# 24 Renal Pathology

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## Introduction

This chapter reviews the usual circumstances in which biopsies are obtained, methods of obtaining the biopsy material and analyzing the tissue, and the distinct characteristic morphologic findings in various diseases. Last, experimental techniques that may provide important pathogenic, prognostic, or diagnostic information are discussed.

## Renal Biopsy Indications

The indications for renal biopsy vary according to the ethnic and age characteristics of the population studied and the geographic location because these factors influence the incidence of various renal diseases. The indications discussed below present the most common settings in children for which renal biopsy is undertaken.

### Hematuria

Isolated hematuria (i.e., without proteinuria and with normal function of the kidney) may be due to hypercalciuria or familial or urologic disease (1–3). Once these disorders are ruled out, a glomerular origin of persistent isolated hematuria should be considered. Red blood cell casts or dysmorphic red blood cells indicate glomerular origin of hematuria. Renal biopsy may define the underlying abnormality in these patients. The most common findings are mesangial proliferative disease or IgA nephropathy (Berger's disease). Less common disorders include hereditary nephritis (Alport syndrome) and thin basement membrane lesion. The latter may be familial (benign familial hematuria) or sporadic. One-quarter to nearly one-half of the patients with isolated hematuria have normal biopsies (1–4). Renal biopsy may, therefore, define the pathology and provide assurance of a benign prognosis in some patients or diagnose a possible hereditary disease, which would initiate screening of other family members. Last, the information obviously can be of importance in avoiding further repeated invasive evaluation in the patient.

### Proteinuria

Isolated proteinuria may be postural or due to tubulointerstitial disease. These possibilities should be evaluated completely over time before renal biopsy is considered. Any glomerular disease may cause mild to moderate proteinuria as the only manifestation, and biopsy may yield the diagnosis even at an early stage.

### Nephrotic Syndrome

Numerous children with nephrotic syndrome (NS) were studied when renal biopsy first became available. The biopsies showed so-called minimal-change disease (MCD) in the vast majority of cases. The efficacy of corticosteroids in this setting has obviated the need for renal biopsies in most of these cases. Therefore, young children with NS will typically undergo a therapeutic trial of corticosteroids without a biopsy. However, in infants with NS, in older children, or in those with evidence of nephritis (hypertension, hematuria, low C3, or decreased renal function) or failing corticosteroid therapy, renal biopsy is often performed. In these patients, disease other than MCD (e.g., focal segmental glomerulosclerosis [FSGS], membranoproliferative glomerulonephritis [MPGN], IgA nephropathy, membranous glomerulopathy, or more rarely, in infants less than 1 year, Finnish-type nephrotic syndrome or diffuse mesangial sclerosis) is often present (4–9). Children with steroid-resistant NS and FSGS on biopsy may have a podocyte gene mutation, such as podocin, as cause of their disease. Genetic testing is important to identify these children, as 10–30% of children with sporadic steroid-resistant NS and FSGS have such mutations, and do not typically respond to continued immunosuppression (10, 11). These genetically-induced lesions do not show specific renal biopsy morphologic findings.

## Acute Nephritis

The child with acute glomerulonephritis may need a biopsy when the course is not typical of acute poststreptococcal disease or if urinary abnormalities persist. Although the primary disease process may be evident in systemic conditions, such as Henoch-Schönlein purpura or systemic lupus erythematosus (SLE), renal biopsy often is indicated to assess severity of injury, to guide therapy and prognosis. Differentiation of specific types of proliferative lesions such as MPGN type I and dense deposit disease (DDD) is made by renal biopsy. This distinction has important implications for eventual treatment because the morphologic lesions of DDD invariably recur in transplants, although the clinical course is less severe than in the native kidney (12, 13).

## Acute Renal Failure

The cause of acute renal failure may be clinically obvious, or there may be multiple potential culprits. When prerenal and obstructive causes are not apparent, renal parenchymal disease should be considered. When acute renal failure is associated with nephritis, NS, or evidence of vasculitis or systemic diseases, biopsy is usually performed. Other common causes include acute tubular necrosis or injury, often caused by drug or ischemic injury, vascular disease, and interstitial nephritis. These conditions can often be diagnosed without renal biopsy. However, when the cause remains uncertain after complete evaluation, renal biopsy may be necessary for diagnosis (14).

## Rapidly Progressive Glomerulonephritis

Renal biopsy may be considered an urgent procedure in the patient with rapidly progressive glomerulonephritis (RPGN). Various systemic vasculitides that may be distinguished only by specific serologic studies (see below) or renal biopsy must be treated urgently to avoid severe chronic renal damage. Although anti-glomerular basement membrane (GBM) antibody or anti-neutrophil cytoplasmic antibody (ANCA) titers may provide useful information, the ANCA test in particular is not diagnostic of a specific condition, rather it is a screening test for necrotizing vasculitides (15, 16). ANCA positivity, whether in a perinuclear (p-ANCA) or cytoplasmic (c-ANCA) pattern was present in approximately 60% of patients with immune-complex glomerulonephritis with crescents in a study of more than 200 renal biopsies (17). Furthermore, specialized confirmatory ELISA ANCA assays may have a longer turn-around time than the renal biopsy, from which preliminary information from immunofluorescence (IF) and light microscopic studies can be available within hours after biopsy.

## Chronic Renal Insufficiency

Patients with chronic renal insufficiency of uncertain etiology are candidates for renal biopsy. Although renal biopsy of the small, shrunken kidney is more risky because of the greater incidence of bleeding complications, the diagnosis of primary disease can be important. This information allows assessment of existing severity of morphologic lesions, determination of risk of recurrence in eventual renal transplant, and suitability of cadaveric vs. living-related donor transplantation. If the disease has a familial basis or recurs frequently with resultant graft loss, cadaveric transplantation may be preferable to living-related donor transplant (13).

## Systemic Diseases

The severity of renal involvement in systemic disease, such as hemolytic-uremic syndrome (HUS), Henoch-Schönlein purpura, diabetes mellitus, or SLE, may not be apparent without renal biopsy. The trend is now toward early biopsy in patients with diabetes and renal abnormalities. Severity of lesions and stage of chronicity and activity impart prognostic information and may affect therapeutic decisions (see below). The most extensively studied disease in this regard is SLE. Differentiation of specific class of lupus nephritis by WHO or International Society of Nephrology/Renal Pathology Society (ISN/RPS) class (see below) may be difficult without renal biopsy (18–20). Overall, evidence indicates that renal biopsy findings may be more sensitive than clinical assessment alone in evaluating the severity of renal involvement in SLE (21, 22).

## Follow-Up of Disease

With improved therapeutic modalities available for intervention in chronic progressive renal disease, sequential or follow-up biopsies is becoming increasingly necessary to evaluate therapeutic efficacy. On the other hand, additional cytotoxic therapy with its side effects may be withheld if the biopsy shows end-stage histology. Intervention with, for example, low-protein diets or angiotensin-converting enzyme (ACE) inhibitors or angiotensin

type 1 receptor blockers (ARBs) has been shown to alter the course of chronic progressive renal disease (23).

### Transplantation

Renal transplant biopsies are useful in assessing episodes of clinically suspected rejection, investigating the cause of decreased renal function or urine output, and detecting the development of de novo or recurrent disease. Occasionally, infection may be diagnosed by renal biopsy. Drug toxicity may be diagnosed by morphologic findings. The absence of lesions in a patient with a rise in creatinine supports calcineurin inhibitor toxicity because this drug commonly causes a decline in the glomerular filtration rate (GFR) by vasoconstriction and not overt structural lesions. The absence of findings of acute rejection in renal biopsy or by needle aspiration (see below) thus can assist in avoiding unnecessary immunosuppressive therapy with its potential for increased morbidity and mortality. Even a diagnosis of chronic allograft nephropathy (CAN) which is not amenable to immunosuppressive therapy, has important therapeutic implications for the patient.

Diseases that recur in the transplant with high frequency include IgA nephropathy, MPGN type I, dense deposit disease (DDD, also known as MPGN type II), FSGS, HUS, and membranous glomerulopathy. The latter two also occur de novo in the transplant. Metabolic diseases such as oxalosis and diabetic nephropathy can also cause recurrent disease in the renal transplant, if liver or pancreas transplantation does not cure the primary abnormality (13). Alport syndrome is caused by mutation in one of the type IV collagen genes, resulting in abnormal GBM assembly and structure. Patients with Alport may develop anti-GBM antibody disease in the transplant because of antibodies against its normal type IV basement membrane collagen (24, 25).

## Obtaining Tissue

### General Considerations

Percutaneous renal biopsy is the most common method for obtaining tissue for the kidney. In large series, major complications are rare. The technique, first done in 1951 by Iverson and Brun, allows tissue yield in 93–95% of biopsies, with more than 87% of these being adequate (23–25). The biopsy findings altered diagnoses in half of the cases in one series, indicating different therapeutic approaches in approximately one-third of those cases (26). Although some renal diseases show diagnostic features by light microscopy (LM) (*Table 24-1*), special studies add to the sensitivity of the study. For the renal biopsy to be most useful, it must be evaluated appropriately by an experienced renal pathologist. Biopsies must be examined by special LM, IF, and electron microscopy (EM) for the most accurate diagnosis (27). If the nephrologist's hospital does not provide these services, arrangements must be made to send tissue in appropriate fixatives (see below) to a reference laboratory with these capabilities. If such services cannot be provided, it is doubtful whether the institution should be undertaking renal biopsies.

### Contraindications

Contraindications to percutaneous biopsy are solitary, ectopic, or horseshoe kidney; bleeding diathesis; abnormal renal vascular supply; and uncontrolled hypertension (26–29). In the era of ultrasound guidance and automated biopsy instruments, solitary kidney may be biopsied safely in selected patients, however (30). Relative contraindications include obesity, uncooperative patients, hydronephrosis, ascites, and small shrunken kidneys, all associated with greater risk for complications. Open biopsy is preferable if the biopsy information is crucial in these conditions. Percutaneous biopsy is contraindicated if the kidney has tumors, large cysts, abscesses, or pyelonephritis because the needle track may facilitate spread of malignant cells or infection. Open biopsy allows selection of specific areas for biopsy in these situations.

### Biopsy Technique

The patient may be brought to the hospital on the day of the biopsy. Laboratory evaluation must include a complete blood cell count with normal platelet count, partial thromboplastin and prothrombin times. On rare occasions, infusions of platelets or fresh frozen plasma may be necessary to allow renal biopsy in critical clinical situations in which histopathologic diagnosis is essential. Adequate control of hypertension before the procedure is important as hypertension is a risk for post biopsy bleeding (31). Before biopsy is done, ultrasound examination must confirm that there are two kidneys in normal position. The biopsy optimally is timed so that an experienced technician or pathologist can attend to ensure prompt processing of the biopsy tissue.

Food and drink should have been withheld for at least 6 h before biopsy, and the child should be lightly sedated. The child lies in the prone position with a sandbag or

**Table 24-1**

**Characteristic abnormalities of glomerular diseases**

| Disease and typical clinical presentation | LM pattern | IF Staining | | | EM, Other Findings |
|---|---|---|---|---|---|
| | | Mesangial | Subepithelial | Subendothelial | |
| *Hematuria/nephritis* | | | | | |
| Alport's syndrome | Early: normal<br>Late: sclerosis | – | – | – | Thin and thick, split GBM |
| Mesangial lupus nephritis ISN/RPS II | Mesangial proliferation | +<br>All Igs, C3, C4 | – | – | Immune deposits by EM, reticular aggregates in endothelial cells |
| Focal lupus nephritis ISN/RPS III | Proliferative, <50% of glomeruli | + | + (few) | + (Scattered) | Immune deposits by EM, reticular aggregates in endothelial cells |
| | | –All Igs, C3, C4– | | | |
| Diffuse lupus nephritis ISN/RPS IV | Proliferative, >50% of glomeruli wire loops | + | + | + (wire loop) | Immune deposits by EM, reticular aggregates in endothelial cells |
| | | –All Igs, C3, C4– | | | |
| IgA nephropathy | Mesangial proliferation | +<br>Predominantly IgA | – | – | Immune deposits by EM |
| Henoch-Schönlein purpura | Mesangial and ± endocapillary proliferation, ± crescents | +<br>– | +/–<br>Predominantly IgA | +/–<br>– | Immune deposits by EM |
| Post-infectious GN | Endocapillary proliferation, PMNs | +<br>Coarsely granular<br>IgG, C3 | +<br>Coarsely granular<br>IgG, C3 | – | Irregular, hump-like deposits on top of GBM by EM |
| Hemolytic-uremic syndrome | Glomerular/arteriolar thrombosis | – | – | – | Increased lamina rara interna by EM, swollen endothelial cells, no deposits |
| MPGN I | Endocapillary proliferation, lobular double contour GBMs | +<br>IgG, C3 | – | +<br>IgG, C3 | Subendothelial immune deposits by EM, cellular interposition |
| MPGN II (dense deposit disease) | Mesangial, ± endocapillary proliferation, ribbon-like capillary wall | ±<br>C3 globular | –<br>Ribbon-like, C3 | Discontinuous | Intramembranous, mesangial non-immune dense deposits by EM |
| *Nephrotic syndrome* | | | | | |
| Minimal change disease | Normal | – | – | – | Effacement of podocyte foot processes<br>No deposits |
| Focal segmental glomerulosclerosis | Segmental glomerulosclerosis, glomerular hypertrophy | +/–<br>IgM, C3 | – | – | Effacement of podocyte foot processes, no deposits |
| Diabetic nephropathy | Increased mesangial matrix, ± nodular, thick GBM, hyalinized arterioles | – | – | – | Thick GBM without deposits |

**Table 24-1 (Continued)**

| Disease and typical clinical presentation | LM pattern | IF Staining | | | EM, Other Findings |
|---|---|---|---|---|---|
| | | Mesangial | Subepithelial | Subendothelial | |
| Membranous lupus nephritis ISN/RPS V | Thick GBM, spikes on Jones' stain | Scattered | + | – | Subepithelial, mesangial immune deposits |
| | | + | –All Igs, C3, C4– | – | Reticular aggregates in endothelial cells |
| Idiopathic membranous GN | Thick GBM, spikes on Jones' stain | – | + | – | Subepithelial immune deposits |
| | | – | IgG, C3– | – | |
| *RPGN* | | | | | |
| Anti-GBM disease | Focal segmental necrosis of glomeruli, crescents | – | Linear staining of GBM | – | No deposits by EM |
| | | | IgG, C3 | | |
| Wegener's granulomatosis | Focal segmental necrosis of glomeruli, crescents | – | – | – | No deposits by EM |
| Microscopic polyangiitis | Focal segmental necrosis of glomeruli, crescents | – | – | – | No deposits by EM |

rolled sheet under the abdomen, and the skin of the flank is "prepped" and draped in sterile fashion. Although the left kidney is usually preferred, either side can be chosen for biopsy. The lower pole of the kidney is marked on the skin with a pen after localization by any of several imaging techniques, such as fluoroscopy, radionuclide scanning, intravenous urography, computed tomography (CT), or ultrasound, the last being the most commonly used method. Local anesthetic is infiltrated first in the skin and then in deeper tissues, taking care not to enter the kidney. In younger children who cannot reliably cooperate in this manner, biopsy is done under anesthesia. Conventional or spring-loaded needles are used for renal biopsies. Most now use the spring-loaded so-called "biopty gun". For conventional needles, the biopsy needle is inserted to the desired position as the patient again holds his or her breath, advancing the cannula over the obturator once the needle is in correct position. The entire needle with the core of tissue is then removed.

The use of an automatic spring-loaded biopsy system has been used widely in the last years because of the simplicity and ease of the technique (32, 33). The kidney is localized with ultrasound guidance, and the depth of the kidney as judged by ultrasound. The biopsy needle is advanced to the kidney capsule under ultrasound observation and guidance. The patient may hold his or her breath for only a few seconds while the spring-loaded needle is activated, causing the obturator to automatically advance into the kidney, and the entire needle is then removed with the tissue core. The speed of automated biopsy needles, however, minimizes the need for the patient to hold respirations, required with conventional needles. It is important to note that the caliber of the needle used with any of these techniques directly impacts the adequacy of the specimen (34). When 18-gauge needles are used with this method, the resulting cores are very small and there is artifact along the edges. The use of a 16-gauge needle thus is more likely to provide an adequate tissue sample without distortion and with fewer passes necessary to obtain adequate tissue.

Usually two cores of tissue are necessary for optimum evaluation, or three cores if 18-gauge needles are used. If tissue cannot be obtained after several passes, the biopsy should be attempted on another day. After biopsy, a dressing is applied, and the child is kept supine in bed for 6 h and monitored with frequent checks of vital signs and urine for hematuria.

Increasingly, percutaneous native and transplant renal biopsies are performed as same day procedures in pediatric patients so that hospital admission is not required. Those with post biopsy perinephric hematomas, post biopsy gross hematuria, or very young children can be observed overnight so that hemostasis is assured (35–37). Open biopsy can be performed under local or general anesthesia. The kidney can be directly visualized even through a small incision. Although a larger sample may be obtained with a wedge biopsy, it is preferable to also perform a needle biopsy to sample deeper cortex and

medulla for assessment of diseases that preferentially involve juxtamedullary glomeruli (see below).

### Aspiration Biopsy

Fine-needle aspiration (FNA) biopsy technique is used most often in the transplant setting for analysis of immunologically activated cells. FNA can be useful in evaluating type 1 acute rejection (38). FNA can also obtain material for culture. A modified FNA technique has been described for collection of glomeruli from either native or transplant kidneys to analyze glomerular lesions. This FNA technique has limitations in sample size and is obviously not suitable for study of vascular or fibrotic processes. The less invasive nature of the procedure makes it amenable to serial monitoring of interstitial cellular infiltrates in transplanted kidneys (39).

### Complications

Important complications occur in 5–10% of patients (26, 40–48). Major complications, usually bleeding, leading to nephrectomy occurred in 5 patients in a review of 8,081 (48), and 1 patient in a series of 5,120 biopsies. In a series totaling 1,820 biopsies in children, 1 nephrectomy resulted (40–45). Transient microscopic hematuria is universal after biopsy, although macroscopic hematuria is seen in only 5% and requires transfusion in up to 2.3% of patients. Perirenal hematoma is most often asymptomatic and can be seen by CT in up to 85% of biopsies. Symptomatic hematoma is rare, occurring in less than 2%. Arteriovenous fistulae are symptomatic with hematuria, hypertension, or cardiac failure in only 0.5% of biopsies, although bruits may be detected in as many as 75% of patients. Most fistulae heal within a few months. Other complications have been reported, including inadvertent puncture of other viscera or major renal vessels, sepsis, renal infection, and seeding of cancer. Death is rare, less than 0.1% in reviews of large series. Complication rates appear to be slightly higher in less developed countries (40, 42). Complications for the spring-loaded needle biopsy system appear to be similar to conventional needles if the same gauge is used (49). Complications relate in part to the number of passes made to obtain tissue. Therefore, the lower rate of complication with an 18-gauge spring-loaded needle in some studies is offset largely by the need for more passes for adequate samples and the distortion of tissue and edge artifact with use of an 18-gauge needle (see above).

## Assessment of the Renal Biopsy

### Adequacy of Sample

Cores of fat and connective tissue will float when placed in saline, but a core of renal parenchyma will sink. The biopsy sample should also be visually inspected with a dissecting microscope or hand lens. Glomeruli are visualized as small red dots in the biopsy core. Scarred glomeruli may be difficult to identify since they are not perfused. In diffuse disease, such as membranous glomerulopathy, one glomerulus may be adequate for diagnosis. However, in other diseases, such as crescentic glomerulonephritis, FSGS, or lupus nephritis, disease may be focal. The greater the number of glomeruli sampled, the lower the probability of missing a focally distributed lesion (50). If only 10% of glomeruli in the kidney are involved by the focal process, a biopsy sample of only 10 glomeruli has a 35% probability of missing the lesion, decreasing to 12% if the biopsy contains 20 glomeruli. When one-fourth of glomeruli are involved in the kidney, there is only a 5% chance of missing the abnormal glomeruli in a biopsy of 10 glomeruli. A biopsy of 20–25 glomeruli is sufficient to distinguish between mild disease (less than 20% of glomeruli involved), moderate disease (20–50% of glomeruli involved), or severe disease (more than 50% of glomeruli involved). Unfortunately, the widespread use of small gauge spring-loaded biopsy needles often results in smaller samples, which make the above assessments difficult or impossible. The sample site must also be considered in evaluating the adequacy of tissue. Even a large biopsy, consisting only of superficial glomeruli, cannot exclude the presence of early FSGS, in which the initial involvement is in the juxtamedullary glomeruli. Likewise, although nephronophthisis is most often diagnosed clinically, a juxtamedullary biopsy would be necessary for its morphologic diagnosis.

### Allotment of Tissue

Renal tissue should be studied by light microscopic techniques with special stains (hematoxylin and eosin, modified silver stain [periodic acid/methenamine or Jones' stain], periodic acid-Schiff), IF, and EM (51–53). The tissue is divided so that glomeruli are present in each portion of the sample. Optimally, the pathologist or an experienced histotechnologist will attend the biopsy and inspect and allot tissue for each study. If this is impossible, tissue may be placed in saline and brought directly to the laboratory for prompt processing. It is important to handle the tissue gently so that artifacts do not occur.

The fresh tissue must not be picked up with forceps because this will crush and distort the morphology. The core can be handled carefully with a wooden stick or pipette. The core should not be placed on a sponge or gauze pad because this may cause a divotlike artifact as the unfixed tissue molds to the holes of the underlying surface. The biopsy specimen is therefore placed on a clean smooth surface, such as a wax board, for cutting.

It may be difficult to identify the cortical end of the specimen even after inspection with a hand lens or dissecting microscope when scarring or severe injury is present. Therefore, we recommend cutting two 1-mm pieces with a sharp blade from each end of the core for electron microscopic studies. The remaining core is then divided into specimens for IF and LM. Of note, use of an 18-gauge needle yields tissue cores that are too thin to divide lengthwise, leading to a greater chance of problems in allocation of tissue for each method of study. This tissue should be cut across into two pieces for IF and LM. When two cores are obtained, we prefer to duplicate this process, rather than allocating one complete core to one study to maximize chances of adequacy of tissue for each study. When the tissue sample obtained is very small, the nephrologist and the pathologist should consider the differential diagnosis and allocate tissue accordingly. For example, in a case of suspected IgA nephropathy, tissue for IF is most important. Although electron microscopic studies can be done on other portions of tissue (as long as mercury-based fixatives have not been used), IF studies on fixed tissue are not as reliable. When tissue for EM is inadequate, portions of the paraffin-embedded tissue left after light microscopic examination may be cut out from the block and processed for EM. Although the quality is not optimal, diagnostic findings can still be discerned. In special circumstances, when no tissue remains in the paraffin block and a focal lesion in one section must be studied, one may attempt to process the tissue section from a glass slide for EM.

## Light Microscopy

Numerous fixatives are used for light microscopic examination, and they vary from institute to institute. Satisfactory results may be obtained with Zenker's, Bouin's, formalin, Carnoy's, or paraformaldehyde. Material for IF studies may be snap frozen immediately at −20°C in solutions of isopentane, dry ice, acetone, or freon and embedded in Tissue-tech, OCT, or other compounds for frozen sections. If tissue cannot immediately be snap frozen, it may be placed in Michel's tissue media, where it may be stored for up to 1 week before freezing. This allows tissue to be sent to reference laboratories for appropriate processing. Tissue for EM may be fixed in glutaraldehyde, formaldehyde, or other appropriate non-mercury fixatives. Tissue placed in glutaraldehyde should be promptly processed, or if stored for future possible processing, should be transferred to an appropriate buffer solution within a week to avoid artifacts. Tissue for LM is routinely processed, embedded in paraffin, and cut into 2- to 3-μ thick sections. Serial sections with multiple levels are then prepared for examination.

If water-soluble compounds are expected, such as urate or uric acid, the tissue should be fixed in ethanol. Lipids are best detected in frozen sections because they are extracted during xylene processing for paraffin sections. Hematoxylin and eosin stains are most useful for overall assessment of the interstitium and crystals. This stain also allows particularly good visualization of infiltrating cells, especially eosinophils. In addition, fibrin may be easily visualized by this stain. Periodic acid-Schiff (PAS) stains glycoproteins and accentuates basement membranes and matrix material and allows definition of the brush border of proximal tubular cells. Areas of hyalinosis and protein precipitation, including cryoglobulin (which usually is dominantly IgM), are also accentuated with PAS. Silver stains, such as Jones' stain, stain basement membrane material but not deposits, thus allowing distinction of these components. Masson's trichrome stain detects areas of collagen deposition by staining bluish. Other special stains, such as immunohistochemistry for specific molecules, may be indicated. Congo red stain detects amyloid. Special stains can detect bacterial or fungal organisms and acid-fast bacilli. Special techniques may also be used on the light microscopic material. These include polarization to detect crystals or foreign bodies and morphometry to assess glomerular size (see below) and severity of interstitial fibrosis quantitatively.

## Immunofluorescence

IF studies are most commonly done by direct IF on frozen tissue sections with application of fluorescein-conjugated antibodies directed against IgG, IgA, IgM, and complement component C3. Additional antisera may be used as clinically indicated. These include antisera to kappa or lambda light chain, antisera to hepatitis B antigen, thyroglobulin, fibrinogen, C1q, C4 cyantisera to type IV collagen chains and C4d. Some of these antigens are recognized by antisera even after fixation and can be detected by immunohistochemical techniques (e.g., immunoperoxidase) on the formalin-fixed tissue sections and then studied

with a light microscope. This technique requires enzyme pretreatment of tissue, which must be tailored exactly depending on length and type of fixation, section thickness, and antigen one wishes to study. Direct observation of the digestion process, stopping when all plasma is removed from capillary loops has been used to achieve reliable results (54). These challenges have prevented widespread use of this technique in the USA.

Frozen tissue sections stained by the commonly used method of fluorescein-conjugated antibodies are viewed by IF microscopy, evaluating staining in glomeruli, vessels, tubules, and interstitium. The pattern of glomerular staining is assessed to define granular or linear capillary wall staining or mesangial deposits. Arteriolar staining may be of diagnostic significance. Tubules may show deposits in lupus nephritis, light chain deposition disease or linear staining in the rare anti-TBM antibody disease. Nuclear staining can be seen in lupus or lupuslike diseases as a tissue manifestation of the patient's positive ANA. IF is more sensitive than EM in identifying immune deposits, although EM provides more detailed information on the exact localization of those deposits (52, 53). Lesions of interest should be photographed because fluorescence fades on storage and with light exposure.

### Electron Microscopy

Tissue for EM is processed with postfixation in 1% osmium tetroxide, which enhances contrast of the tissue, and then dehydrated and embedded. With new, more rapidly polymerizing embedding media, such as Spurr, tissue may be ready for examination within 1 day. Electron microscopic study was found to add information in 6–11% of renal biopsies in a study from 1983 (27). In a 1997 study, EM was needed to make a diagnosis in 21% of cases, and provided important confirmatory data in approximately 20% of cases (52). EM showed diagnostic pathological abnormalities in 18% of patients with "normal" light microscopic findings. Larger, so-called thick scout sections (1 μ) are stained with toluidine blue to select smaller areas for thin sectioning for the electron microscope. Usually, the glomerulus containing the most representative lesion is chosen, but sclerotic glomeruli are not useful to examine by EM. If there are irregular or focal proliferative lesions, several glomeruli may be sampled, including the proliferative ones. The 60–90 Å thin sections are stained with uranyl acetate and lead citrate before viewing in the EM scope, to enhance contrast.

Immune complex deposits are nonmembrane-bound and denser than basement membrane or matrix materials. In specific diseases, such as cryoglobulinemia, amyloid, immunotactoid or fibrillary glomerulopathies, or lupus nephritis, specific substructure of deposits may be seen. Specific localization of immune complexes is done by EM examination, indicating whether deposits are subendothelial, subepithelial, mesangial, or in all of the above compartments. In some diseases, such as light chain deposition disease or lupus nephritis, deposition may also be seen in vessels and tubules. So-called fingerprint deposits, with substructure reminiscent of fingerprinting, are present in some cases of lupus nephritis. Reticular aggregates (or so-called tubular arrays) of membrane material in endothelial cell cytoplasm throughout the body are characteristically seen in large numbers in patients with SLE or HIV infection, thought to reflect a response to high levels of interferon (53, 55).

EM also delineates specific basement membrane abnormalities. For instance, small subepithelial deposits without surrounding new basement membrane material do not result in spikes, and therefore cannot be visualized by Jones' stain on LM, but can still be detected directly by EM. The lamina densa of the GBM is markedly thickened in diabetic nephropathy. Circumferential cellular interposition is defined by extension of monocytes and mesangial cell cytoplasm into the subendothelial space, with newly formed basement membrane interpositioning between the advancing infiltrating cells and the endothelium with intervening basement membrane material, thus giving rise to the classic double contours seen with silver stain by LM in MPGN. Increased lucent material is present in the expanded lamina rara interna in transplant glomerulopathy, HUS, ecclampsia and other diseases presumed to involve endothelial injury/coagulopathy. In these conditions, deposition of fibrin, fibrinogen and their degradation products may also occur. Fibrin is recognizable by its dense, coarse sheaflike structure by EM, with periodicity observed in favorable sections. Morphometry of the GBM from EM prints is used to diagnose thin basement membranes in hereditary nephritides. EM also allows structural assessment of changes of specific cells (see below). Diagnostic inclusions are seen in various storage/metabolic disease, such as Fabry's disease.

## Basic Renal Lesions

### Normal

During fetal maturation, the glomerular capillary tufts are initially covered by large, cuboidal, darkly staining epithelial

cells with only small lumina visible (Fig. 24-1). The cells lining Bowman's space undergo similar change from initial tall columnar to cuboidal to flattened epithelial cells, except for those located at the opening of the proximal tubule, where cells remain taller. Immature nephrons may occasionally be seen in the superficial cortex of children up to 1 year of age. Glomerular growth continues until adulthood, with average normal glomerular diameter approximately 95 μ in a group of patients less than 5 years old (average age 2.2 years) and 140–160 μ in adulthood (57, 58).

The normal mature glomerulus consists of a complex branching network of capillaries originating at the afferent arteriole and draining into the efferent arteriole. The glomerulus contains three resident cell types: mesangial, endothelial, and epithelial cells (Fig. 24-2). The visceral epithelial cells (podocytes) cover the urinary surface of the GBM with pseudopodlike extensions called foot processes, with intervening filtration slits. Endothelial cells are opposed to the inner surface of the GBM and are fenestrated. At the stalk of the capillary, the endothelial cell is separated from the mesangial cells by intervening mesangial matrix. The term *endocapillary* is used to describe proliferation filling up the capillary lumen, contributed to by proliferation of mesangial, endothelial and infiltrating inflammatory cells. In contrast, *extracapillary* proliferation refers to proliferation of the parietal epithelial cells that line Bowman's capsule.

The mesangial cell is a contractile cell that also has phagocytic properties. It lies embedded in the mesangial matrix in the stalk region of the capillary loops, attached to anchor sites at the ends of the loop by thin extensions of its cytoplasm. Normally up to three mesangial cell nuclei per lobule are present. The basement membrane consists of three layers distinct by EM, the central broadest lamina densa and the less electron-dense zones of lamina rara externa and interna. Thickening occurs with maturational growth. Most investigators have found thicker basement membranes in boys, with normal range from 220 to 260 nm at 1 year of age, 280 to 327 nm at age 5 years, 329 to 370 nm at age 10 years, and 358 to 399 nm at age 15 years (59, 60). In our laboratory, we found a range of GBM thickness in children with normal kidneys from approximately 110 nm at age 1 year to 222 ± 14 nm at 7 years of age.

The glomerulus is surrounded by Bowman's capsule, which is lined by parietal epithelial cells. These are continuous with the proximal tubule, identifiable by its

**Figure 24-1**

**Schematic illustration of glomerulus, with glomerular capillary attached to a mesangial stalk area. The glomerular endothelium *(E)* is fenestrated and lines the glomerular basement membrane *(GBM),* which covers the mesangium. The outside of the GBM is covered by the epithelial cell and its foot processes *(Ep)*. The mesangial cell *(M)* is embedded within the mesangial matrix *(MM),* with processes connecting to the GBM (Provided courtesy of Professor Wilhelm Kriz with permission from (56).**

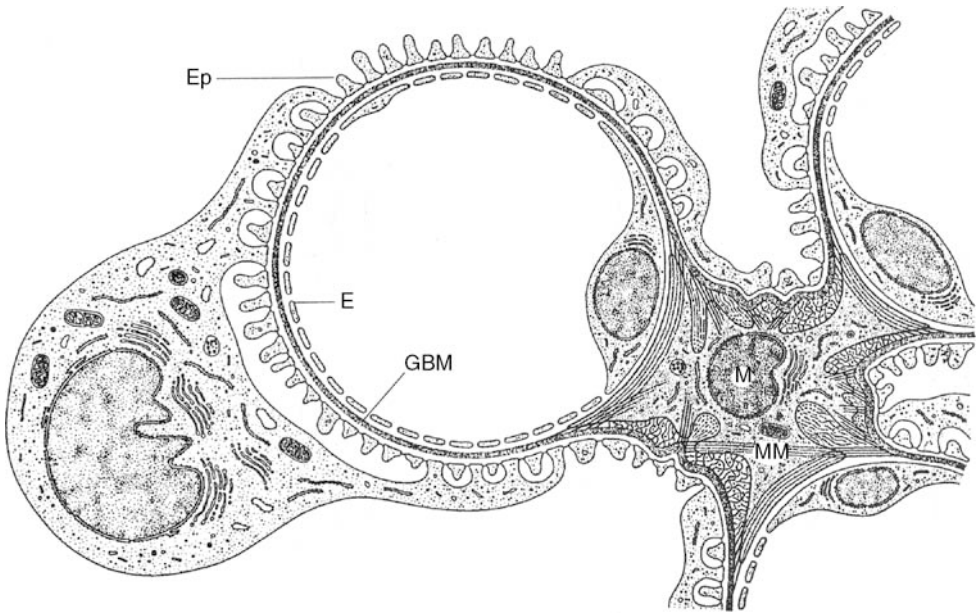
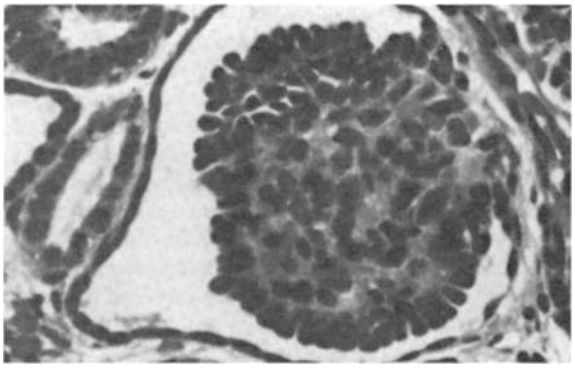


**Figure 24-2**
**Immature glomerulus with plump, dark epithelial cells from biopsy of a 29-week-gestation baby (PAS, ×670).**



PAS-positive brush border. The efferent and afferent arterioles can be distinguished morphologically in favorably oriented sections or by tracing their origins on serial sections. Segmental, interlobular, and arcuate arteries may also be present in the renal biopsy specimen. The cortical biopsy also allows assessment of the tubules and interstitium. Proximal tubules are readily identified by their PAS-positive brush border, lacking in the distal tubules. Collecting ducts show cuboidal, cobblestonelike epithelium. Tubules are normally back-to-back with minimal interstitial cells and the peritubular capillaries intervening. The medulla may also be included in the biopsy.

## Overall Pattern

Assessment of the biopsy specimen must include inspection of all sections from different levels because additional glomeruli may be sampled on deeper cuts of the biopsy core and many diseases are characterized by focal lesions. The severity and patterns of lesions are assessed, and normal and affected glomeruli are counted. Lesions are classified as focal if only some (less than half) glomeruli are involved, diffuse if all (or most) glomeruli are involved, segmental if only portions of glomeruli are involved, and global if entire glomerular tufts are involved. Characteristic glomerular disease patterns include lobular proliferation in MPGN, nodular proliferation of mesangial cells and abundant matrix material in characteristic Kimmelstiel-Wilson lesions, focal and segmental sclerosis, intraglomerular/arteriolar fibrin thrombi, necrotizing lesions and crescent formation (*Table 24-1*). The crescent, a lesion due to proliferation of mostly parietal epithelial cells, owes its name to its shape in well-established lesions.

Glomeruli are assessed for alterations in size (see below). It is important to compare with a normal control for a given age group because marked glomerular maturational growth occurs in children. Glomerular hypertrophy may be an important predictor of increased risk of FSGS in children with apparent MCD (see below). Maturational pattern of glomeruli (see above) should be noted. Occasional fetal glomeruli may be found in children beyond infancy and are not of specific diagnostic significance.

Glomeruli are assessed for glomerulosclerosis, that is, the presence of segmental obliteration and scarring of glomerular capillary tufts. Sclerosis may be in a segmental or global pattern (*Table 24-2*). Previous studies suggested that up to 10% of glomeruli may be normally globally sclerosed in people younger than 40 years of age (61). This number may be even smaller in children, with less than 1–3% global sclerosis expected normally up to age 40 or 56, respectively (62, 63). These occasional globally sclerotic glomeruli are thought to represent errors of nephrogenesis. The percentage of global sclerosis increases even with normal aging, up to half the patient's age, minus 10 (63). Globally sclerosed glomeruli in greater percentage indicate the possibility of renal disease (focal global sclerosis) (64).

The pattern of tubulointerstitial fibrosis, whether proportional to glomerular sclerosis or not, whether diffuse or present in a "striped" pattern following the medullary rays, or in broad patchy zones, has diagnostic significance (see below).

## Specific Glomerular Cells

### Podocytes

The podocytes (glomerular visceral epithelial cells) may show vacuolization in various diseases with severe proteinuria. Although more extensive vacuolization of podocytes has been seen in FSGS compared with patients with MCD (65), these changes are only seen after established sclerotic lesions are identifiable by LM and do not permit distinction of these two disease processes in the early phase in which segmental sclerosis may be undetected. Hypertrophy of podocytes is prominent in MCD and FSGS. Effacement of the foot processes of the podocytes by EM is common to any disease with marked proteinuria, and the podocyte may also show microvillous transformation, with long, attenuated pseudopods. Podocytes are limited in their ability to proliferate. However, the early sclerotic lesion of FSGS is characterized by prominence and apparent increase of the overlying podocytes

**Table 24-2**

**Definitions of common morphological terms**

| | |
|---|---|
| *Light microscopy* | |
| Focal | Involving some glomeruli |
| Diffuse | Involving all glomeruli |
| Segmental | Involving part of glomerular tuft |
| Global | Involving total glomerular tuft |
| Lobular | Simplified, lobular appearance of capillary loop architecture (MPGN) |
| Nodular | Acellular areas of mesangial matrix (diabetic nephropathy) |
| Sclerosis | Obliteration and scarring of capillary loop |
| Crescent | Proliferation of parietal epithelial cells |
| Spikes | Projections of glomerular basement membrane intervening between subepithelial immune deposits (membranous GN) |
| Endocapillary proliferation | Increase in mesangial and/or endothelial cells |
| Hyaline | Descriptive of glassy, smooth appearing material |
| Hyalinosis | Hyaline-appearing insudation of plasma proteins (focal segmental glomerulosclerosis) |
| Mesangium | Stalk region of capillary loop with mesangial cells surrounded by matrix |
| Subepithelial | Between podocyte and glomerular basement membrane |
| Subendothelial | Between epithelial cell and glomerular basement membrane |
| Tram-track | Double contour of glomerular basement membrane due to deposits and/or CMIP (see below) |
| Wire loop | Thick, rigid appearance of capillary loop due to subendothelial deposits |
| Activity | Score of possible treatment sensitive lesions, based on e.g., extent of crescents, cellular infiltrate, necrosis, proliferation |
| Chronicity | Score of probable irreversible lesions, based on e.g., extent of tubular atrophy, interstitial fibrosis, fibrous crescents, sclerosis |
| *Immunofluorescence microscopy* | |
| Granular | Discontinuos flecks of staining along capillary loop producing granular pattern |
| Linear | Smooth continuous staining along capillary loop |
| *Electron microscopy* | |
| Foot process effacement | Flattening of foot processes so that they cover the basement membrane |
| Microvillous transformatiom | Small extensions of epithelial cells with villus-like appearance |
| Circumferential mesangial interposition (CMIP) | Extension of cell cytoplasm with interpostion between endothelial cell cytoplasm and basement membrane and underlying new basement membrane formation |
| Reticular aggregates | Organized arrays of membrane particles within endothelial cells |
| Immunotactoid GP | Large, organized microtubular deposits, >30 nm diameter |
| Fibrillary GP | Fibrils 14–20 nm diameter without organization |

*GP* glomerulopathy

("capping" lesion), often associated with endocapillary foam cells. This cellular variant of FSGS may be more common in children than in adults with FSGS (66). The idiopathic collapsing variant of FSGS and HIV-associated nephropathy both show prominent hyperplasia and protein droplets of the podocytes, with overlying segmental collapse of the glomerular capillary tuft. In the situation of recurrent FSGS in the renal transplant, NS and foot process effacement may be seen within weeks after biopsy, with sclerosis becoming apparent at a later date (67). In Fabry's disease, there is accumulation of glycosphingolipid because of deficiency of alpha-galactosidase. Podocytes show marked vacuolization by LM with characteristic whorled, laminated electron-dense myelin bodies by EM. In Fabry's disease, these inclusions may also be present in endothelial cells, tubular epithelial

cells, some interstitial cells, and the vessels in early lesions in children (27, 53, 68).

### Mesangial Cells

Hyperplasia of mesangial cells is recognized by LM when more than three mesangial cell nuclei are present per mesangial region. Increased mesangial prominence may be due to increased cellularity, increased matrix, deposits, or a combination. Large mesangial deposits appear on Jones' stain as pinkish areas surrounded by the light silver-staining areas of mesangial matrix. So-called interposition results when the monocyte or mesangial cell cytoplasm extends outward between basement membrane and endothelial cells and new matrix accumulates between the mesangial and endothelial cell bodies.

### Endothelial Cells

Extreme proliferation and swelling of endothelial cells can obliterate capillary lumina in conditions characterized by abnormalities of coagulation. Endothelial cells usually contain characteristic reticular aggregates in lupus nephritis and HIV-associated nephropathy (53, 55). Endocapillary cell proliferation is characteristic of for example diffuse proliferative lupus nephritis and MPGN type I.

### Crescents

Crescents consist primarily of proliferating parietal epithelial cells with some infiltrating macrophages and are a manifestation of severe glomerular injury. The name reflects the often crescent-shaped sheet of cells filling up part or nearly all of Bowman's space. Crescents result from injuries that break the GBM, leading to exudation of plasma protein and formation of fibrin within Bowman's space, which then induces proliferation of the parietal epithelial cells and infiltration of macrophages. When crescents are a prominent histologic feature, the patient most often presents clinically with a rapidly progressive glomerulonephritis.

Crescents may occur in a variety of diseases. Diseases with crescents as a primary manifestation include antibody-mediated injury (anti-GBM antibody disease), severe immune-complex diseases (e.g., lupus nephritis) and pauciimmune diseases. The latter are often, but not invariably, associated with positive ANCA tests, and may be associated with systemic disease, or be renal limited. The p-ANCA (anti-myeloperoxidase) pattern is most often associated with microscopic polyangiitis, whereas the c-ANCA (anti-proteinase-3) pattern is typical in Wegener's granulomatosis. Of note, positive ANCA tests are not sensitive in distinguishing these categories (15–17). Renal biopsy is therefore critical for accurate diagnosis. Diagnosis and appropriate treatment must occur rapidly in this clinical situation to optimize chances of recovery of renal function. The early lesion of cellular crescents is responsive to cytotoxic therapy. Biopsy indications of irreversible renal damage include breaks of Bowman's capsule and fibrous transformation of the cellular crescents, periglomerular fibrosis, and scarred glomeruli and tubulointerstitium.

## Glomerular Basement Membrane

GBM abnormalities are best evaluated by EM. The basement membrane is abnormally thick in diabetic nephropathy (53). Diffuse abnormally thin GBMs, less than 250 nm in adults, are seen in familial hematuria (59, 60). GBM thinning cannot be accurately from EM processed from paraffin tissue, as this back-up technique causes artifactual and variable thinning of GBMs (69). In children, the diagnosis of thin basement membranes is more difficult than in adults because GBM increases in thickness with normal maturation. Glomerular basement membrane thickness should be compared with normal for age and sex (see above; 59, 70, 71). In Alport syndrome, the basement membrane in established lesions is characterized by irregular areas of very thin and very thick, with splitting and splintering of the basement membrane (25, 72). The GBM in nail-patella syndrome is irregular, thickened, and split, with electron-lucent areas containing banded collagen type I fibers (53).

Immune deposits may localize on either side of the GBM. Subepithelial immune deposits are characteristically seen in membranous glomerulopathy. Subendothelial immune deposits are seen for example in proliferative lupus nephritis or MPGN type I.

The basement membrane may appear split by LM in diseases other than MPGN type I or dense deposit disease. In transplant glomerulopathy, the split appearance results from varying degrees of cellular interposition and widening, with increased lucent material in the lamina rara interna. This is also a characteristic finding in preeclampsia, transplant glomerulopathy and chronic HUS or other chronic thrombotic microangiopathies (53).

## Tubules

Morphologically evident tubular necrosis correlates poorly with the clinical extent of acute kidney injury (AKI).

The changes vary from nondiagnostic vacuolization to frank necrosis with sloughing of tubular epithelial cells (toxic type) and flattened epithelium characteristic of regeneration (ischemic type). In cortical necrosis, zones of cortex, including glomeruli, are necrotic. In chronic kidney disease, tubules are atrophied with dilation and flattened epithelium, presumably secondary to lesions affecting the glomerulus, although primary tubular and interstitial injury mechanisms may also be involved in these changes. Tubular atrophy is also present in primary tubulointerstitial diseases. Tubulointerstitial fibrosis is an important manifestation of calcineurin inhibitor toxicity. The fibrosis occurs along the medullary rays due to the more severe ischemia occurring in these areas, resulting in a striped, rather than diffuse, pattern of fibrosis, with intervening preserved tubules.

Nonspecific casts of Tamm-Horsfall protein are seen in chronic kidney disease. Other casts may have a diagnostic appearance, such as the giant cells surrounding tubular casts in light chain cast nephropathy (so-called myeloma kidney). Casts of myoglobin with characteristic reddish-brown appearance are seen in rhabdomyolysis, often with associated acute tubular necrosis. Crystals, for example oxalate or cysteine, may be identified by examination under polarized light (73). Tubules contain characteristic inclusions in Fabry's disease (68).

Polymorphonuclear neutrophils (PMNs) within collecting ducts and proximal tubules are diagnostic of acute pyelonephritis. In chronic pyelonephritis, there is tubular atrophy and interstitial fibrosis, characteristically in a patchy, regional distribution (geographic or "jigsaw" pattern). The combination of segmental glomerular sclerosis with ischemic changes of corrugation and thickening of the GBM and periglomerular fibrosis, and patchy, regional interstitial fibrosis and tubular atrophy is also characteristic of reflux nephropathy (74).

Cysts may be demonstrated by biopsy, although the diagnosis of specific cystic diseases is usually made by combination of clinical and ultrasound findings. The segment of the nephron giving rise to the cysts can be identified by histochemical stains (75). Areas of low cuboidal epithelial-lined structures surrounded by a cuff of immature mesenchyme are present in the dysplastic kidney, often with cartilage, fat, or abnormal blood vessels in the interstitium. The deep medullary cystic dilation with thickened, lamellated TBM characteristic of nephronophthisis can be identified with a deep biopsy. Dilation of proximal tubules with microcyst formation in conjunction with extensive foot process effacement by EM is characteristic of congenital nephrotic syndrome of Finnish type. FSGS in a collapsing patten with podocyte hyperplasia, numerous reticular aggregates by EM, and with tubular cystic dilation and interstitial fibrosis out of proportion to the severity of glomerular lesions are highly suggestive of HIV-associated nephropathy (55).

Viral infections, including BK and CMV, result in characteristic nuclear inclusions in tubular epithelia. Specific diagnosis is made by immunostaining for viral proteins. Characteristic viral particles may also be detected by EM (76).

## Interstitium

Interstitial edema is a nonspecific change, present, for example, in early acute transplant rejection, renal vein thrombosis, or inflammatory processes. Identification of eosinophils in an infiltrate is suggestive of drug-induced interstitial nephritis, although eosinophils are also present in some cases of idiopathic interstitial nephritis (77). Eosinophils can also be part of acute cellular rejection in the transplant. Nonnecrotizing granulomas, with or without eosinophils, most often reflect drug-induced hypersensitivity reaction. A pleomorphic infiltrate of lymphocytes, plasma cells and PMNs suggests possible BK nephropathy in the transplant, confirmed by finding viral nuclear inclusions and positive immunostaining in tubules (see above) (76). Fibrosis results in increased spacing of tubules because of the accumulation of PAS-positive collagenous material. The collagen also stains specifically blue with Masson's trichrome stain. Fibrosis in a striped pattern suggests calcineurin inhibitor toxicity (78, 79). Occasionally, the interstitium is infiltrated by malignancy. Hematopoietic neoplasms are especially prone to involve the kidney. In cystinosis, characteristic rectangular or trapezoid crystals are present mostly in monocyte/macrophages, and can be recognized by EM or when polarized on frozen sections (73).

## Vessels

Arterioles and larger segmental and interlobular arteries are evaluated for changes in the intima and media; the presence of deposits, fibrin, hyalin, amyloid, or other material; or the presence of vasculitis. Arterioles show inclusions early in children with Fabry's (68). Larger vessels typically are not sampled by a biopsy, and diseases such as classic polyarteritis nodosa that affect these large vessels are therefore best evaluated by other methods (e.g., arteriography). Intimal fibrosis and medial thickening with hyperplasia and hypertrophy of media are characteristic of hypertensive injury. Concentric medial necrosis

with nodular protein deposition suggest acute calcineurin inhibitor nephrotoxicity (79). Fibrin thrombi, when present in glomeruli and/or arterioles, are the essential lesion of the thrombotic microangiopathies caused by e.g. HUS (53). Fibrin localizes predominantly within glomerular lumina in disseminated intravascular coagulation and hyperacute rejection.

## Clinical Pathological Correlations

After evaluation of the structural changes of the renal biopsy in conjunction with the clinical history, a diagnosis may be obvious. In some cases, the biopsy specimen may show overlap features, or there may be elements that do not correlate clearly with the clinical setting. Close collaboration by nephrologists and pathologists is essential in arriving at the diagnosis. The pathologist must be familiar with clinical manifestations of renal disease, and the nephrologist should be familiar with the terminology used by the pathologist to describe the biopsy findings (*Table 24-2*).

When lesions are evaluated, the balance of all elements must be considered. When a typical disease pattern is not present, one must consider whether more than one process is taking place. For instance, drug-induced interstitial nephritis may be superimposed on other glomerular disease. This is especially true in the transplant setting, where multiple disease processes may occur at one time. In some instances, the biopsy findings do not correlate with the patient's renal function. When such apparent discrepancies are found, one possibility is that the biopsy specimen is not representative of all the nephrons of the kidney. The number of glomeruli necessary to estimate the severity of diseases that show focal distribution has been discussed above. However, the extent of glomerulosclerosis may in and of itself not correlate with renal function. Tubular atrophy and interstitial fibrosis may be more closely correlated with extent of renal damage and renal function (80–82). One must also consider the elements other than structure that influence the patient's renal function (i.e., blood pressure, filtration properties of the GBM, and the glomerular filtering surface area). Patients with enlarged glomeruli, either caused by compensatory hypertrophy or by a primary pathological process, may show less deterioration of renal function than expected based on the extent of glomerular scarring. Similarly, treatment of the patient with antihypertensive agents that affect glomerular filtration rate (e.g., ACE inhibitors, which preferentially dilate efferent arterioles) may actually increase serum creatinine levels in the short run. Compensation by remaining nephrons may mask ongoing severe disease processes such that creatinine levels may remain near normal until late in the course of disease when therapy is less likely to have an impact on chronic progressive injury.

## Diagnostic Findings in Selected Renal Diseases

### Minimal Change Disease/Focal Segmental Glomerulosclerosis

MCD is diagnosed only after the exclusion of abnormal findings at the light microscopic level, with diffuse foot process effacement as the only abnormality by EM. The disease is characteristically sensitive to glucocorticoid therapy. However, repeated renal biopsies in patients with apparent MCD initially have shown progression to FSGS, which has a high incidence of progression to end-stage renal disease (ESRD) (83, 84). As discussed above, a small sample may not include the segmentally sclerotic glomerulus, diagnostic of FSGS. In FSGS, there is often also hyalinosis, an exudation of plasma proteins and lipids with a glassy, smooth (hyaline) appearance on LM. There are no immune deposits, and foot process effacement is present in all glomeruli by EM (*Fig. 24-3*). Mesangial expansion in the native kidney FSGS biopsy may be associated with increased risk for recurrence in the transplant (85).

The presence of IgM by IF without deposits by EM in a biopsy that otherwise appears to be MCD (so-called IgM nephropathy) does not have prognostic value (86). Some variants of FSGS may have prognostic value (87). A recently proposed working classification of FSGS aims to examine whether morphological patterns of FSGS have prognostic implications (*Table 24-3*) (88). The usual type is diagnosed when no special features are present. The collapsing type of FSGS, characterized by collapse of the glomerular tuft, either segmental or global with associated podocyte hypertrophy/hyperplasia, shows a rapid progression to end-stage disease (89). The cellular lesion, with endocapillary proliferation with frequent foam cells and often with podocyte hyperplasia, may represent an early stage of FSGS, and appears to occur more often in children with FSGS than adults (66, 90). The tip lesion, that is adhesion, sclerosis or endocapillary foam cell lesion localized to the proximal tubular pole, appears to have better prognosis (91–93). The perihilar variant, with sclerosis and hyalinosis localized to the vascular pole, likely more often represents a secondary sclerosing process (88).

**Figure 24-3**

**Minimal change disease. The foot processes are effaced (×11,000).**

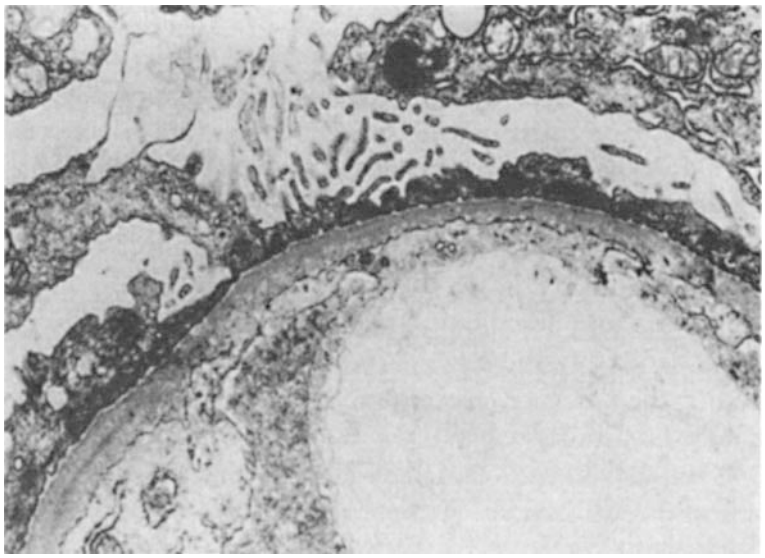


**Table 24-3**

**Working classification of FSGS**

| Type | Key histologic feature | Possible prognostic implication |
|---|---|---|
| FSGS, nos | Segmental sclerosis | Typical course |
| Collapsing FSGS | Collapse of tuft, GVEC hyperplasia | Poor prognosis |
| Cellular FSGS | Endocapillary proliferation, often GVEC hyperplasia | ?Early stage lesion |
| Tip lesion | Sclerosis of tuft at proximal tubule pole | ?Better prognosis |
| Perihilar variant | Sclerosis and hyalinosis at vascular pole | ?May reflect a secondary type of FSGS |

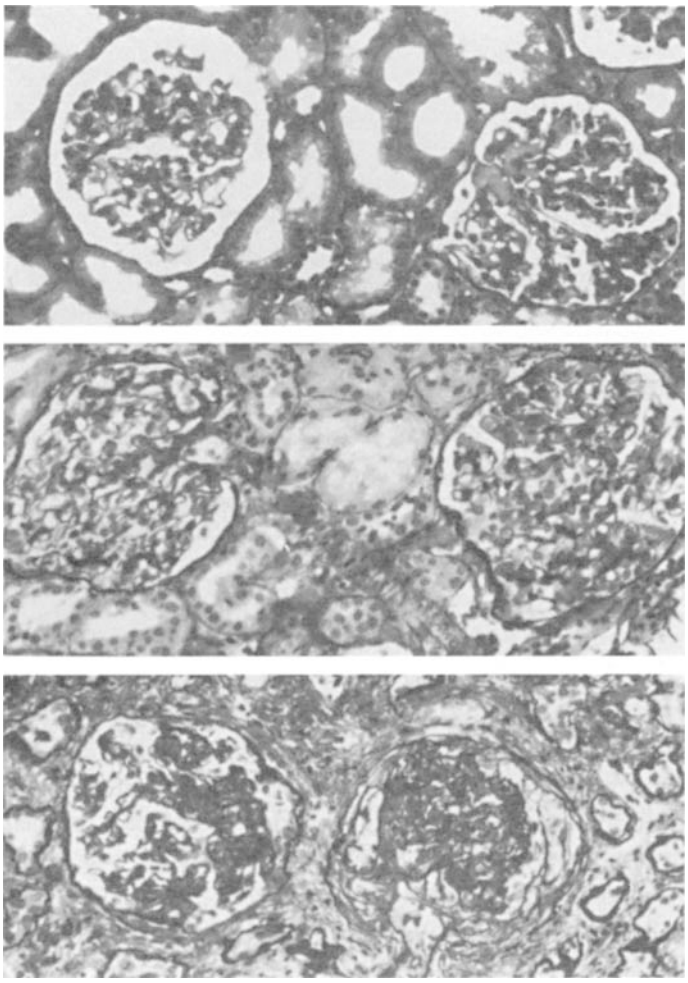
*nos* not otherwise specified; *GVEC* glomerular visceral epithelial cell

C1q nephropathy is characterized by either no sclerosis or segmental glomerulosclerosis by LM, with mesangial C1q deposits and lesser immunoglobulin components without dominant or codominant IgA (94). EM shows mesangial and paramesangial dense deposits but a lack of reticular aggregates. Patients typically are adolescents and have steroid-resistant NS and do not have clinical evidence of SLE. Although these findings suggest a distinct clinicopathologic entity, the prognostic significance of these lesions has not yet been established. Progression to ESRD has occurred in some patients with sclerosis at biopsy, but long-term outcome of those without sclerosis at presentation has not yet been established.

Because therapy and prognosis are different for MCD vs. FSGS, early distinction of these two entities is of primary interest. We studied pediatric patients with steroid-resistant nephrotic syndrome and MCD on renal biopsies and compared to patients with apparent MCD on biopsy who subsequently progressed to overt FSGS (57). Morphometric analysis of initial biopsies showed that glomerular size at the onset of disease, before sclerosis was apparent, was remarkably larger in patients who subsequently progressed to FSGS (*Fig. 24-4*) (95, 96). There was a higher risk for development of FSGS in patients <5 years old with glomerular area greater than 1.5 times that of normal age-matched controls. On the other hand, glomerular size equal to or less than normal controls in this group of patients indicated a good prognosis. Calculated glomerular diameter for increased risk of FSGS in these patients less than 5 years old was more than 118 μ vs. 95 μ glomerular diameter in age-matched controls. Depending on processing and fixation,

**Figure 24-4**

**Apparent minimal change disease (MCD) with subsequent progression to focal segmental glomerulosclerosis (FSGS). The first biopsy from this 5-year-old girl, *middle panel,* was indistinguishable from MCD, except for marked glomerular hypertrophy vs. age-matched typical MCD with subsequent benign clinical course *(top panel).* The patient's later biopsy, *bottom panel,* 50 months later, showed segmental sclerosis, diagnostic of FSGS (Jones' stain, ×160).**



these values may vary (our values are based on paraffin-embedded tissue fixation in formalin or Zenker's fixative). Normal ranges should be established in each laboratory assessing glomerular size.

From these studies, abnormal glomerular enlargement suggests a high probability of development of FSGS in pediatric patients with apparent MCD. Causes of abnormal glomerular enlargement other than idiopathic FSGS, such as diabetes mellitus, cyanotic cardiovascular disease, and massive obesity, must be excluded before such inferences can be made. Interestingly, the incidence of FSGS may be increased in these diseases. The association of abnormal glomerular growth with development of glomerulosclerosis may reflect a pathogenic linkage, in that processes leading to excess matrix and sclerosis may be manifested as glomerular growth. This view is supported by the coexistence of these two processes in many other diseases, including sickle cell diseases, HIV infection, and reflux nephropathy (97).

Alterations of dystroglycans, specific proteins that are expressed along the GBM, may be of use in differentiating MCD in FSGS. α- and β-dystroglycans were decreased in MCD, but preserved in the nonsclerotic areas of FSGS in a small study (98). Recently, molecular studies also indicate distinct gene expression profiles in MCD vs FSGS, with much higher ratio of podocin to synaptopodin in mRNA in the former (99).

Recently, specific gene mutations of podocyte-specific genes have been identified in some forms of familial FSGS. The slit diaphragms are crucial for regulation of permselectivity, and are decreased in density in proteinuric conditions. Mutations of several slit diaphragm genes cause proteinuria and FSGS. The gene for autosomal dominant FSGS has now been localized to ACTN4, at chromosome 19q13. ACTN4 encodes alpha-actinin-4, and a gain-of-function mutation with possible altered actin cytoskeleton interactions has been proposed. The prognosis of this form of familial FSGS has been poor, with progression to renal disease in 50% of patients by age 30. Recurrence of NS in the transplant has been very rare, presumably related to immune events, as the transplant does not carry the mutated gene (100). Autosomal recessive FSGS with early onset and rapid progression to end stage is caused by mutations in NPHS2, which encodes podocin (101). Podocin is expressed only in podocytes and is an integral stomatin protein family member. Its function is not determined. Mutations in NPHS2 have been described in sporadic steroid resistant FSGS (11). TRPC6, a receptor, is mutated in other kindreds with FSGS, with variable penetrance and adult onset (102). Phospholipase C epsilon mutation (NPHS3) recently was found to underly many cases of DMS or rare cases of FSGS, with rare steroid response (103–105). These familial forms do not have specific morphological features of the segmental sclerosis.

Congenital nephrotic syndrome (CNS) of Finnish type shows mesangial hypercellularity or no glomerular lesions and dilated proximal tubules by light microscopy. The gene mutated is nephrin and is also in the 19q13 region (106). Nephrin localizes to the slit diaphragm of the podocyte, and is tightly associated with CD2 associated protein (CD2AP). Nephrin is thought to function as a zona occludens type junction protein. CD2AP plays a crucial role in receptor patterning and cytoskeletal polarity, and its absence resulted in sclerosis and foot process effacement in mice, supporting a role for CD2AP in the function of the slit diaphragm. Rare case reports of CD2AP mutation in human FSGS exist (107). Laminin-β2 (LAMB2) is mutated in Pierson syndrome, often with microcoria and CNS abnormalities, with mesangial sclerosis (108, 109).

FSGS associated with mitochondrial cytopathy, due to a mutation of mitochondrial DNA in tRNAleu(UUR), may show multinucleated podocytes and have abnormal mitochondria by electron microscopic examination. Patients also have unusual hyaline lesions in the arterioles. Some patients with FSGS without full-blown features of mitochondrial cytopathy (i.e., myopathy, stroke, encephalopathy, occasionally diabetes mellitus, hearing problems, cardiomyopathy) have also been reported to have this mitochondrial mutation (110). For further discussion of MCD and FSGS, see Chapters 45 and 46.

## Hemolytic-Uremic Syndrome

HUS is the most common disease in children that is manifested by injury to the microvasculature and is typically due to E. coli 0:157 verotoxin and associated with diarrhea. In adults, thrombotic thrombocytopenic purpura, related to ADAMTS13 deficiency, and postpartum renal failure may produce similar morphologic changes. The characteristic lesion of these conditions is thrombotic microangiopathy (TMA). By LM, thrombi in glomeruli and arterioles are present (*Fig. 24-5*). The renal biopsy findings, rather than clinical parameters, have recently been found to best predict long-term prognosis (111). Patients with cortical necrosis have a particularly ominous prognosis. The extent of glomerular vs. arteriolar involvement is also of prognostic significance. Generally, both the long-term prognosis and the clinical presentation are more severe if larger vessels are involved. Arterial involvement was not seen in biopsies performed during the first 2 weeks of hospitalization (112). The glomerular endothelium is markedly swollen, nearly occluding capillary lumina. Fibrin thrombi are visualized easily. With chronicity, these areas may progress to segmental ischemic collapse with sclerosis, especially when arterioles are involved. The arterioles can become completely occluded by thrombi, with necrosis of vessel walls. IF shows occasional nonspecific entrapment of C3 and IgM in injured areas with fibrin and fibrinogen. EM shows extreme swelling of the glomerular endothelium with increased lucent material in the lamina rara interna of the basement membrane with entrapped platelets, fibrin, and red cell fragments, without immune deposits (*Fig. 24-6*). De novo thrombotic microangiopathy in the renal transplant is indistinguishable morphologically from HUS in the native kidney. Cyclosporine and FK506 have both been implicated in its pathogenesis (113, 114). Mutations of complement regulatory genes are implicated in familial and some diarrhea-negative sporadic cases. The morphologic characteristics of the TMA lesion are largely the same regardless of etiology. For further discussion of HUS, see Chapter 48.

## Henoch-Schönlein Purpura/IgA Nephropathy

Henoch-Schönlein purpura is often viewed as the systemic variant of IgA nephropathy (Berger's disease) (4, 115).

**Figure 24-5**

**Arteriolar fibrin thrombi with minor areas of thrombi in capillary loops in hemolytic uremic syndrome (Jones' stain, × 270).**

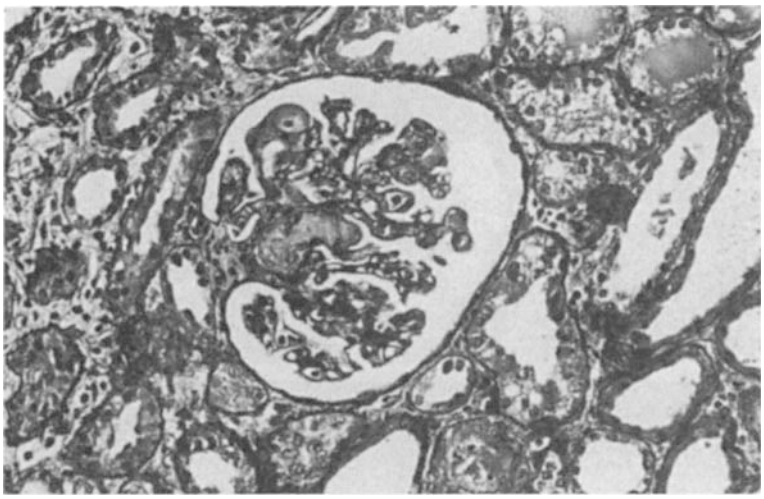
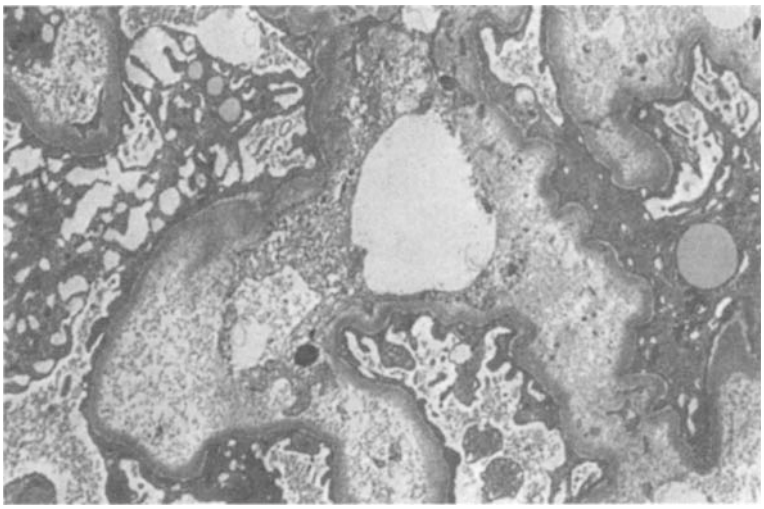


**Figure 24-6**

**Hemolytic uremic syndrome. The lamina rara interna (endothelial side of the glomerular basement membrane) is widened with increased lucent material. No immune deposits are present (× 3,300).**



The glomerular manifestations are similar, and vary in both, likely depending on extent and location of deposits. In some cases, there is only focal or even diffuse mild to moderate mesangial proliferation (*Fig. 24-7*) (116). In more severe cases, there is focal or even diffuse endocapillary proliferation. In severe cases, there may also be necrosis of glomerular tufts with crescents in Bowman's space. IF by definition shows predominance or codominance of mesangial IgA, with capillary loop deposits in more severe cases. IgG, IgM, and C3 deposits may also be detected. The immunoglobulin deposits are present diffusely, even in glomeruli that appear normal by LM. By EM, electron-dense mesangial deposits are present, with occasional "spill-over" of deposits to subendothelial regions in the regions adjacent to the mesangium, particularly in cases with endocapillary proliferative lesions (*Fig. 24-8*). Deposits are decreased when clinical remission occurs (117). In Henoch-Schönlein purpura, deposits are often present in subendothelial as well as mesangial areas, associated with more severe glomerular lesions, including crescents, and worse outcome (116). Classification schemas analogous to those for lupus nephritis have

**Figure 24-7**
**Mesangial prominence, segmental sclerosis, and small organizing crescent with adhesion in Henoch-Schönlein purpura. Immunofluorescence demonstrated IgA mesangial deposits (Jones' stain, × 430).**

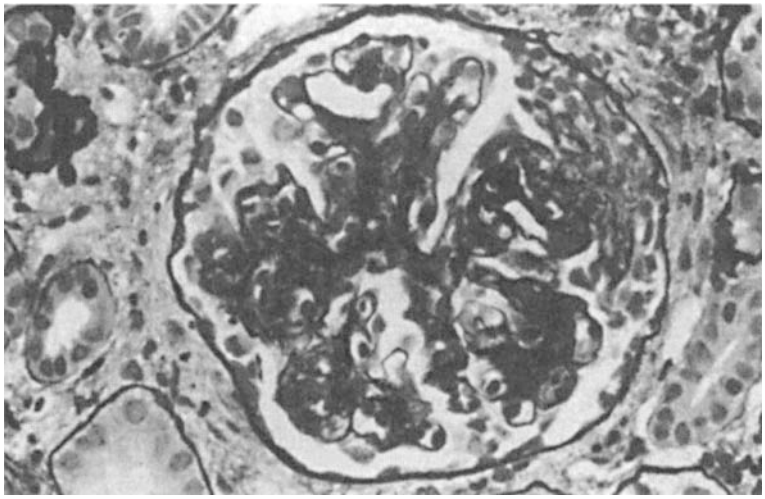
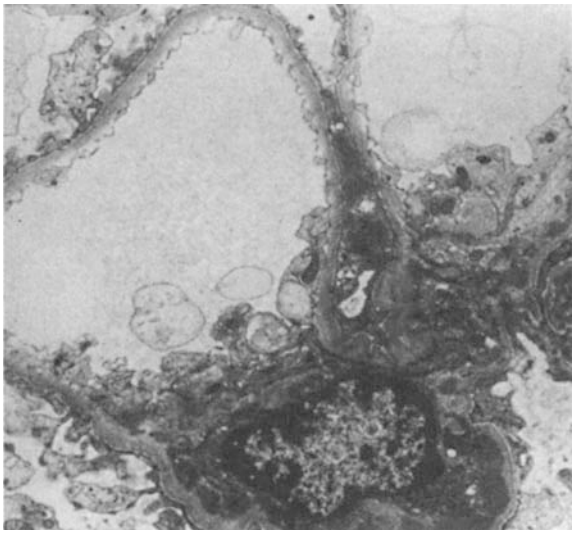


**Figure 24-8**
**Immune complex deposits surrounding mesangial cell in Henoch-Schönlein purpura (× 3,400).**



been proposed, but have not had widespread use (118, 119). Therefore, a recent International Study Group of IgA Nephropathy composed of nephrologists and pathologists reviewed a large number of cases to determine which biopsy lesions have prognostic implications (120). Mesangial hypercellularity, i.e., more than half the glomeruli with more than 3 nuclei in a mesangial area, proliferation (either endo- or extracapillary), segmental sclerosis and increasing interstitial fibrosis were each associated with worse long-term outcome. For further discussion of Henoch-Schönlein purpura/IgA nephropathy, see Chapter 42.

## Membranoproliferative Glomerulonephritis Type I

Type I MPGN is characterized by a tram-track appearance of the GBM on silver stain, because of duplication around intramembranous/subendothelial deposits and interposition of mesangial cells and macrophages (*Fig. 24-9*). The glomeruli are enlarged and hypercellular with a lobular appearance by LM (*Fig. 24-10*). There is marked mesangial and endocapillary hypercellularity and occasional PMNs and mononuclear cells may be present. By IF, C3 predominates in a coarse granular pattern along basement membranes, with moderate amounts of IgG and usually lesser IgM. Subendothelial and mesangial immune deposits are seen by EM (4, 53). MPGN may be idiopathic or secondary to any of numerous chronic infections. Hepatitis C positivity, often with associated cryoglobulins, was present in about one fourth of adult cases of MPGN type I in adults in Japan and the United States (121, 122). This association has not been demonstrated in children with apparent idiopathic MPGN (123). MPGN type I recurs in 20–30% of grafts, and may lead to graft loss (13). Secondary MPGN, more common in adults, more often demonstrates a focal

**Figure 24-9**

**Split basement membrane (''tram-tracking'') in MPGN type I (*arrows*), caused by subendothelial/intramembranous deposits and cellular interposition (Jones' stain, × 1,125).**

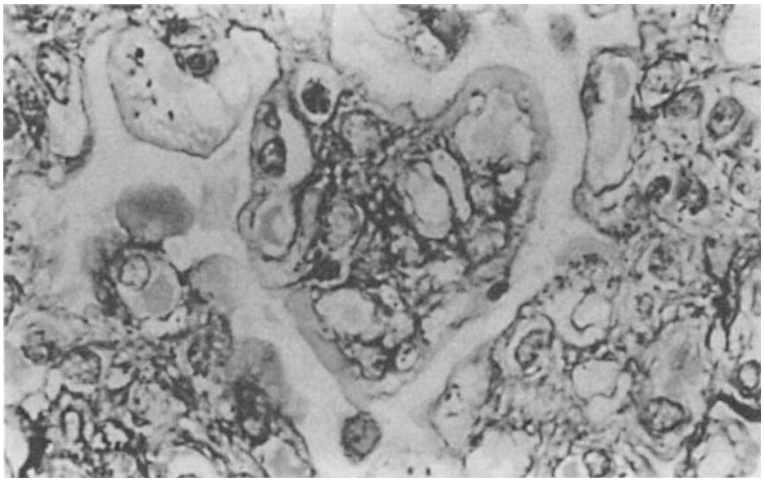
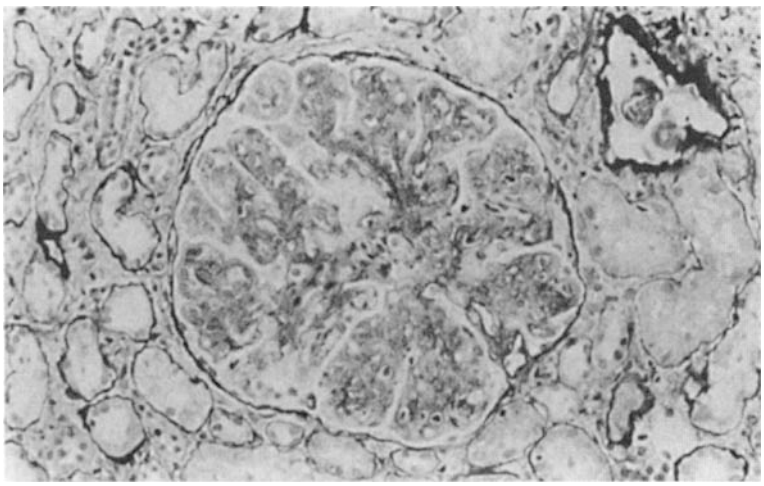


**Figure 24-10**

**Lobular appearance of glomeruli in MPGN type I (× 430).**



segmental pattern of proliferation, contrasting the more diffuse involvement seen in idiopathic MPGN, more common in children.

## Dense Deposit Disease

In dense deposit disease (DDD), the glomeruli may appear similar by LM to those of type I MPGN, and this disease has therefore also been called type II MPGN. However, the pathogenesis is entirely different. These patients often show circulating IgG autoantibodies, also known as C3 nephritic factor (12, 124, 125). The basement membranes are deeply eosinophilic, often with a ribbon garland or sausage-shaped contour. By IF, discontinuous smooth linear deposits of C3 along the GBM and round globular mesangial deposits are found, typically without immunoglobulin staining. The disease is named dense deposit disease because of the characteristic appearance by EM with strongly electron-dense deposits within the basement membrane. Studies of the dense deposits indicate that these are likely an alteration of basement membrane material and not deposition of circulating immune complexes. Although less specific than

electron microscopic diagnosis, deposits can also be identified by their staining with the fluorescent dye thioflavin T in cases where electron microscopic examination cannot be performed (125). A recent large international study of 69 cases of DDD by the Renal Pathology Society showed mesangial proliferation was most common, followed in order by membranoproliferative appearance with endocapillary proliferation, crescentic or acute proliferative patterns (126, 127). Renal survival may be worse in DDD than in type I MPGN (median survival 8.7 vs. 15.3 years) (128). The distinction between these two diseases is also important since dense deposit disease invariably recurs in renal transplantation, although loss of graft is not always the outcome (12).

## Lupus Nephritis

Lupus nephritis is not a single disease but rather there is a spectrum of severity of involvement of the kidney by the immune complexes characteristic of SLE. Most patients with SLE will have morphologic manifestation of renal immune deposition. However, patients who undergo renal biopsy most often have clinical renal manifestations and will have more pronounced changes. Lupus nephritis is characterized by deposits in all anatomic compartments of the glomerulus (i.e., mesangial, subepithelial, and subendothelial regions) (19–21) (*Figs. 24-11* and *24-12*). All immunoglobulin classes with dominant IgG, C3, and smaller amounts of C4/C1q are usually found in lupus nephritis deposits (*Fig. 24-13*), and immune complex deposits are seen by EM. Reticular aggregates (see above) are typically seen in endothelial cells in any class of lupus nephritis (4, 53) (*Fig. 24-14*).

The WHO classifications, either the original or modified, were previously most commonly used (18, 19). However, the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) put forth a revised lupus nephritis classification to clarify some areas of difficulty in the previous versions (20, 129, 130). Greater interobserver reproducibility occurs with the ISN/RPS classification (129). In this new ISN/RPS classification, Class I has minimal mesangial deposits with normal LM. Class II is characterized by mesangial expansion visible by LM and mesangial deposits, with only scattered peripheral loop deposits. In class III, focal lupus nephritis, deposits are present in mesangial areas and there are lesions of either active endocapillary proliferation, necrosis, cellular crescents, sclerosis, fibrocellular or fibrous crescents. These focal lesions, by definition, involve less than 50% of glomeruli. The subendothelial deposits can result in thick, rigid-appearing capillary basement membranes by LM, the so-called wire-loop lesions. "Hyaline" thrombi (aggregates of immune complexes) may fill capillary lumina. When these lesions affect more than 50% of glomeruli, lupus nephritis is characterized as class IV diffuse lupus nephritis. The proliferative lesions of both class III and IV are typically associated with subendothelial deposits in addition to mesangial deposits, with only rare or scattered subepithelial deposits. IF demonstrates

**Figure 24-11**

**Diffuse lupus nephritis (ISN/RPS Class IV) with massive dense mesangial and subendothelial deposits, and fewer deposits in subepithelial areas (× 5,600).**

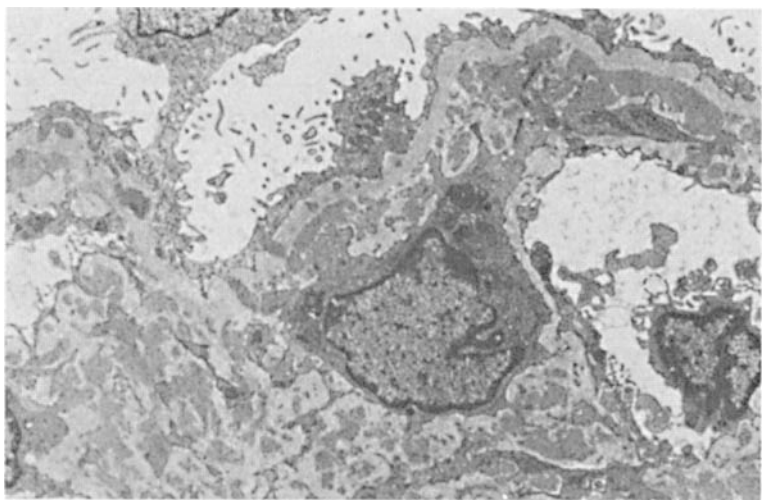


**Figure 24-12**
**Membranous glomerulopathy with subepithelial deposits and intervening lamina densa (seen as spikes by silver stain on LM) (× 15,580).**

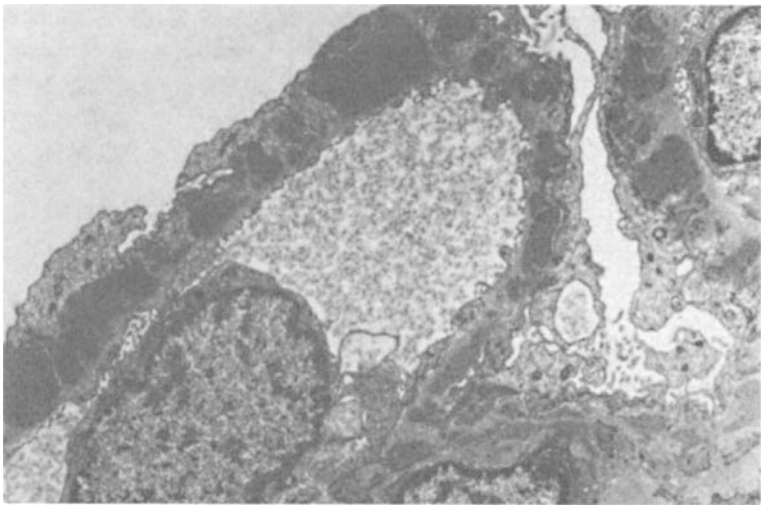
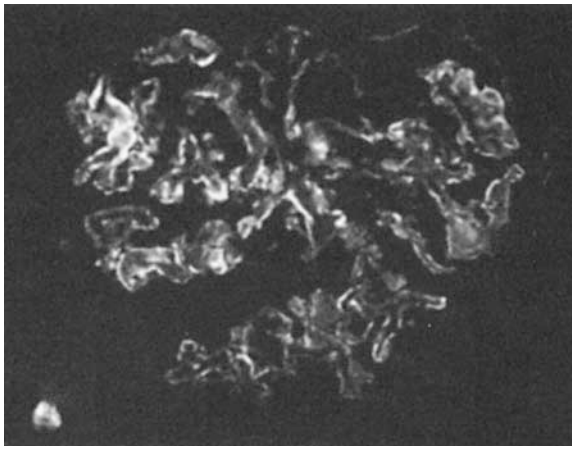
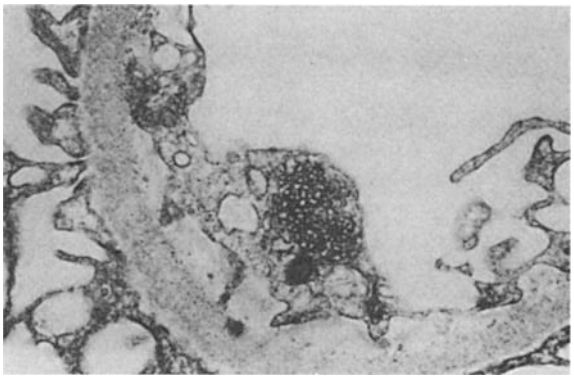


**Figure 24-13**
**Immunofluorescence of granular capillary and small mesangial IgG deposits in diffuse lupus nephritis (ISN/RPS Class IV). The larger segments of capillary loop staining correspond to subendothelial deposits, with a smooth outer edge where deposits are molded underneath the GBM (× 250).**



widespread distribution of immune complexes. EM confirms the massive and extensive immune complex deposition (*Fig. 24-11*). For class III and IV, the extent of active vs chronic lesions is specified. The segmental necrotizing lesions in class IV lupus nephritis may have worse prognosis, and thus the presence of these lesions vs global endocapillary proliferation is noted (20, 130).

**Figure 24-14**
**Endothelial cell containing tubular-shaped reticular aggregates in lupus nephritis (× 20,000).**



Class V membranous lupus nephritis is characterized by predominance of subepithelial deposits in a pattern similar to that of idiopathic membranous glomerulopathy, with added mesangial deposits (*Fig. 24-12*). Subendothelial deposits are minor components in class V. When there are superimposed focal or diffuse proliferative lesions in addition to membranous changes, both processes are diagnosed, e.g., combined focal or diffuse lupus nephritis and membranous lupus nephritis, ISN/RPS Class III + V or Class IV + V, respectively. Widespread chronic sclerosing lesions in a nonspecific pattern in a case of lupus nephritis are defined as Class VI. Tubular basement membrane deposits can occur in any class of lupus nephritis and

may account in part for the tubulointerstitial injury. Vascular lesions include immune deposits, or thrombotic microangiopathy, often related to anti-phospholipid antibodies. Vasculitis occurs rarely. For further discussion of lupus nephritis, see Chapter 47.

## Anti-Glomerular Basement Membrane Antibody Disease

Light microscopic examination shows crescentic glomerulonephritis with focal necrotizing lesions. Patients may not always show detectable serum levels of anti-GBM antibodies, especially after the acute phase of illness. Serology is positive in 95% of patients in the first 6 months after onset. However, in all patients, even those with negative serology, linear IgG staining by IF of glomerular basement membranes is present (*Fig. 24-15*). Standard EM does not visualize the immune deposits, perhaps because of the diffuse distribution of the antigen (α3 type IV collagen NC-domain). Patients with more than 50% crescents have a worse prognosis (131). For further discussion of anti-GBM antibody disease, see Chapter 29.

## Wegener's Granulomatosis

By LM, the appearance of Wegener's granulomatosis is the same as for other non-immune complex crescentic necrotizing glomerulonephritides, such as microscopic polyangiitis or anti-GBM antibody disease (*Fig. 24-16*). The lesions are focal and segmental. Granulomas are rare in the kidney, and arteritis is rarely found in the small sample inherent to the renal needle biopsy. IF studies allow differentiation of the lesion from anti-GBM antibody disease. It shows fibrin and fibrinogen in areas of necrosis, and nonspecific trapping of immunoglobulin, especially IgM. By EM, immune deposits are not identified. Distinction from microscopic polyangiitis cannot usually be made by renal biopsy findings. Clinical manifestations must be used to distinguish between these two disorders. For further discussion of Wegener's granulomatosis, see Chapter 45.

## Postinfectious Glomerulonephritis

Patients with typical postinfectious glomerulonephritis due to streptococcal infection do not usually undergo renal biopsy. Infectious agents other than streptococci can also cause postinfectious glomerulonephritis. When the diagnosis remains in question, when abnormalities persist, or when the initial disease is severe, renal biopsy may be done. Glomeruli are enlarged and hypercellular with prominent endocapillary proliferation and infiltration by neutrophils and mononuclear cells (*Fig. 24-17*) (132). In severe disease, crescents are present. Occasionally,

**Figure 24-15**

**Anti-glomerular basement membrane antibody disease with linear staining of GBMs by immunofluorescence for IgG. A crescent is present in the glomerulus on the *right* (× 125).**

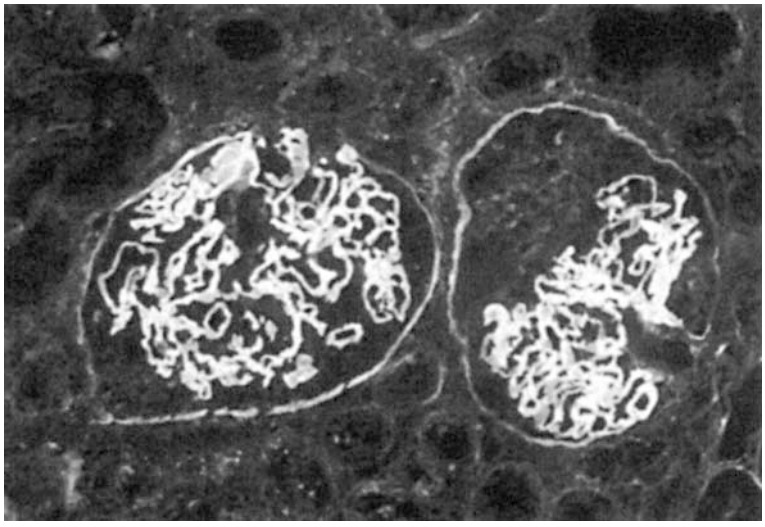


**Figure 24-16**

**Segmental necrosis and crescent in Wegener's granulomatosis. Immunofluorescence was negative (Jones' stain, × 430).**

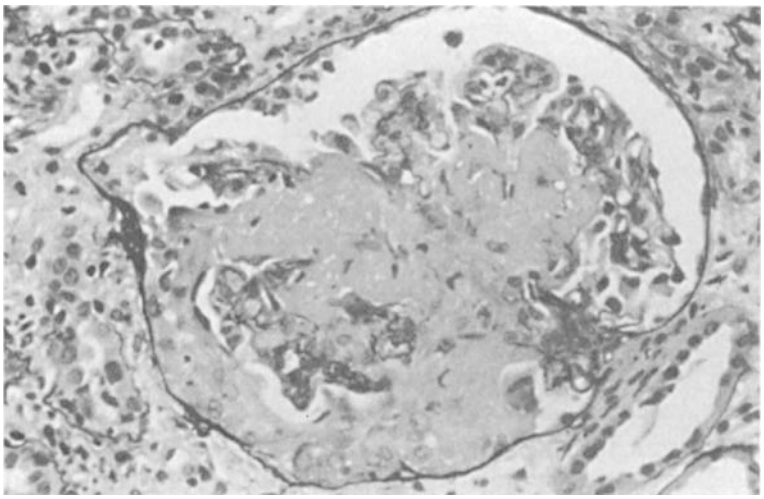
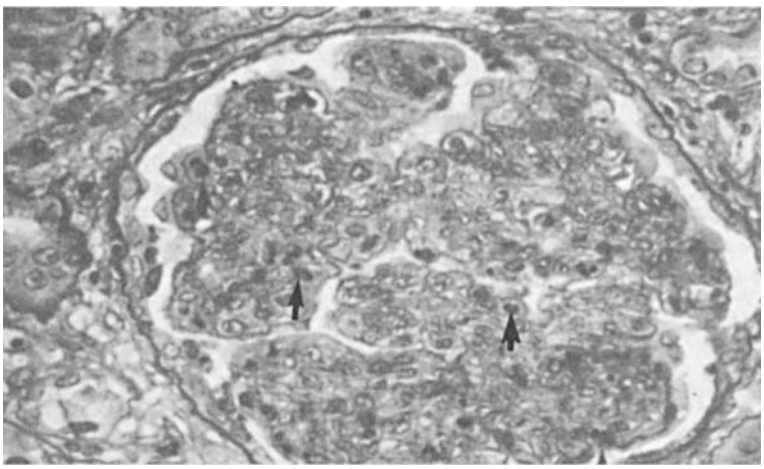


**Figure 24-17**

**Postinfectious glomerulonephritis with endocapillary proliferation and PMN infiltration (*arrows*) (PAS, × 430).**



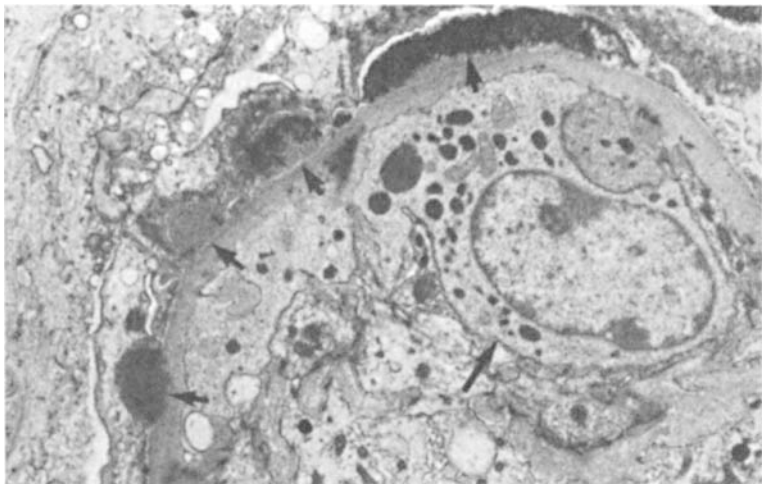
large subepithelial deposits can be visualized by LM. These differ from those typical of membranous glomerulopathy in being more unevenly distributed along the capillary basement membrane and larger in size. The deposits lie on top of the basement membrane, rather than being embedded within it (as in membranous glomerulopathy), and therefore spikes are not usually present. By IF, there are coarsely granular, discontinuous areas of IgG and prominent C3 along the capillary wall and in the mesangium. Postinfectious glomerulonephritis due to staphylococcal infection may have dominant IgA rather than IgG, and can be distinguished from IgA nephropathy by EM appearance of deposits (133). Electron-dense subepithelial deposits are large, variegated, hump- or dome-shaped and irregularly spaced (*Fig. 24-18*). Occasional mesangial deposits are present in many biopsies. For further discussion of postinfectious glomerulonephritis, see Chapter 30.

## Diabetic Nephropathy

Diabetic nephropathy affects 30–40% of patients with diabetes mellitus, either type 1 or type 2, with overt clinical nephropathy manifest 15–20 years after onset of diabetes. Therefore, diabetic nephropathy has been

**Figure 24-18**

**Electron micrograph of subepithelial large, irregularly spaced, hump-shaped subepithelial deposits in postinfectious glomerulonephritis. The deposits are variegated and lie on top of the GBM (*small arrows*). There is endocapillary proliferation with PMN (*long arrow*) infiltration (×7,000).**



considered a disease of adults. However, adolescents may have diabetic renal lesions even after short duration of disease (134). In addition, obesity and type 2 diabetes mellitus are increasing in children. The structural changes in these diabetic children include GBM thickening and mesangial expansion and were associated with proteinuria, hypertension, and decline in GFR. Overt diabetic nephropathy with nodular glomerulosclerosis and afferent and efferent arteriolar hyalinization was present in several of these young patients. For further discussion of diabetic nephropathy, see Chapter 50.

## Alport Syndrome/Thin Basement Membrane Lesion

Early in life in males with classic Alport syndrome, and in female carriers, the renal biopsy may show no significant light microscopic abnormalities. At later stages, glomerulosclerosis, interstitial fibrosis and prominent interstitial foam cells are typical. These foam cells are not specific for this disease, and are found in numerous proteinuric states. Glomeruli can show segmental sclerosis. Immunofluorescence may show non-specific trapping of IgM. By electron microscopy, the diagnostic lesion consists of irregular thinned and thickened areas of the glomerular basement membranes with splitting and irregular multilaminated appearance of the lamina densa, so-called "basket weaving". In between these lamina, granular, mottled material is present. Similar GBM changes by EM, but with normal collagen chain staining (see below) have been reported in Frasier syndrome. This entity is due to WT-1 mutation, and manifests as NS with FSGS by LM, no deposits, pseudohermaphroditism and increased risk of gonadoblastoma (135). At early stages of Alport, i.e., in children or carrier women, the glomerular basement membrane may show only thinning. Some males with classic Alport syndrome only have glomerular basement membrane thinning even at advanced clinical stages (25, 136). Rarely, Alport patients may have proliferative lesions with IF and EM deposits within lamellated GBM areas, suggesting trapping of immunoglobulins rather than a superimposed immune complex disease (137).

Immunostaining for type IV collagen chains can aid in the interpretation of thin basement membranes (136). Heterotrimers of α3, α4 and α5 type IV collagen are a key normal component of the GBM. Mutation of α5 type IV collagen in X-linked Alport syndrome, or of α3 or α4 subchains in autosomal forms, prevents incorporation of the other chains into this heterotrimer of the GBM. Thus, in kidney biopsies, about 70–80% of males with X-linked Alport syndrome lack staining of GBM, distal tubular basement membrane and Bowman's capsule for α3, α4 and α5 (IV) chains. In autosomal recessive Alport syndrome, due to mutations of either α3 or α4, the GBMs also usually show no expression of α3, α4 or α5 type IV collagen. In contrast to X-linked cases, there is normal expression of α5 type IV collagen in Bowman's capsule, distal tubular basement membrane and skin, where the α5 collagen chain are part of other heterotrimers. Female

heterozygotes for X-linked Alport syndrome show mosaic staining of GBM and distal TBM for α3, α4 and α5 type IV collagen chains, and skin mosaic staining for α5 type IV collagen due to the Lyonization effect. Patients with autosomal dominant Alport syndrome have not been studied immunohistochemically. Of note, occasional cases with Alport syndrome clinically and by renal biopsy showed apparent normal α5 type IV pattern of skin IF staining, and about 20% of male X-linked Alport patients show faint or even normal staining of the GBM for α3 and α5, likely because the antigenic site recognized by the antibody has not been altered by the mutation (136).

Thinning of the GBM is also the characteristic finding in benign familial hematuria (70, 71). The diagnosis of thin basement membranes is based on morphometric measurements from electron microscopic prints, and was present in 1.9% of a large native kidney biopsy series (138). LM and standard IF are normal. The GBM thickness normally increases with age. Normal thickness in adults in one series was 373 ± 42 nm in men vs. 326 ± 45 nm in women. GBM thickness < 250 nm has been used as a cutoff in many series (139). In another series, average was 330 ± 50 nm in males and 305 ± 45 nm in women (138). In children, the diagnosis of thin basement membranes must be made with caution, establishing normal age-matched controls within each laboratory. In our laboratory, we found a range of GBM thickness in normal children, from approximately 110 nm at age 1 year to 222 ± 14 nm in seven year olds. As mentioned above, thin GBM (without lamellation) is also the early, or may be the only, manifestation in some kindreds with Alport syndrome and in female carriers of X-linked Alport. Thus, the presence of thin GBM cannot per se be taken to categorically indicate a benign prognosis. Some patients with a clinical diagnosis of benign familial hematuria and α4 or α3 type IV collagen, suggesting that they may represent a carrier state of autosomal recessive Alport (140).

## Prognostic Implications of Biopsy Findings

When the biopsy sample is adequate, extensive, severe, and irreversible lesions signify a dismal prognosis for the patient. Globally sclerotic glomeruli are not amenable to treatment, although evidence from human diabetic nephropathy and animal studies indicates that the earlier stages of sclerosis may be affected by some therapeutic interventions, and may even be reversible (141–143). Similarly, active lesions with ongoing cellular crescents, necrosis, and inflammatory infiltrate are potentially dramatically modulated by therapy, allowing subsequent healing. There may be minimal irreversible damage to glomerular structures when intervention occurs early.

Although the renal biopsy may yield a diagnosis, there is less information of prognostic indicators in diseases that have a variable course. Extensive analysis aimed at determining histologic features associated with poor prognosis has been done in some diseases discussed below.

Classification schemes, especially for lupus nephritis and membranous glomerulopathy, imply progression from one stage of disease to the next. Although sequential biopsies have illustrated progression from focal to diffuse proliferative glomerulonephritis in lupus nephritis, there is not clear-cut evidence that progression occurs among all ISN/RPS classes (18, 19, 21, 130). In lupus nephritis, patients with less severe proliferative disease, especially segmental necrotizing lesions, appear to have better prognoses.

Although the presence of cellular crescents is associated with activity of disease clinically, the renal biopsy offers additional prognostic information beyond that gleaned from the clinical presentation (144). Focal and diffuse proliferative lesions (ISN/RPS III and IV) may present very similarly clinically, but only the latter appears to require intense, long-term immunosuppression. Lesions of activity in lupus nephritis include endocapillary proliferation, necrosis, cellular crescents, and interstitial inflammatory cells. Lesions that indicate chronicity include tubular atrophy, interstitial fibrosis, glomerular sclerosis, and fibrous crescents.

Although assessment of activity and chronicity indices is useful for population groups, these appear to have less absolute information to guide assessment in individual patients. Nonetheless, in large series, assessment of indices of activity and severity in patients with lupus nephritis or other diseases has shown some correlation with prognosis and response to therapy. Diffuse proliferative lesions, extensive crescents, segmental necrosis and tubulointerstitial fibrosis are associated with progression to ESRD (144, 145). The best prognostic indicator in a recent study was the proportion remaining of intact glomeruli in follow-up biopsies (146).

IgA nephropathy was previously thought to have a benign prognosis. In a large series of adult patients with IgA nephropathy, poor prognosis was indicated by segmental glomerulosclerosis, adhesions or crescents, and tubulointerstitial fibrosis (147). Progression occurs in 11–15% of pediatric patients (4, 97, 116, 148). Scoring of activity and chronicity of lesions has been correlated with clinical course. Activity is assessed by degrees of

crescent formation, mesangial proliferation, and interstitial infiltrate. Chronicity is scored by degrees of fibrous crescents, segmental and global sclerosis, tubular atrophy, and interstitial fibrosis (149). High indices of chronic injury and focal segmental glomerular changes were associated with a worse prognosis (150, 151). Histologic features that predicted progression in a recent multicenter study in children were crescents, tubulointerstitial fibrosis, and glomerulosclerosis in 20% or more of glomeruli (141). Predominance of matrix expansion appears to be a later stage of injury, associated with a higher percentage of sclerosis and persistent proteinuria (118). Extension of deposits to glomerular basement areas has also been reported as a poor prognostic indicator (149). A recent International Study Group of IgA nephropathy identified four lesions associated with worse long-term outcome, namely mesangial hypercellularity, i.e., more than half the glomeruli with more than 3 nuclei in a mesangial area, proliferation (either endo- or extracapillary), segmental sclerosis and increasing interstitial fibrosis (120).

Focal glomerulosclerosis superimposed on membranous glomerulopathy has been associated with more severe tubulointerstitial nephritis and a worse outcome. This lesion was present in 20% of children with hepatitis B-associated membranous glomerulopathy (152).

## Renal Transplant Biopsy

The primary use of biopsy in the renal transplantation is to uncover the reason for altered renal function. Causes of renal dysfunction in the transplant can be broadly divided into those related to rejection, drug toxicity, recurrent or de novo disease, and those related to the procedure itself, such as acute tubular necrosis.

### Rejection

Acute rejection is diagnosed by the presence of either interstitial inflammation with lymphocytes and plasma cells infiltrating tubules (tubulitis, the hallmark of acute interstitial type rejection, *Fig. 24-19*), or when more severe, by extension of this process to vessels, with subendothelial arterial or arteriolar infiltration by lymphocytes (endothelialitis, the hallmark of acute vascular rejection, *Fig. 24-20*). The interstitial changes of acute rejection are not pathognomonic. In contrast, the finding of endothelialitis is highly specific for acute vascular rejection. Appropriate stains, such as PAS, must be used to allow visualization of the tubular basement membrane and identification of tubulitis. An adequate specimen for evaluation of possible rejection should contain at least two cores, with at least seven glomeruli and two arteries (153).

Several schemes have been used to diagnose and classify rejection: The Banff scoring system, based on detailed scoring of various components of injury; and the Cooperative Clinical Trials in Transplantation (CCTT) criteria (153, 154). In both classification schemes, acute rejection is based on the presence of tubulitis or endothelialitis, i.e., lymphocytes in the tubule under the tubular basement membrane or underneath the endothelium of arteries. Other inflammatory cells (e.g., eosinophils, neutrophils, and plasma cells), although much fewer in number than T lymphocytes, may also contribute to the infiltrate in acute rejection. Type I rejection in both schemas is diagnosed when interstitial lymphocytic infiltrate and tubulitis are present (>25% of parenchyma infiltrated in Banff, >5% in CCTT) (*Fig. 24-20*). Infiltrate and tubulitis less than specified for type I is called "borderline" by Banff criteria (154). Type II acute vascular rejection is diagnosed in both schemas when there is mild or moderate endothelialitis (arteritis). Severe acute vascular rejection, type III, is diagnosed when there is transmural vascular inflammation and/or fibrinoid necrosis. These types are differentiated not only based on histologic pattern, but also on differences in underlying mechanisms and response to therapies: type I and II are likely T-cell dependent processes and are separated based on the likely greater clinical severity of any rejection when endothelialitis is present, whereas antibody-mediated mechanisms contribute to type III changes.

Identification of acute rejection at earlier stages and thus initiation of treatment at milder levels of injury appear to be clinically important. Thus, mild tubulitis that is borderline by Banff criteria, even in normally functioning grafts, was found to be predictive of higher serum creatinine at follow-up. In contrast, treatment of such subclinical rejection in the early time period after transplantation resulted in better preserved renal function at 24 months (155).

There are no specific IF or electron microscopic immune complexes associated with acute rejection. The recent surge of exciting molecular studies indicates the possibility of earlier, more sensitive, and specific diagnosis of acute rejection using these techniques (see below) (156). In particular, presence of C4d, a complement breakdown product which binds covalently to tissue, in peritubular capillaries, is highly associated with anti-donor antibodies (humoral rejection) (157). Diagnosis of humoral, antibody-mediated rejection has important

**Figure 24-19**

**Acute rejection, classified as type I by Cooperative Clinical Trials in Transplantation criteria. There is interstitial lymphocytic infiltrate with tubulitis, activated lymphocytes, tubular cell injury, and interstitial edema (Jones' stain, × 220).**

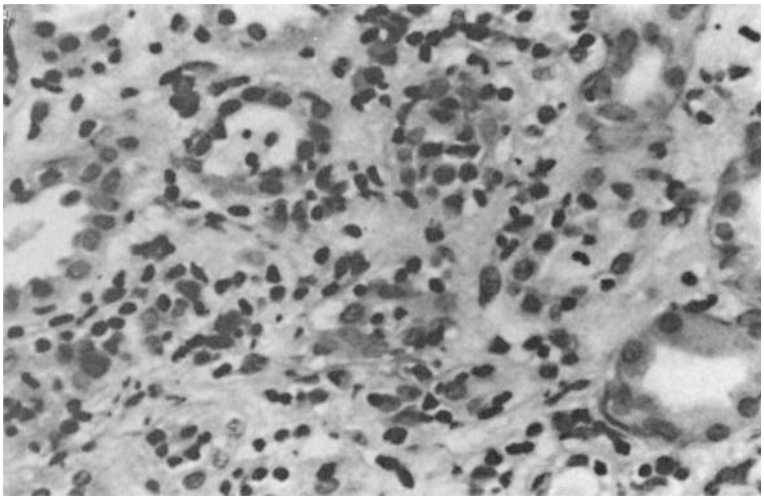
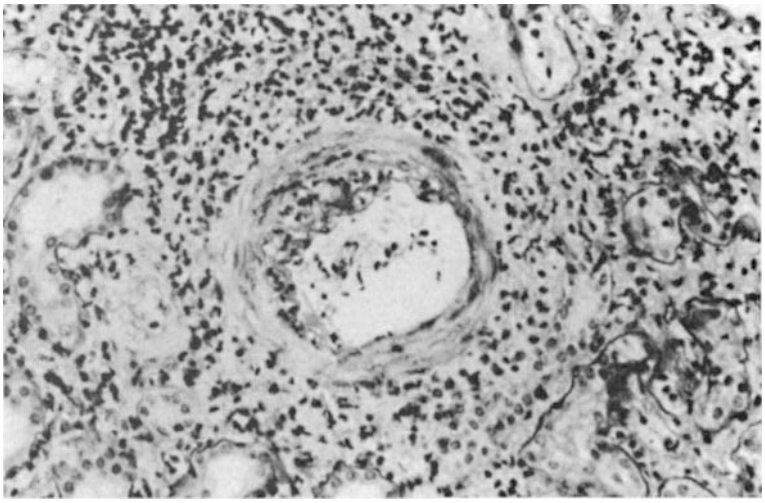


**Figure 24-20**

**Acute vascular rejection, classified as type II by Cooperative Clinical Trials in Transplantation criteria. There is subendothelial infiltration by lymphocytes in this artery, so-called endothelialitis (Jones' stain, × 220).**



therapeutic and prognostic implications. C4d staining can be done on frozen or fixed paraffin-processed tissue, although the latter is less sensitive (158).

The changes of chronic rejection include intimal fibrosis of arteries, interstitial fibrosis, and transplant glomerulopathy (154, 159). A previous or baseline biopsy is necessary to prove that intimal fibrosis is de novo and potentially represents chronic rejection, rather than a preexisting, nonspecific change in the graft. Interstitial fibrosis is also a nonspecific finding and may result from various injuries. Transplant glomerulopathy is a more specific lesion indicative of chronic rejection, i.e., scarring injury related to previous immune injury. By LM, the glomeruli show basement membrane splitting and corrugation, and even segmental sclerosis with hyalinosis. The latter lesion likely resulted in erroneous reports of de novo "idiopathic FSGS" in the transplant. However, in transplant glomerulopathy, there is widening of the

lamina rara interna of the GBM with cellular interposition and new basement membrane formation by EM. Reduplication of basal lamina of peritubular capillaries is suggested to be more specific of transplant glomerulopathy but may also occur in some other glomerular diseases and HUS (160).

## Calcineurin Inhibitor Toxicity

Calcineurin inhibitor toxicity may manifest in various ways. Tacrolimus (FK506) has much the same spectrum of toxicity as cyclosporine (159, 161). The most common morphologic lesion in patients with a clinical diagnosis of cyclosporine toxicity, as verified by clinical follow-up, is that of a normal kidney biopsy morphologically. In these patients, renal dysfunction is due to reversible, calcineurin inhibitor-induced vasoconstriction and hypofiltration. Morphologic changes of calcineurin inhibitor toxicity include arteriolopathy with injury to the endothelium and vascular smooth muscle cells. In its classic form, this injury results in nodular IgM IF positivity along the apical side of the arteriole, with necrosis and smooth muscle cell injury demonstrated by EM (159, 161). By LM, concentric hyalinosis is present, whereas typically eccentric, more segmental hyalinosis is associated with hypertension. Isometric tubular vacuolization in a patchy distribution, although not specific, is also indicative of cyclosporine toxicity. Chronic calcineurin inhibitor toxicity results in a striped distribution of interstitial fibrosis caused by injury along the medullary rays (159). This pattern often cannot be gleaned in small needle biopsies. FSGS with ischemic, corrugated GBMs in remaining glomeruli may also result from calcineurin inhibitor toxicity and can be associated with significant proteinuria (161).

Calcineurin inhibitors have also been associated with thrombotic microangiopathy lesions (see above) (159, 161). Of note, thrombotic microangiopathy can occur in patients who are recipients of transplants of kidneys or other organs and following radiation, with or without calcineurin inhibitor treatment. In some patients, collapsing type glomerulosclerosis may be associated with cyclosporine toxicity, likely representing a response to severe vascular injury and ischemia (162).

## Recurrent and De Novo Disease

Recurrent and de novo disease are important causes of renal allograft injury, affecting approximately 10% of renal allografts (159). Of all graft loss, 2–4% is due to recurrence of disease. IF microscopy should be performed in all transplant biopsies to rule out this possibility. When IF or LM findings in conjunction with the clinical setting indicate an undetermined lesion, electron microscopic study should also be performed.

In children the most common recurrent diseases include IgA nephropathy and Henoch-Schönlein purpura, MPGN, dense deposit disease, and FSGS (163). Although SLE has been reported to recur only rarely, our experience indicates a recurrence rate of approximately 30% (164). However, morphologic recurrence of disease does not necessarily lead to graft loss (159, 163). Dense deposit disease recurs morphologically in nearly all patients, but with only 10 to 20% resultant graft loss. In contrast, HUS has a recurrence rate of 15–25% and 40–50% of these experience graft loss. Although IgA nephropathy recurs in approximately 50% of patients, only 10% of grafts with recurrent disease are lost. MPGN type I recurs in 20–30%, with 10–40% graft loss. FSGS recurs in 20–30% of cases, resulting in graft loss in 30–50% of these. Of note, in recurrent FSGS, the only morphologic change found in the first weeks after recurrence of proteinuria is foot process effacement, with early segmental sclerosis detectable at 6–8 weeks after recurrent NS.

De novo disease may also affect the transplant. Membranous glomerulopathy is the most common de novo glomerulonephritis in the transplant. The etiology remains unknown (159). Glomerulonephritis related to infections, such as hepatitis C-related MPGN, also can occur in the transplant. Early changes of diabetic nephropathy develop much more rapidly in the transplant than in the native kidney and may occur within a few years, whether diabetes preexisted or is corticosteroid-induced (165). Thrombotic microangiopathy may be related to drug toxicity (see above) or be idiopathic in the transplant.

Posttransplant lymphoproliferative disease (PTLD) is due to the unrestrained proliferation of B lymphocytes, most often because of transformation by Epstein-Barr virus, and is an aggressive process, which if untreated, disseminates and may cause death (166). PTLD may respond to decreased immunosuppression. An expansile lymphoid infiltrate with atypical, transformed lymphocytes and serpiginous necrosis are features suggestive of PTLD (166). Immunohistochemical studies can be used to detect Epstein-Barr virus to further support this diagnosis. Typing studies of the lymphocytic infiltrate are not often helpful because most PTLD is polytypic, rather than clonal. Of note, acute rejection and PTLD may be present concurrently.

Polyoma virus nephropathy (PVN), most often due to the BK virus, has increased in the last years in the transplant, perhaps related to increased immunosuppression (76). PVN occurs in both adult and pediatric transplant recipients (167). The biopsy shows a pleomorphic infiltrate with lymphocytes, plasma cells, PMNs and occasional eosinophils, with enlarged tubular cells with smudgy nuclei. Early lesions (stage A) with minimal inflammation, fibrosis or injury have better prognosis than stage B with marked viral changes and inflammation and moderate fibrosis, or stage C, with extensive fibrosis (76). BK infection is confirmed by immunostaining. In some BK nephropathy cases, there may be associated tubular basement membrane deposits staining with IgG and C3 and visualized by EM (168). There may be some response to decreased immunosuppression and antiviral therapy.

## New Methods for the Future

With the recent surge of application of molecular biology techniques to the study of renal disease, candidate factors involved in pathogenesis and progression of disease are being studied in animal models. Studies in human beings have also commenced. With further development of such studies, we may identify specific abnormal processes and thus target therapy more specifically. Research techniques that have been advantageously applied to elucidate pathogenesis of disease include immunostaining; identifying specific antigen in deposits of membranous glomerulopathy in some patients (thyroglobulin with Hashimoto's disease, hepatitis B, C antigen); light chains or paraproteins in plasma cell dyscrasia-associated diseases; identification of specific type IV collagen abnormality in Alport syndrome; C4d as a marker of humoral rejection and elucidation of pathogenesis of specific *E. coli*-associated toxins in some forms of HUS.

Current studies are aimed at understanding disease etiologies and mechanisms at a molecular and proteomic level, studying renal biopsies by laser capture microdissection (LCM), with real time reverse transcription polymerase chain reaction (RT-PCR), and in situ hybridization techniques (99, 169–171). Recent efforts have expanded use of these techniques to study mechanisms of injury and progression. Competitive and real time RT-PCR have been used successfully on small cores and even single isolated glomeruli from human biopsies (99, 169). Modulation of growth factors, collagens, cytokines, chemokines and their receptors has been investigated molecularly in renal biopsies. Cytokines and chemokines and their receptors were upregulated in diseases with macrophage influx and mesangial cell proliferation, supporting an important role in initiating and perpetuating injury. Such approaches can offer exciting new mechanistic insights into renal diseases.

In parallel, genetic studies are targeted both at diagnosis and identifying patients at risk for progression in diseases with a variable course, such as diabetes and IgA nephropathy. Genetic screening for podocin mutations is often done in children with FSGS. Polymorphisms of the renin-angiotensin system genes have been implicated as risk factors for progression and also as indices for response to therapies that target this system (172, 173). Noninvasive urinary proteomic studies are also explored to identify particular signature patterns indicative or predictive of specific lesions or risks (174, 175).

Diagnostic use of RT-PCR and in situ hybridization has focused on detection of viruses, including hepatitis B and C, cytomegalovirus, polyoma virus and Epstein-Barr virus. Increased expression of various immune-activated genes, such as perforin, granzyme and fas ligand, quantified by competitive RT-PCR showed initial high predictive value for acute rejection, but subsequent studies have not been as clear-cut (149).

Together these approaches promise to map risks and mechanisms of disease initiation and progression and point to targets to achieve resolution of injury. Sequential biopsies with evaluation of changes in structure and patterns of abnormal factors and modulation by therapy may be necessary to fully understand pathogenesis. Instead of diagnoses of morphologic patterns recognized by current techniques in the renal biopsy specimen, molecular techniques may allow more precise diagnosis of the specific diseases and identification of injury mechanisms.

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