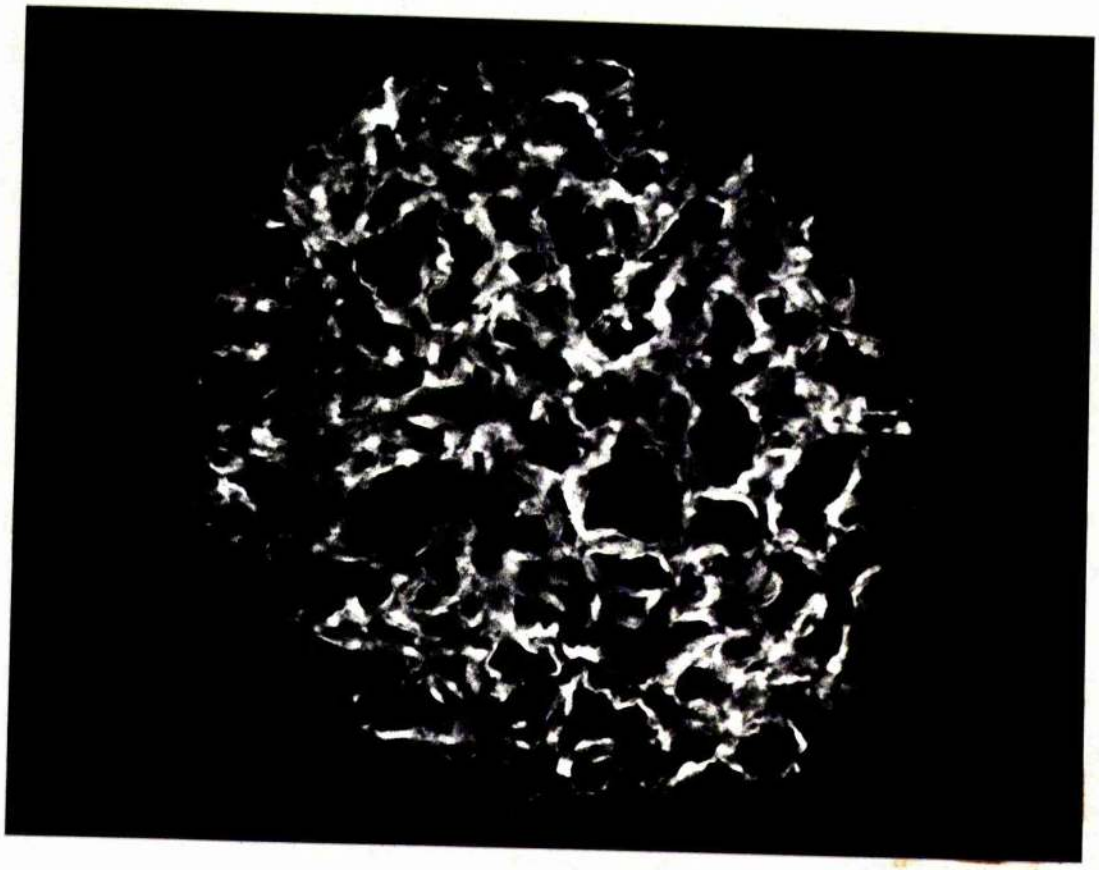


Fig. 101 NTS Nephritis, 3 days, Case 85

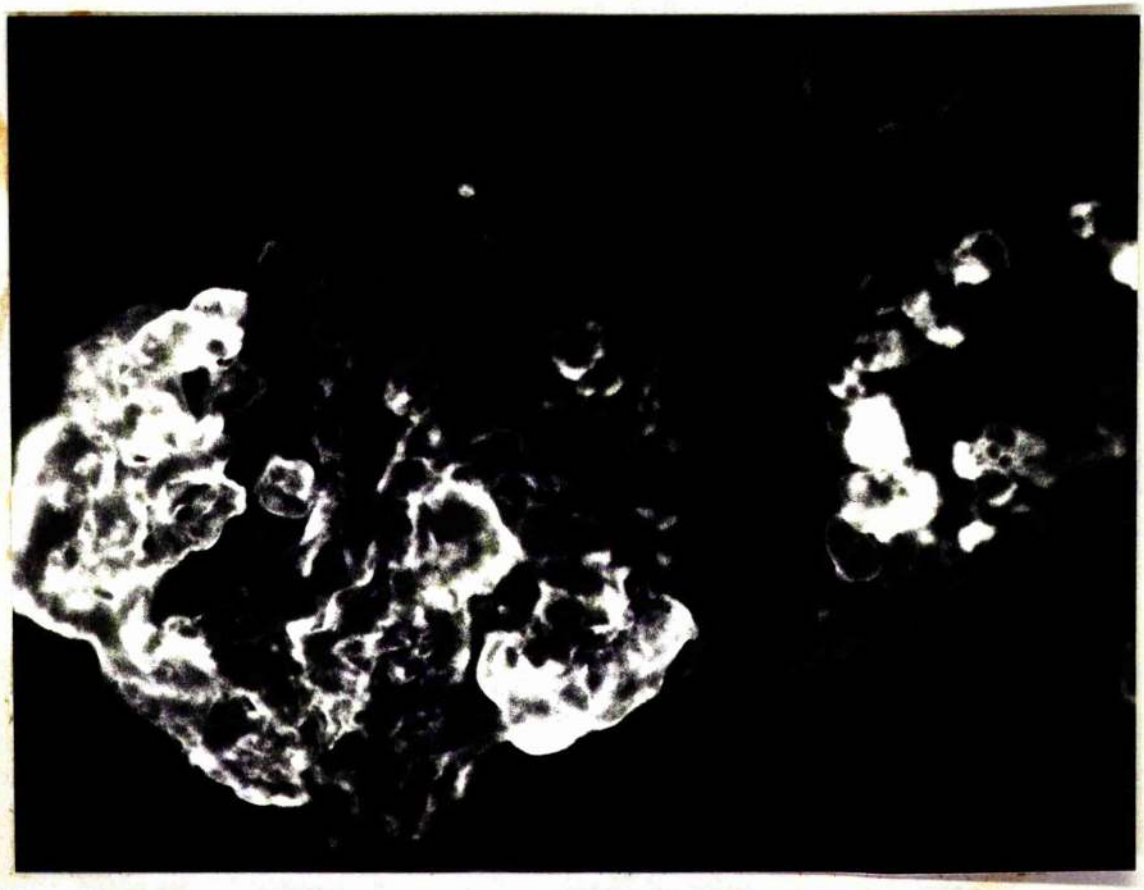
Large deposits of fibrin are present in the glomerulus (on the left) in the capillaries and urinary space. Smaller amounts are also present in a tubule (on the right).

(Immunofluorescence x 300)

Fig. 102 NTS Nephritis, 3 days, Case 85

A lake of fibrin is present in the urinary space of this glomerulus. This was a common finding.

(Immunofluorescence x 450)



DISCUSSION

It is now well known that the GN mediated by the injection of NTS falls into two distinct phases which can be identified with immunofluorescence microscopy. The first (heterologous) stage, which lasted in this experiment for 6 days, is mediated by the interaction of rabbit antibody with canine GBM. The second (autologous) stage results from the production and fixation of canine immunoglobulin to this bound rabbit antibody. Corresponding to these different immunological mechanisms are differences in the pathology. The heterologous phase was characterized by extensive glomerular fibrin deposition and necrosis, while in the autologous phase glomerular scarring and "crescent" formation predominated. Similar findings have been reported before in the dog (Wright et al. 1973b).

However, the mechanisms by which these immune reactions actually produce glomerular injury are not fully understood. Work with this type of nephritis in other species has revealed several pathways of injury. Firstly, it has been shown in the guinea pig that binding of anti-GBM antibodies produces proteinuria in the absence of any glomerular damage probably by reducing the net negative change of the membrane (Couser et al. 1977). Secondly, there is complement dependent polymorphonuclear leucocyte mediated injury. This has been shown to be the predominant mediator system in the heterologous phase of NTS nephritis in rats and rabbits (Cochrane et al. 1965). Thirdly, there is polymorphonuclear leucocyte mediated injury which is independent of complement, the actual chemotactic factor(s) involved being at present

unknown. This system has been identified in the autologous phase of NTS nephritis in rabbits (Naish et al. 1975, Thomson et al. 1976b). It is possible that other known mediators of immunological injury are also involved e.g. vasoactive amines, kinins, prostaglandins. Recently it has been shown in NTS nephritis in the rat, that glomerular injury also results from cell mediated immunity (Bhan et al. 1978). Cell mediated reactions were clearly shown to increase the severity of the glomerular damage. However, the mechanisms by which this occurred are at present unknown, although proliferation of glomerular cells may result from the release of lymphokines.

Finally though, (in the rabbit at least) it is the activation of the blood coagulation system that is responsible for much of resultant glomerular scarring and crescent formation. Not only is fibrin deposition very prominent in the disease in rabbits, but in addition, fibrinolytic drugs, serum defibrination (with Ancrod) and, in some instances, anticoagulants afford protection to the glomeruli while fibrinolytic inhibitors give the reverse affect (see page 156). Several mechanisms involving complement, platelets and polymorphonuclear leucocytes by which the coagulation cascade may be activated by immune reactions in glomeruli are known (see page 141). It is known for certain that polymorphonuclear leucocytes are important in the autologous phase in rabbits; although they are not prominent in tissue sections their removal from the blood system produces a marked reduction in fibrin deposition and crescent formation (Naish et al. 1975). However, whether the polymorphonuclear leucocytes lead to fibrin deposition by release of

procoagulant substances or by tissue injury exposing GBM to the circulation is not known (Naish et al. 1975).

Unfortunately none of this work has been carried out on, or applied to, the dog. Therefore, the mediators of injury in this species are at present unknown. However, the prominence of fibrin in this and a previous study (Wright et al. 1973b), and the presence of certain similarities with liquoid nephropathy, suggests that activation of the coagulation cascade plays an important role in the genesis of permanent glomerular injury in canine NTS nephritis. Differences between NTS nephritis and liquoid nephropathy were also apparent. At least 2 factors account for this: the longer period of fibrin deposition with the exudation of large quantities of fibrin into the urinary space, and the involvement of immunological processes.

Initially two main lesions were present in the glomeruli: fibrin deposition and hypercellularity. Where large amounts of fibrin had built up, the resultant ischaemia had led to cellular degeneration and necrosis of the glomerular cells and disintegration of the mesangial matrix and GBM. Thus, the lesions were identical to those seen in acute liquoid nephropathy. However, capillary loops where only a little fibrin was present were different from those from a liquoid treated animal, due to a marked increase in cellularity. The major component of this was infiltration by cells closely resembling circulating monocytes. These cells were very similar to those described previously in NTS nephritis in other species (Kondo and Shigematsu 1972, Morita et al. 1976, Schreiner et al. 1978). Their presence may be analogous to inflammation elsewhere in the body;

monocytes enter from the circulation, transform into macrophages which multiply and so account for some of the mitoses seen with the light microscope (Boss and Rosenmann 1976). However, their absence from liquoid nephropathy suggests that infiltration was a specific result of the immune reaction. Shigematsu and Kobayashi (1971) suggested, on the basis of an electron microscopic study, that they were specifically concerned with the removal of immunoglobulin deposits. However, in light of the recent discovery by Bhan et al. (1978) described above, it is more likely they are a reflection of cell mediated immunity. In addition, hypercellularity also reflected an increase in the numbers of endothelial and mesangial cells. Presumably proliferation of these cells had taken place, for although mitoses were seen in every case under the light microscope they were never positively identified in these cells with the electron microscope. The increase in the numbers of these cells was greater than in liquoid nephropathy. This was possibly a reflection of the longer period of fibrin deposition and hence the more prolonged need to clear fibrin by phagocytosis and repair necrotic areas. In addition, however, endothelial proliferation could have resulted from cell mediated immune reactions (Bhan et al. 1978).

As in liquoid nephropathy fibrin deposition was eventually followed by glomerular scarring. Similarly 2 processes appeared to be involved but differences were apparent. Firstly, the gradual merging of some granular fibrin deposits into basement membrane-like material suggested the transformation of one to another by some

unknown process. However, unlike liquoid nephropathy the discrete foci of brightly staining collagen material with a very fibrillar ultrastructure, which appeared to be formed from persisting fibrin deposits, were not seen. The reason for this was not ascertained. Secondly, the glomerular cells were active in producing new mesangial matrix and GBM. All cells, epithelial, endothelial and mesangial had an increased amount of endoplasmic reticulum indicating protein synthesis. In addition, excess numbers of both endothelial and mesangial cells were present. It was further seen in this study that the canine glomerulus was capable of some degree of recovery from severe damage (cases 95, 96). Although the reduced glomerular injury in these cases could be explained on the basis of less severe initial damage, similar findings have been reported before (Wright et al. 1973b).

The liberation of large quantities of fibrin into the urinary spaces was also an important event in the process of glomerular scarring. The association of fibrin with capsular adhesions, noted in CIN and CGN, was again seen. Adhesions were very prominent in NTS nephritis reflecting the widespread exudation of large amounts of fibrin into the urinary spaces.

A major difference between NTS nephritis and liquoid nephropathy, CIN and CGN, was the presence of crescent shaped cellular infiltrations around the glomeruli. Possibly this reflected the much greater amounts of fibrin that reached the urinary spaces in NTS nephritis than in the other nephropathies. In Human and certain experimental animal

nephropathies, crescent formation is widely believed to be a result of exudation of fibrin into the urinary space (see page 146). Part of the evidence for this concept comes from NTS nephritis in other species, where anti-coagulants, serum defibrination and fibrinolytic drugs have, in certain instances, ameliorated fibrin deposition and subsequent crescent formation (see page 156). However, this may not be relevant to the dog as the cellular infiltrates seen in this study do not strictly conform to the definition of a crescent; cells were invariably present in the interstitium as well as the urinary spaces. The relationship between these infiltrates and the exudation of fibrin into the urinary spaces was not clear. Immunofluorescence showed that large amounts of fibrin were still present in the urinary spaces of glomeruli in cases 91-94. This was rarely seen with the light microscope but cellular infiltration was at its most prominent in these cases. Possibly the masses of cells obscured the presence of fibrin although only a little was identified amongst the cells with the electron microscope.

On the other hand, the predominantly macrophage-like nature of the cells suggests that cell mediated immunity may also have been involved. This finding correlates well with those, in rabbit NTS nephritis, of Kondo et al. (1972) and Holdsworth et al. (1978). The former workers identified many macrophage and monocyte-like cells in crescents with the electron microscope, while the latter authors cultured macrophages from glomeruli with crescents. The route by which these macrophage-like cells reached the urinary spaces

and periglomerular interstitium was not clear in this study. It is likely many were derived from the monocytes present in the tuft, but migration through the capillary wall was never actually seen. In addition, some infiltrates appeared to be present solely in the interstitium suggesting an additional route by-passing the glomeruli. In the past it has been thought that crescents were primarily the result of proliferation of the epithelial cells, in particular the parietal epithelial cells (Churg et al. 1973, Meadows 1973, Min et al. 1974). In this study, although both visceral and parietal cells were present in the crescent shaped cellular infiltrates, there did not appear to be excess numbers of them and mitoses were never seen in the urinary spaces.

In NTS nephritis, in addition to immunoglobulin bound on the GBM, there may also have been deposition of circulating immune complexes. The control cases show that circulating immune complexes were formed, presumably by the production of antibody to rabbit serum proteins, and then deposited in the mesangium and GBMs. This may have accounted for some of the dense deposits and granules of immunoglobulin and complement seen with electron and immunofluorescence microscopy respectively in the later cases (90-96) of NTS nephritis. However, this deposition is a relatively unimportant event, as at the worst it only produced very mild focal glomerular lesions (case 99) against the diffuse severe damage typical of NTS nephritis.

CONCLUSIONS

This study showed that chronic nephropathies of the dog characterized by diffuse renal scarring fall into two distinct groups, CIN and CGN. It was impossible to accurately differentiate them on morphological grounds, although renal scarring tended to be less severe and glomerular fibrin deposition more severe in CGN. Accurate classification relied on techniques (such as immunofluorescence) that revealed the immune complex aetiology of CGN, and the virtual or complete absence of such complexes in CIN.

However, the diagnosis of CIN probably covered a heterogeneous group of dogs. Most (24 of the 30 cases in this study) resembled those thought to be a result of previous L.canicola infection, with renal scarring concentrated around the cortico-medullary junction. The elution of anti-L.canicola antibodies from many of these cases, sometimes at high titres added further support to this concept. Other cases (6 in this study) had diffuse even fibrosis throughout the kidney, a pattern less likely to result from L. canicola infection. Indeed anti-L.canicola antibodies were not found in eluates from these cases. However, there was no positive evidence as to a different aetiology of these cases. Two other organisms, CAV and L.icterohaemorrhagiae, (Bush and Evans 1972, Timoney et al. 1974) have been proposed as a cause of CIN but elution and immunofluorescence studies failed to show any evidence of infection by either in the 30 cases described.

It is reasonable to presume that CIN follows a non-fatal episode of AIN, but the mechanisms which produce the progressive renal scarring that eventually leads to chronic renal failure have not been fully elucidated. Two processes have been suggested: the stimulation of hypertension and immunological mechanisms (Anderson 1968b). The latter was investigated in this study by a combination of immunofluorescence and elution techniques. Anti-kidney antibodies were never identified and an immunofluorescence pattern suggestive of immune complex deposition was seen in only 3 cases where scanty deposits were present in just an occasional glomerulus. Thus, the results of this study indicate that neither autoantibodies nor immune complex deposition are the cause of the severe progressive renal scarring that characterizes CIN.

In contrast, CGN is an immunologically mediated nephropathy. In 9 cases granules of immunoglobulin with bound complement were present in the glomeruli - a pattern widely accepted as indicating immune complex deposition. The nature of the antigen in these complexes remains unknown. There was no association with any particular extra-renal lesion and immunofluorescence and elution studies failed to implicate L.canicola, L.icterohaemorrhagiae, CAV or renal antigens. In the remaining case a linear deposition of immunoglobulin and complement along the capillary walls was seen, a pattern suggestive of anti-GBM antibodies but these were not found in the eluate.

Despite this difference in the pathogenesis of CIN and CGN, cases of both were characterized by very similar

lesions of glomerular scarring. This was found to involve the progressive obliteration of the glomerulus by 4 materials: basement membrane, mesangial matrix, fibrin and material derived from fibrin, and collagen. In the early stages of scarring localized narrowing or obliteration of capillaries was seen, caused by a combination of GBM thickening, wrinkling and duplication, axial and circumferential expansion of the mesangial cells, an increase in the amount of mesangial matrix and an increase in tuft cellularity. In some capillaries fibrin deposits were also present and exudation of fibrin into the urinary space appeared to lead to the formation of capsular adhesions. These changes were often accompanied by thickening, wrinkling and duplication of the CBMs. The end result was a shrunken non-functional mass of collapsed, thickened CBM enclosing matrix but few, if any, patent capillaries and atrophic cells. In most of these obsolescent glomeruli the CBM had collapsed around the tuft and the urinary space obliterated by material containing collagen fibres. These glomerular remnants appeared to progressively shrink and disintegrate. In what was usually a minority of instances however, the CBM remained intact and filled with fluid, and so persisted as a cyst.

The impression was gained from both light and electron microscopy that fibrin was of major importance in the genesis of glomerular scarring. Not only did fibrin appear to lead to the formation of capsular adhesions, but more importantly intra-capillary deposits appeared to lead to mesangial expansion and GBM thickening. This concept received further support from two experimental renal

diseases, one immunologically mediated (NTS nephritis) and the other non-immunologically mediated (liquoid nephropathy). In the latter, a short period of severe glomerular thrombosis is followed in those cases that survived by mild focal glomerular scarring. In the former, a prolonged period of fibrin deposition was followed by widespread and severe glomerular scarring. The morphological details of scarring were similar in these two experimental nephropathies and, moreover, closely resembled those described above in CIN and CGN. Obviously this does not prove that fibrin deposition alone leads to glomerular scarring in the dog, and it must be remembered that other mediators of tissue injury are active in NTS nephritis and possibly in liquoid nephropathy as well. However, previous studies of these nephropathies in other species have given more conclusive evidence to link scarring with fibrin deposition. Anticoagulants, fibrinolytic drugs and serum defibrination have, in some but not all studies, ameliorated both fibrin deposition and subsequent scarring whilst anti-fibrinolytic agents have given the opposite effect.

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